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Comparative Study between Variable Computer Printed Documents to Identify the Type of the Producing Output Devices

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Abstract

Background: Within the last years, forgery has markedly increased and expanded to encompass computers and printers' usage, their wide evolution facilitated the production of fraudulent printed documents by even non-experts. The printers and photocopiers leave their imprints on the produced documents. Examination of printed output of a questioned document is essential as it provides information about the producing device.

This study aims to compare the characteristics of different computer printed documents to identify the type of the producing output devices (photocopier, inkjet, and laser printers).

Methods: 132 documents were typed and printed using different models of inkjet, laser printers and photocopiers. The produced documents were examined with direct light digital microscope and ultraviolet light and the printed markings were compared.

Conclusion: Our results revealed that each class of the studied printing devices induced specific criteria on the examined printed documents. These criteria are class characteristics but differ from one type of device to another.

Keywords: *Forgery, inkjet printers, laser printers, photocopiers, questioned printed document.*

Introduction

Documents are any material containing marks or signs that convey message to someone. Therefore,

adding, deleting information on it or replacing an entire page is considered forgery⁽¹⁾. Forensic examination of these questioned documents to determine their genuineness is considered a critical and legal issue⁽⁶⁾.

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The examination of the printed matter in questioned documents can provide information about its authenticity and genuineness. It also provides clues for several criminal cases and sources of secure documents leak age⁽³⁾.

The examination of photocopied, laser and inkjet-printed documents provides unique characteristics corresponding to their operating mechanisms. These imprints produced by these devices are the main evidence in examining printed document⁽¹⁴⁾.

The present study aims to compare the characteristics of different computer-printed documents to identify the type of the producing output devices (photocopier, inkjet, and laser printers).

Materials and Methods

Study design and setting:

This is a comparative cross-sectional study conducted at Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Ain Shams University.

Materials:

§ Printing medium: white blank paper (80 GSM, Allam Premium, size A4, 210 X 297mm)

§ Documents were typed using the same computer (Toshiba, Microsoft Office version 2007).

§ Four models of inkjet printers (IJ) were used and labeled:

(i) IJ1: HP Deskjet, ink advantage 2135.

(ii) IJ2: Brother T300.

(iii) IJ3: HP Officejet 4500.

(iv) IJ4: Brother 1100.

§ Four models of laser printers (L) were used and labeled:

(i) L1: Xerox, 5024.

(ii) L2: Ricoh, Aficio, SP C430DN.

(iii) L3: HP 5000N.

(iv) L4: Samsung, ML-1865W.

§ Four models of photocopiers (PC) were used and labelled:

(i) PC1: Xerox 5645.

(ii) PC2: Ricoh, Pro907EX.

(iii) PC3: Ricoh, Pro1107EX.

(iv) PC4: Gestetner, Aficio, MP1100.

§ All printed documents were examined with:

- Paper Thickness Measuring Device, Mitutoyo 7360 Dial Thickness 0-10mm, Japan.

- USB Powered 50-500X Digital Microscope, China.

- Ultraviolet (UV) lamp 365 Mila T5 Electronic Fixture, China.

Samples:

· 132 sample documents were typed on Arabic language. Font used is "Black Simplified Arabic", size "16".

· *The samples were subdivided into 3 subgroups: Subgroup (IJ), (L) and (PC):* 44 samples were collected from every group, 11 samples were collected from each of the chosen models.

· The printed markings were examined and compared with that produced by their same group or other groups.

· **Exclusion criteria:**

- Paper of different type than the selected.

- Documents typed in fonts, sizes, colors, or languages different from the selected.

- Different devices' models other than the selected.

Methods:

1) Documents were examined by the Paper Thickness Measuring Device to get the mean thickness of letters as follows:

(i) Measuring the thickness of the studied document at 4 unprinted areas, and 4 printed areas then their mean thickness (in μm) was calculated.

(ii) Difference between the mean thickness of printed and unprinted areas was calculated to get the mean thickness of letters (in μm).

2) Documents and printed markings were magnified and examined by digital microscope and ultraviolet rays to compare between their characteristic features: brightness, homogeneity, and edges of the printed markings. Scattering of ink or toner, effect of folding and scratching of the paper over the printed markings.

Statistical analysis:

The data was collected, tabulated, and statistically analyzed where:

- Qualitative variables were expressed as percentages.
- Excel computer program was used to tabulate the results of paper thickness and calculate their means.
- Mean and standard deviation were used for description of the variables.
- Analysis of variance (ANOVA) was used to detect and confirm the significant difference between the quantitative variables (mean thickness of papers) for the tested groups.

Ethical consideration: This study was conducted after getting approval of the ethical committee of Faculty of Medicine, Ain Shams University, code

number (FWA000017585).

Results and Discussion

The current study showed that documents produced by different models of the same type of printing devices either (inkjet, laser, or photocopier) displayed no significant difference when the studied printed markings' characteristic were compared. On the other hand, this research suggested that each class of the chosen printing devices induced specific criteria that can be identified over the examined documents as class characteristics which differ from one type of device to the other.

The general criteria induced by different types of the studied printers (Inkjet, laser, and photocopier) over the documents examined by UV rays and direct light digital microscope revealed that inkjet-printed markings were dull, not homogenous, with hairy-like, ill-defined edges as shown in figures (1) and (4). Laser-printed markings were bright, homogenous, with sharp and well-defined edges as shown in figure (2). Photocopied markings were moderately bright, moderately homogenous, with slightly sharp edges as shown in figure (3). These results are shown in table (1).

The dullness of the inkjet-printed marking can be attributed to the chemical composition of the inkjet's ink which is composed of coloring substances dissolved in solvents⁽¹⁰⁾. According to *Shang et al.*⁽¹⁴⁾, the inkjet-printed markings were dull and non-glossy.

The bright glossy appearance of laser-printed markings can be related to the chemical composition of the laser's toner which contains different components like polymers, dyes, surfactants, and wax⁽¹⁵⁾. The toner particles become permanently fixed onto the paper surface when it travels between a fuser and a pressure roller. The polymers and wax are melted by pressure and heat then sprayed on the paper producing images with a shiny surface⁽⁷⁾. The

studies of *Noronha et al.*⁽⁸⁾ reported that laser-printed markings are glossy, homogenous, with well-defined edges.

The non-homogeneity of the inkjet-printed markings can be referred to the mechanism operating inkjet printers as well as ink's liquid nature. Upon computer instructions the ink is jetted in the form of tiny liquid dots from the printer's nozzles over the paper⁽¹⁷⁾. Both viscosity and surface tension of the liquid ink determine drop's behavior on the paper substrate. Viscosity in all cases reduces dot area, liquid penetration, and dot surface coverage; leading to breaking of the ink stream into droplets with tiny spaces between⁽⁴⁾. *Noronha et al.*⁽⁸⁾ found lack of uniformity of inkjet-printed markings and the ink was not consistent throughout the letter.

The hairy like ill-defined edges of the inkjet-printed markings with scattered ink around them could be referred to the printing-head that emit tiny ink droplets onto the printing paper. As the paper is drawn through the printer, the print-head moves back and forth horizontally and sprays the ink directly, leading to irregularities of the markings' edges with scattering of ink around them⁽¹³⁾. At the same time there was scattered ink around these inkjet-printed markings. *Shang et al.*⁽¹⁴⁾ found tails or satellites of dropped ink near the inkjet-printed markings, and their contours were rough with ups and downs. *Noronha et al.*⁽⁸⁾ observed that letters' edges in inkjet-printed documents were rough and looked degraded upon magnification, with apparent smudging in the background. *Krainer et al.*⁽⁴⁾ found that inkjet-printed markings were asymmetrical with unrealistic drop spreading.

Concurrently, the homogenous, sharp, well-defined appearance of edges of laser-printed markings can be explained by the processing technique of laser printers. The computer sends a vast stream of electronic data to the laser printer;

this data carries the figures to be printed. Then a laser beam scans the optical photoconductor drum inside the printer to draw the image of the page and discharges specific locations on the drum, which attract toner particles the toner will be attracted to specific locations on the paper carrying opposite charge. Next, the paper with the toner particles on it passes through the fuser to be fixed. This makes the electronic devices in a laser printer accurately transfer the image signal corresponding to the document. As a result, the printed characters will have clear contours with regular and well-defined edges⁽¹⁸⁾. *O'Connell*⁽⁹⁾ related the regularity of edges to the physical state of the toner as it is a solid fine powder. The physical properties of solids, with closely packed molecules fixed in position, dominate the behavior of the toner leading to its regular definite shape. *Schulze et al.*⁽¹³⁾ recorded that laser-printed letters typically show sharp contours, regular edges with homogeneity of markings. *Shang et al.*⁽¹⁴⁾ confirmed that the laser-printed words have clear contour with regular edges and higher printing quality. Furthermore, *Noronha et al.*⁽⁸⁾ observed that letters' boundaries in laser-printed samples are sharp and significantly refined. The toner deposition in the letters was uniform giving them smooth borders with a considerable thickness and higher resolution.

Additionally, to the previous results, examination of photocopied markings revealed that they were moderately bright and moderately homogenous with slightly sharp edges. *Schulze et al.*⁽¹³⁾ confirmed that it is very challenging to distinguish between laser-printed and photocopied documents as both devices use indirect electrostatic digital imaging to transfer the printing substrate. However, the present study noticed deterioration in quality of photocopied characters in comparison to those printed by laser. This can be explained by two factors. **First**, is how the image is formed on a photosensitive drum: A copier uses a bright light and lens to focus the image of the

original document onto the drum. So, any dimness of light, scratch, or dirtiness over the lens causes reduction of the photocopied markings' brightness and haziness of their edges. While laser printer uses a low power sharply focused laser beam to scan one line on the drum resulting in sharp bright markings. **Second factor** is the developer material (powdered iron) which are attracted by a magnet, if it is of low-quality cheap material, it will produce less bright characters⁽¹⁵⁾. *Shang et al.*⁽¹⁴⁾ observed that toner used in photocopied documents has glossy appearance and the letters had clearer contour than the inkjet-printed characters but had less clear contour than that printed by laser printer.

Scattering of toner on the front of the paper despite its absence around laser-printed markings was due to the incomplete removal of the toner that must be swept using an electrically neutral soft plastic blade that scrapes it off the photosensitive drum into a debris hopper. When this process is not completed, a small portion of the toner' particles remain on the drum surface and are transferred to the paper and form "trash marks" the next time that the printer is used⁽¹⁶⁾.

Scattering of toner on the front and back of plain paper, and its absence around photocopied markings may occur due to the lack of commercial, cheap machines to the 'drum blade' which scrapes residual used toner from the drum, resulting in its precipitation over the drum' surface that gave rise to impressions of residual toner scattered as spots over the paper⁽⁵⁾. *Saroa and Saini*⁽¹²⁾ found that the toner of photocopiers was scattered around the photocopied markings.

Furthermore, folding and scratching had no effect on inkjet-printed markings as shown in figure (1), on opposite to photocopied and laser-printed markings which were broken, removed, and disrupted by folding and scratching, as shown in figures (2) and (3). These results are displayed in table (1). The

mean thickness of letters in documents produced by the different studied printing devices was compared using ANOVA one-way statistical analysis, it showed significant difference between the letters' thickness of computer-typed documents produced by different devices (printers and photocopiers) with a degree of probability of ($P < 0.05$) as shown in table (2).

The non-disruption of the inkjet-printed marking scan be explained by the interrelation between the ink's liquid nature and the paper texture. The projected ink droplets over the paper surface spread and absorbed upon their impact. The complex arrangement of the paper fibers as a network of layers over each other forms a complex porous media with a 3D network of pores. When the ink is injected, it is entangled in between these pores then completely absorbed and stained the paper tissues. So, it cannot be removed either by folding or scratching⁽²⁾. Concurrently, the injected ink was embedded and absorbed by capillarity leading to slight displacement and separation of the fibers' molecules with slight increase in the intermolecular space which will give relative increase in the paper thickness⁽¹¹⁾.

Folding or scratching the paper over laser-printed and photocopied markings resulted into their breaking and disruption. Furthermore, their mean thickness was found to be significantly higher than the unprinted areas. These phenomena resulted due to the physio-chemical properties of the toner used in these machines⁽¹⁵⁾. During the printing process, toner is bonded to the paper by the principle of attraction of opposite charges. Then, it is fused to it by the effect of heat and pressure forming a layer of blocks that increase the thickness of the printed areas⁽⁵⁾. This also facilitated the removal and disruption of the conjoined toner upon folding or scratching of the printed papers^(18, 19).

Table (1): Comparison between the general criteria induced by different types of the studied printers (Inkjet, laser, and photocopier) on the produced computer typed documents Samples were examined by UV rays and direct light digital microscope(N= 132 samples).

Examined criteria over the different printed markings	Inkjet printers		Laser printers		Photocopiers	
	Criteria	%	Criteria	%	Criteria	%
Brightness	dull	100	bright	100	moderately bright	100
Homogeneity	not homogenous	100	homogenous	100	moderately homogenous	100
Edges	hairy-like & ill defined	100	sharp & well defined	100	slightly sharp	100
Ink or toner scattered on the paper	absent	100	present on the front	100	present on the front& the back	100
Ink or toner scattered around printed markings	present	100	absent	100	absent	100
Effect of paper folding	no effect	100	printed markings broke	100	photocopied markingsbroke	100
Effect of scratching	no effect	100	printed markings removed & disrupted	100	photocopied markingsremoved & disrupted	100

Table (2): ANOVA one-way statistical analysis for comparison of the measured mean thickness of letters of computer typed documents produced by the studied printers (Inkjet, laser, and photocopier) Samples were examined by the Paper Thickness Measuring Device(N= 132 samples).

Mean thickness of letters in (µm)	Inkjet	Laser	Photocopier
Mean ± SD	4.926 ± 0.3811	17.14 ± 0.4124	10.82 ± 0.1852
P F	<0.0001 (S) 1280		

SD= standard deviation S= Significant P = probabilityFc means calculated variance ratio

The following figures show photomicrographs displaying the criteria induced by the printer devices on the computer-typed documents. Samples were examined by UV rays and direct illumination of digital microscope. (Mic.Mag 125X).

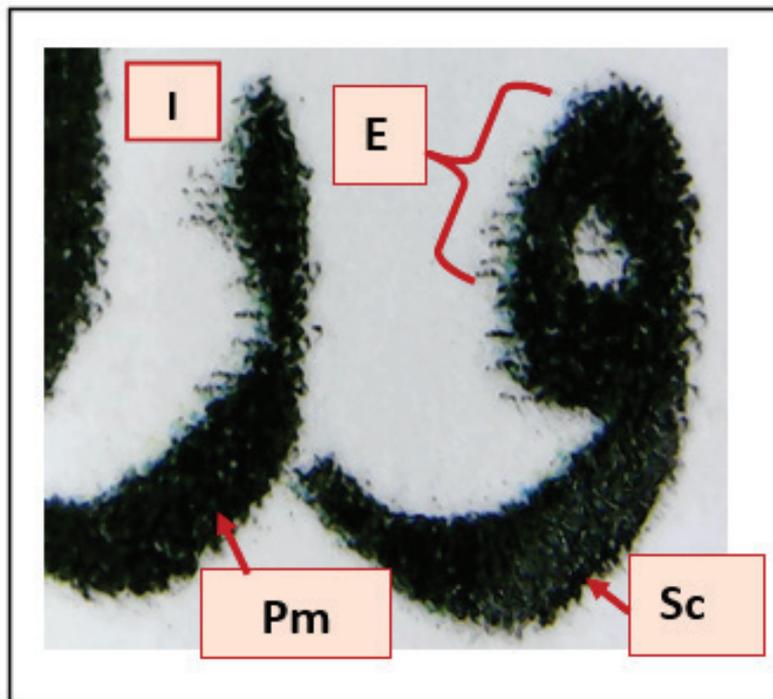


Figure (1): photomicrograph of document produced from an inkjet printer showing:E: edges of inkjet-printed markings are hairy-like & ill-defined, Pm:inkjet-printed markings are dull and not homogenous, I:ink is scattered around the inkjet-printed markings and Sc:scratching or folding the paper over the inkjet-printed markingshadno effect on it.

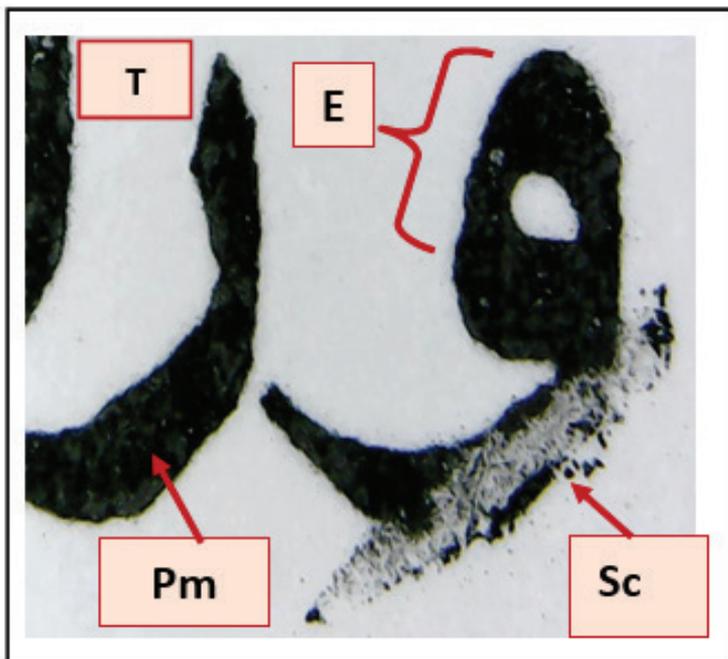


Figure (2): photomicrograph of document produced from a laser printer showing: E: edges of laser-printed markings are sharp and well-defined, Pm: laser-printed markings are bright and homogenous, T: toner is absent around laser-printed markings and Sc: scratching or folding the paper over the laser-printed markings induced its disruption.

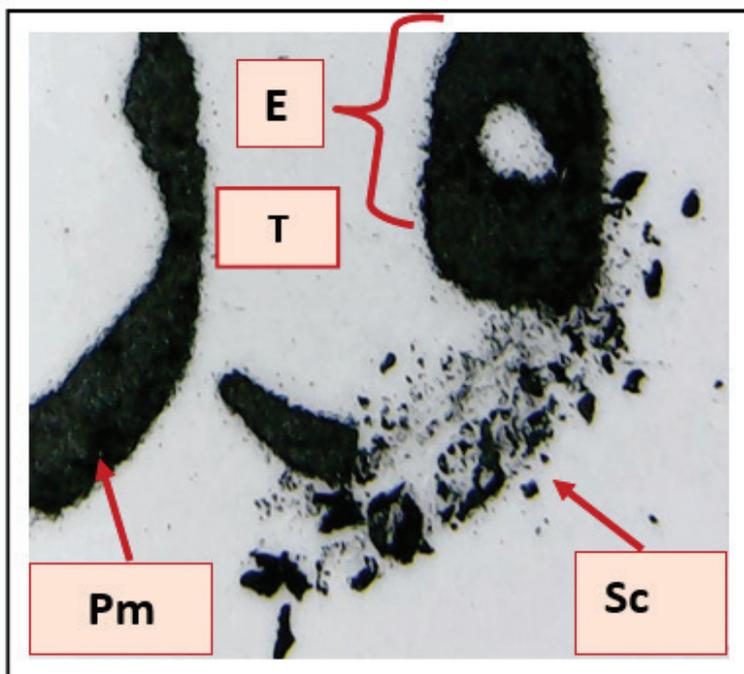


Figure (3): photomicrograph of document produced from a photocopier showing: E: edges of photocopied markings are slightly sharp, Pm: photocopied markings are moderately bright and moderately homogenous, T: toner is absent around photocopied markings and Sc: scratching or folding the paper over the photocopied markings induced its disruption.

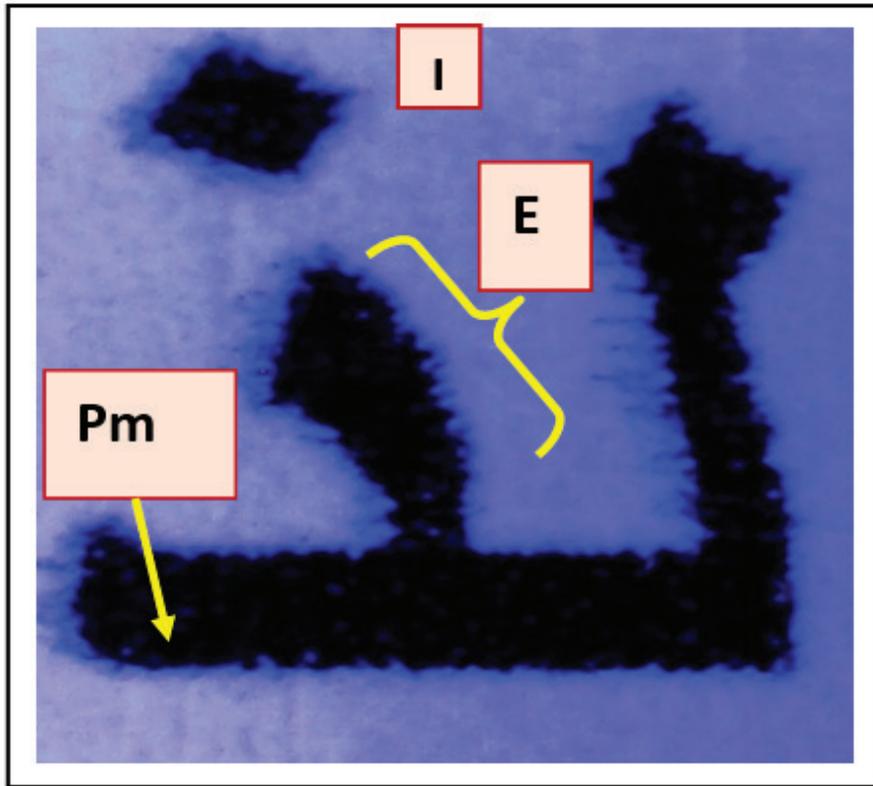


Figure (4): photomicrograph of document produced from an inkjet printer showing examined criteria by using UV light: E: edges of inkjet-printed markings are hairy-like & ill-defined, Pm: inkjet-printed markings are not homogenous and I: ink is scattered around the inkjet-printed markings.

Conclusions

In conclusion, the current study shows that the printed documents produced by different models of the same type of printer devices either (inkjet, laser, or photocopier) displayed no significant difference as regards the studied criteria which are class characteristics but differ from one type of the printing devices to another.

Recommendations:

According to the findings of the present study the following suggestions could be recommended: application of the results of this research to a questioned printed document could help in identification of added word, or statement. Further studies on larger sample size to validate the findings, and more analysis of

the class and individual characteristics of printed documents using other sophisticated techniques.

Conflict of Interest: The authors declare that there is no conflict of interest.

Source of Funding: Self.

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A Correlation Study between Fingerprints and Lip Prints among Twins

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Abstract

Introduction: Fingerprints and Lip prints has its reliability for its uniqueness and can be used solely as an aid in identification for civil and criminal cases. Hence a correlation between the fingerprints and lip prints can bring up a new approach or idea in the field of Forensic Medicine for solving medicolegal cases. The present study was conducted on 25 pairs of twins with the main objective to find any existing correlation between fingerprints and lip prints and to determine the most predominant finger print and lip print pattern among Twin A and Twin B individually.

Materials and Methods: The study was done is subjects age ranging from 6-18 years. A proforma with subject particulars and consent form was prepared. Lip prints were obtained in a drawing chart, the middle part of the lower lip was analysed based on Suzuki and Tsuchihashi classification. Fingerprint of left thumb finger was obtained in a white paper and analysed based on Henry's system of classification.

Results: The present study showed that there was no significant correlation between the Lip prints and Fingerprints in Twins with p value >0.001. Among twin A and twin B the most predominant Lip print pattern was Type I' and the Fingerprint pattern was Loop pattern respectively.

Conclusion: Lip prints and Fingerprints are two important parameters for an individual in identification. There are various studies till now in individuals but very few in twins. So, in this study we made an attempt to find the correlation between the parameters, whether they were existing or not and found that there was no significant correlation yet can be used as a separate tool. Hence it is essential to perform further studies on a larger group and create a database for getting accurate results.

Keywords: Lip prints, Fingerprints, Twins, Correlation, identification.

Introduction

Identification of a person be it living or dead

is based on the fact that each one are unique in their own way. Generally, based on the physical characteristics the identity of an individual can be established, but when it comes to legal issues which requires additional information, various parameters are required to give a confirmatory result. There are many successful and experimental methods which are used to identify the person based on the unique characteristic they possess. It includes anthropometry,

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odontology, dermatoglyphics, fingerprints, age and sex determination, estimation of stature, handwriting, bite marks and DNA analysis¹. These evidences are crucial in both civil and criminal cases to aid the forensic expert to give a conclusion and at times help the police personnel in investigation. The most common physical evidence that stands as a gold standard for identification is Fingerprints. Though, it is an age old method, it has been useful in identification in the illiterates also. Based on its apparent permanence and uniqueness of the epidermal ridges, in every medicolegal work involved and the courtroom acceptance it claims to be a valuable one.

The study of fingerprints is known as Dermatoglyphics or Dactylography or Galton-Henry system³. Since the ridge patterns formed are unchanged, after death even in highly decomposed bodies, with the help of migration of chloride ions, the age of the fingerprint can be assessed whereas in the living the fingerprints can be traced by examining the crime scene, the objects handled. But in the present scenario, criminals are more aware and knowledgeable about the evidences they leave behind especially the fingerprints and try to conceal them. So, an alternative and useful technique which is not much in vogue are Lip prints, left on the materials used, the surfaces touched, the places in contact with and also over the skin of the victim in sexual offence cases.

Cheiloscopy is the study of grooves and furrows present on the zone of human lip³. This method of analysis is similar to the concept of fingerprints hence it serves as an ancillary method. To establish the identity, it is not mandatory to collect direct lip prints, the prints which are produced by the secretions of sebaceous and sweat glands on the edges of the lips, produces latent prints which can be developed later by various techniques using magnetic powder, aluminium powder, Sudan III stain etc. There are many successful studies and detailed research done

on fingerprints and lip prints individually, whereas a combined studies with the two parameters are very few. Moreover, the target study involving the tools of identification among twins, is relatively very less and has left a void in the research field. So, this study focuses on the twin population using fingerprints and lip prints as the source of identification. Since both Cheiloscopy and Dermatoglyphics play a vital role in identification, a correlation study between both, may help in investigations when either of the evidence or both were found, as studies with their association is very scanty, especially in twins is rare in literature. Reference data including various parameters are of paramount importance in solving forensic cases². The main objective of the study is to find the correlation between the fingerprint and lip print pattern among Twin A and Twin B, also to determine the commonest fingerprint and lip print pattern among them and to know the most frequent the rarest combination.

Materials and Methods

The present study was conducted on 25 pairs of twins with age ranging from 6-18 years. The elder one was considered as Twin A and the younger one was taken as Twin B. A proforma was made with particulars like Name, Age, Sex, Date of birth, address, phone number along with a consent form, a white paper and a drawing chart attached. Before heading with the procedure informed consent was taken from the parents in case of minors. Then the procedure was explained verbally in detail, regarding imprinting the fingerprint and lip prints. For obtaining the fingerprint the subject was asked to wash hands thoroughly with soap and water, dry them using the towel. Then they were asked to press the left thumb on to the stamp pad and then keep it over the white paper to imprint the fingerprint. Necessary precautions were taken from sliding of fingers to avoid smudging of the prints. The subjects were asked to relax the arm and not to try to help in rolling the fingers as it may cause smearing.

The fingerprints were obtained and were studied with the magnifying lens. The findings were based on the Henry's system of classification.

1. Loop

2. Whorl

3. Arch

4. Composite



Arch (A)



Loop (L)

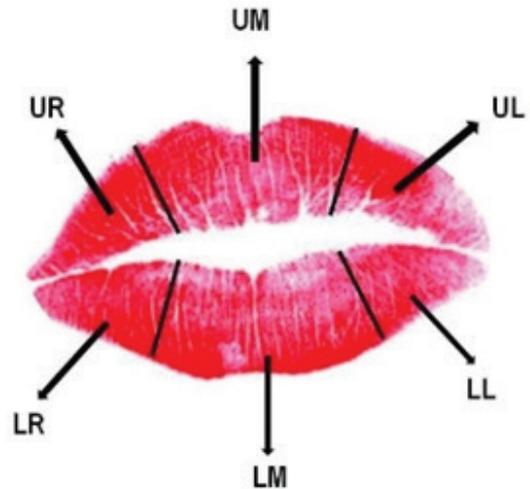


Whorl (W)

In composite variety, there is a combination of more than one pattern either a loop or whorl pattern or two different loop patterns. To avoid confusion, the subjects with composite pattern of fingerprints were excluded from the study.

(10mm wide) was considered as the study area as this was suggested by *Sivapathasundaram et al⁴*. Since this part of lip is mostly the visible area in any trace, based on the numerical superiority of properties of lines the observations are made to determine the pattern.

For taking the lip prints the subject was made to sit on a stool in front of a table and was advised not to move so that recording of lip prints will be accurate. The subject was asked to keep the mouth closed, lip muscles relaxed and record the lip print. A dark colour lip stick was applied all over the lips up to the lip line and the individual was made to bend forwards and imprint the lip on the drawing chart, press it firmly forwards and roll it sideways to right and left side respectively. The drawing chart was air dried for few minutes and then visualised using the magnifying lens for the grooves and wrinkles on lip prints. The findings were based on KazuoSuzuki and Tsuchihashi Classification⁵. The middle part of the lower lip (LM)



Type I	A clear-cut line or groove running vertically across the lip
Type I'	Straight grooves that disappear half way into the lip instead of covering the entire breadth of the lip or partial-length groove of Type I
Type II	Grooves that fork in their course or a branched groove
Type III	An intersected groove
Type IV	A reticular groove
Type V	Grooves that do not fall into any of the above categories and cannot be differentiated morphologically

After recording the fingerprint and lip print patterns from the subjects, the data was entered in MS Excel 2010 and then analysed using statistical package SPSS version 22 for windows and considering p value <0.001 statistically significant.

Results

TABLE 1: DISTRIBUTION OF LIP PRINTS IN TWIN A AND TWIN B

LIP PRINT PATTERN	TWIN A	TWIN B
I	4(16%)	6(24%)
I'	10(40%)	7(28%)
II	5(20%)	6(24%)
III	4(16%)	5(20%)
IV	2(08%)	1(4%)
V	0	0

Table 1 shows the overall distribution of lip prints observed in the study subjects on the middle art of lower lip.

TABLE 2: DISTIRBUTION OF FINGERPRINTS

PATTERNS OF FINGERPRINT	TWIN A	TWIN B
Loop	11(44%)	13(52%)
Whorl	08(32%)	10(40%)
Arch	06(24%)	02(08%)

Table 2 shows the overall distribution of fingerprints in the study subjects of the left thumb finger.

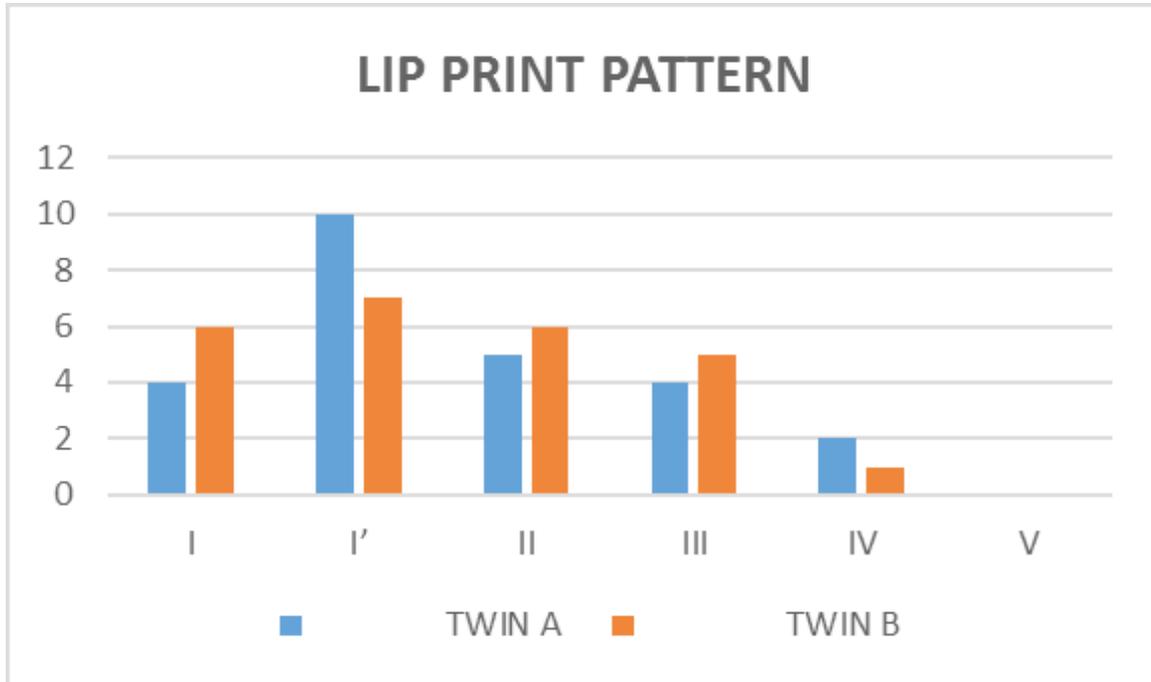


FIGURE 1: COMMON LIP PRINT PATTERN IN TWIN A AND TWIN B

Figure 1 shows the predominant lip pattern among both Twin A and Twin B is Type I' while the less common being the Type IV pattern and the rarest in Type V pattern.

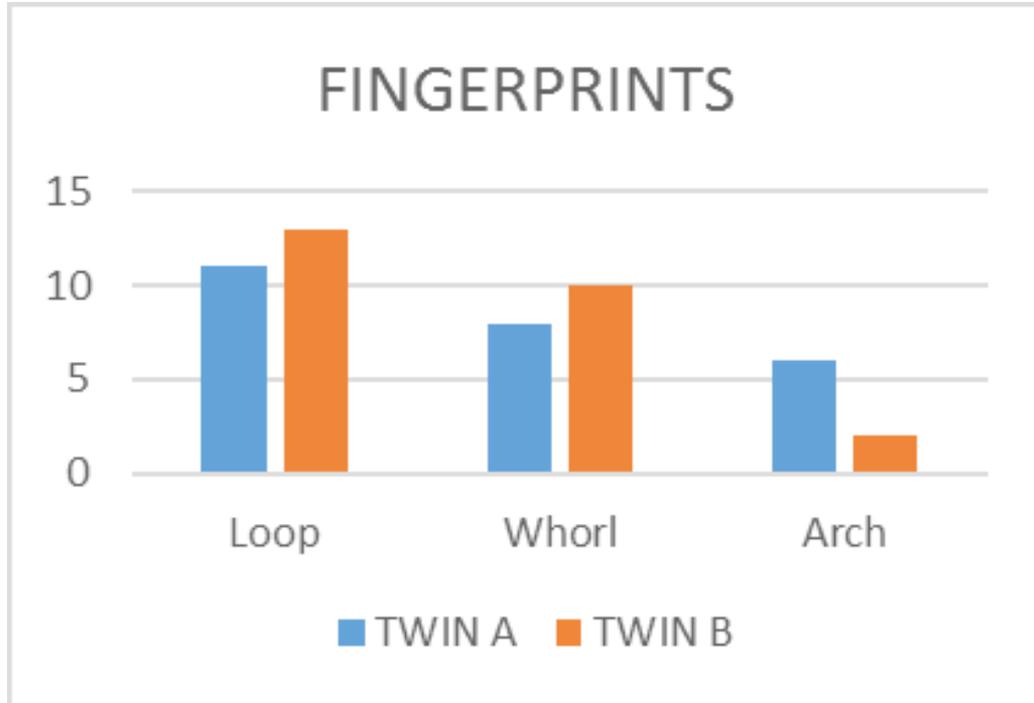


FIGURE 2: COMMON FINGER PRINT PATTERN IN TWIN A AND TWIN B

Figure 2 shows the predominant fingerprint pattern observed in Twin A and Twin B is Loop pattern and the less common is the Arch pattern.

TABLE 3: CROSS TABULATION OF LIP PRINT PATTERN - FINGER PRINT PATTERN IN TWIN A

LIP PRINT PATTERN		FINGERPRINTPATTERN			Total
		LOOP	WHORL	ARCH	
	I	2	2	0	4
	I'	6	0	4	10
	II	1	4	0	5
	III	2	1	1	4
	IV	0	1	1	2
Total		11	8	6	25

TABLE 4: CORRELATION OF LIP PRINTS WITH FINGERPRINTS IN TWIN A

	Value	df	p value
Pearson Chi-Square	13.442 ^a	8	0.098
			(>0.001)

TABLE 5: CROSS TABULATION OF LIP PRINT – FINGERPRINT IN TWIN B

LIP PRINT PATTERN		FINGERPRINTPATTERN			Total
		LOOP	WHORL	ARCH	
	I	5	0	1	6
	I'	4	2	0	6
	II	2	3	1	6
	III	2	4	0	6
	IV	0	1	0	1
Total		13	10	2	25

TABLE 6: CORRELATION OF LIP PRINTS WITH FINGERPRINTS IN TWIN B

	Value	df	p value
Pearson Chi-Square	9.455a	8	0.305
			(>0.001)

Discussion

In the present study, the overall distribution of lip print pattern was observed to be in the order of the sequence showing Type I' being the most common pattern followed by Type II, the least common was Type IV and Type V was not present in any of the subjects. In Twin A, the predominant lip print pattern was found to be Type I' with 40% followed by Type II with 20%, Type I and Type III with 16% distributed equally, and the least common was Type IV pattern respectively. Among Twin B Type I' pattern was the most predominant pattern with 28% followed by Type I and Type II with 20% equally distributed, the least commonly seen pattern was Type IV with 4%. However, the study conducted by Suzuki and Tsuchihashi on 18 pairs of twins showed Type III was the most common pattern⁵. Another study done by Bhavna Thakur et al on 40 pairs of twins showed Type III was the predominant pattern⁶. The study done by Fakir Mohan Debta et al on 30 pairs of twins resulted with Type II being the predominant pattern followed by Type IV pattern⁸. But our observations were based on analysis of only the middle part of lower lip whereas, the above mentioned studies followed the four quadrant method to analyse all the quadrants of the lips. Hence our results are not concordant with the previous studies.

On observation of the fingerprints of the left thumb, the present study showed the most common finger print pattern is Loop, followed by Whorl pattern and the least being arch pattern. Among Twin A the predominant fingerprint pattern was loop with 44% followed by whorl with 32% and arch pattern with 24%. In Twin B the most common pattern is Loop with 52% followed by whorl with 40% and the least pattern observed is arch with 8%. These observations were similar to the study conducted by Murad Ali et al on 30 pairs of twins, in which they found the loop pattern was common with 62.3% followed by whorl with 29.93% and the least being the arch pattern with 7.6%⁷. On reviewing the literature it showed that the frequency of distribution of fingerprint pattern seemed to follow the same order of predominance irrespective of age and sex.

On assessing the relationship between the Lip print and Fingerprint patterns in twins by using chi square test it is found that there is no significant correlation with the two parameters since p value > 0.001. However there are few studies which have been done in twin population to assess the inheritance from the parents to the twins by correlation using multiple parameters and have found out mixed results with both positive correlation and negative correlation for few. There are many studies which are based on correlation between fingerprints, lip prints, palm

prints, blood groups done on males and females. But to our best knowledge and on reviewing the literature this is the first study of its kind to study the correlation of fingerprints and lip prints in twins.

Conclusion

The results revealed that fingerprints and lip prints are unique structures so they are considered a never failing tool for identification individually. Though the present study resulted in an insignificant correlation between lip prints and fingerprints in twins, it is still suggestive that they can be used as individual parameters or a aid in identification. In case of very few evidences left behind it can definitely be used to support certainty in crimes though the correlating factor may not be precise. It is a simple attempt to establish correlation between fingerprints and lip prints in twins which is uncommon, yet can also be done in other subjects as well for further clarity and better understanding. Nevertheless, further studies in twins with a larger sample size, using a basic database for the tool of identification, will be essential in interpreting the results accurately.

Conflict of Interest: Nil.

Source of Funding: Self.

Ethical Clearence: Not Required.

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Type of Article- **Original Research Article**

Demographic Study of Blunt Trauma Chest in Varanasi Region

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Abstract

The mortality rate of chest trauma is 10%. Incidence has significantly increased due to development of rapid mode of transport, new high speed vehicles, ignorance of road safety measures, fall from height, fall on road injuries due to construction activities violence, fall from under construction building. Human fatality associated with chest injuries worsening each year.

This drew the impact on reducing burden of chest trauma, prevention and its measures at social, medical, economical, and governmental. So that understanding the problem is the one of the first step towards awareness and averting the problem.

Present study has been undertaken to analyse the most neglected aspect of human suffering. It is an effort to know the pattern of blunt trauma chest and to elucidate the multi-factorial causations leading to rise in everyday blunt trauma cases.

Here in this paper 300 cases of chest injuries by blunt forces are studied for their epidemiological, demographic aspects. Majority of them are adult/middle ages male between 41 -50 years of age, most of them are caused by road traffic accidents. Male to female ratio is 7.1:1. Majority of the cases are happened on highway.

Keywords- Blunt Trauma Chest, Demographic Data, Death

Introduction

Leading cause of death in India is trauma. The third most common cause of traumatic death is thoracic trauma after head and spinal injury. The incidence of thoracic injury cases is reported 10% of trauma admissions and mortality rate varies from 10%-60 %⁽¹⁻⁵⁾. Although the trauma related injuries

can involve many part of body, but one out of four trauma patient die mainly due to thoracic Injuries or its complications⁽⁶⁾.

It is hard to evaluate the mortality rate as the cause of death of blunt trauma may be due to pulmonary or non-pulmonary complications and associated injuries⁽⁷⁾. 10% or less of blunt trauma cases requires surgical treatment and the remaining patient can be managed conservatively^(8,9).

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Blunt trauma is defined as injuries where organ and structures are injured without disrupting tissue

integrity⁽¹⁰⁾.

Road traffic accident like endemic disease which mainly affect our youth or young populations, currently motor vehicle accident rank 9th in relation of disease burden and they are projected to be ranked 3rd by 2020⁽¹¹⁾.

Approximately 16000 deaths per year occur alone are the result from chest trauma in India⁽¹²⁾

Here in this study the epidemiological, demographic data aspects of blunt trauma chest are studied in the cases brought to mortuary of department of forensic medicine IMS, BHU, Varanasi for post mortem examination.

Material and Methods

The present study was done on the cases selected from dead bodies brought into mortuary of department of forensic medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi for medico legal postmortem examination in the year of 2018-2020. Badly decomposed where significant injuries are not visible not included in these study.

The data of the materials were sourced from 300 cases of blunt trauma chest. The victims information and history of circumstances of injury sustained were gathered from the interviews of relatives and of persons accompanying them if they had the first hand information of the sequence of events leading to such fatalities.

Observations and Results

The study includes 300 cases and its aims to study the demographic data of blunt trauma chest in Varanasi region. Out of 300 cases studied, 254(88%) were males and 36(12%) females, commonest age group involved was 41-50years(20.3%), followed by 21-30 and 31-40 years of age had second highest toll list. Thus all these age group combined, 21-50 age had

a 181(60.33%) cases out of 300cases.

TABLE-1 indicates age wise distribution in male and female sex.

Male : female ratio was 7.1:1. Females in the age group of 41-50years comprised 10(27.78%) cases followed by 31-40 years of age. This signifies that 20-50 years of age-group most dependent part and base of our economy and their tragic lives lost of most active, productive and economically promising group of population. A higher incidence of fatalities in adult age group (21-50years) because the people from these age group are more often required to move out in pursuit of their work and studies this age group is most active phase of life and they have a tendency to take risk. The preponderance of male over female in blunt trauma chest may be explained by the fact that mostly males are generally doing work for earning their family requirement. Males have a more percentage of doing job in comparison to that of female.

TABLE -2 indicates habitat of victims. It is observed that 184 (61.3%) cases were from urban area and 116 (38.7%) cases from rural area. Most of the people used their own vehicle in urban areas.

TABLE -3 indicates the religion wise distribution of victims. Most of the victims belonged to Hindu religion that was 284(94.66%) and 16(5.3%) cases were from Muslim. In Varanasi district, there is dominance of Hindus over Muslims population, so the Hindus were most commonly involved in incidence in present study.

Table- 4 indicates the Month Wise Occurrence of Cases. Highest number of accident were observed in the month of August 68(22.67%), 42(14.00%) in September, 39 (12.0%) in November and least number of accident occurred during the month of February 1(0.33%).

Highest number of accidents occurred in the duration of august to December month.

TABLE-5 shows place of accident type of Road used workplace where accident mostly happened. Fatalities on highways were dominated my heavy motor vehicles .Highway mainly involved with 95 (31.67%) cases. Light motor vehicles cause brutality maximum in inside cities 58(19.33%) cases ,single lane was involved in 65 (21.67%) cases ,people who work in under construction building for earning their livelihood and workplaces , injuries occurred in this place was 40 (13.33%)cases ,house was involved in

15 (5.00%) cases. People not taking any precaution while crossing railway track, railway track involved in 27 (9.00%) cases.

TABLE-6 indicates the time of accident. Fatal accident in peak hours that was 9-122 hours.100(33.33%)cases occur in morning peak hours, afternoon (12-16hours) and early morning peak hours (5-9hours) had 60(20%) and 56(18.67%).On single lane road too evening peak hours(16-20hours)also had 41(13.67%) case thus risk of traffic accident was more during morning and evening peak hours in night hours 20-1hours there was 24(8%)cases.

TABLE1: AGE WISE DISTRIBUTION IN MALE AND FEMALE SEX

Age in year	Male		Female		Total	
	No.	%.	No.	%.	No.	%.
0-10	1	0.38%	0	0%	1	0.3%
11-20	31	11.74%	3	8.33%	34	11.3%
21-30	55	20.83%	5	13.89%	60	20.0%
31-40	53	20.08%	7	19.44%	60	20.0%
41-50	51	19.32%	10	27.78%	61	20.3%
51-60	44	16.67%	5	13.89%	49	16.3%
61-70	28	10.67%	5	13.89%	33	11.0%
71-80	0	0.0%	1	2.78%	1	0.3%
80-70	1	0.38%	0	0%	1	0.3%
Total	254 88.0%		36 12.0%		300	100%

TABLE 2: HABITAT

Habitat	Frequency	Percentage
Urban	184	61.3
Rural	116	38.7
Total	300	38.7%

TABLE 3: RELIGION WISE DISTRIBUTION

Habitat	Frequency	Percentage
Urban	184	61.3
Rural	116	38.7
Total	300	38.7%

Table 4: MONTH WISE DISTRIBUTION OF CASES

Month	Count of month of accident	Count of month of accident
August	68	22.67%
September	42	14.00%
October	31	10.33%
November	39	13.00%
December	22	7.33%
January	3	1.00%
February	1	0.33%
March	7	2.33%
April	27	9.00%
May	24	8.00%
June	31	10.33%
July	5	1.67%
Grand Total	300	100%

Table 5: PLACES OF ACCIDENT

Place of accident	Frequency	Percentage
Highway	95	31.67
Single lane	65	21.67
Inside city	58	19.33
Under construction building/workplace	40	13.33
House	15	5.00
Railway tract	27	9.00
Total	300	100

TABLE-6 TIME OF INCIDENT

Time of incidence	Frequency	Percentage
1-5hours	19	6.33%
5-9hours	56	18.67%
9-13hours	100	33.33%
13-17hours	60	20%
17-21hours	41	13.67%
21-1hours	24	8%
Total	300	100

Discussion

Chest injuries are fatal when it involves solid organ and more than one –third of blood loss occur.

It involves major vital organ heart, lungs, due to blunt force injuries circulation and respiration is obstructed.

Driving and walking was in haphazard manner, there is no provision of left side walking ,Vehicle

occupants not driving in their lanes, that's the reason collision of motor vehicle with light motor vehicle and pedestrian vehicle accidents.

There should be strictly follow the traffic rules and taking safety measures. There should be heavy amount of fine on vehicle occupants so there is no repetition of mistakes and safety saves life.

Kochar MP et al (2013) concluded that most of the patient of under the age group of 11-30year. This result is different in our study 21-50years of age⁽¹³⁾.

Gummadi et al (2017) in their result maximum number of cases occur in august month same as in our study mentioned, but least during October month but in our study least in February.⁽¹⁴⁾

Olaejirinde O. Olaefe et al (2000-2009) in their study maximum accident in intercity road (88%) as compared to intra city (21.3%).it is different from our study⁽¹⁵⁾

Conclusion

The highest numbers of death were in the 21-50 years of age group 60.33%. The incidence were lower in older age group above 70 year 0.6% followed by below 10 years of age group (0-10years) was 0.3%. Male comprises majority of cases 88.0% of the cases and only 12% were female. Most of the cases were Hindu 94.66%. The Muslims were involved in 5.3% of total cases.

Maximum number of blunt trauma cases occurred in urban area 61.3% and in rural area was 38.7%. Maximum number of blunt trauma cases occurred in the month of August 22.67% followed by September 14%, least number of cases occurred in February i.e.0.33%. Maximum number of accident risky since morning peak hours 9-12hour in 33.33% cases and least blunt trauma cases happened in 1-4hours in night hours in 6.33% cases. Maximum number of cases occurred on highway 31.67%, least number

of cases occurred in house 5.00%.

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Legal Aspects of Maternity Nurses performing Childbirth Assistance Measures in the Framework of Carrying Out Doctor's Duties Against the Birth Process in Breach Locations

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Abstract

Health is a human right, meaning that everyone has the same rights in obtaining access to health services and professionalism from health workers. Nurses are the most numerous health workers in Indonesia. Nursing staff who perform nursing actions must be following the competence of nurses and Law Number 38 of 2014 concerning Nursing. The purpose of writing this article is to find out how the legal aspects of maternity nurses who carry out delivery assistance actions carry out the doctor's duties in the Breach delivery process. By using the normative juridical research method, it can be concluded that the competence of the maternity nurse is to assist childbirth but does not have the authority to provide delivery assistance, especially in cases with fatal risks that can cause death to the mother and fetus. When referring to the criminal provisions of Law Number 36 of 2009 concerning Health Workers, Article 84 paragraph (1), any Health Worker who commits serious negligence resulting in serious injury to the Health Service Recipient shall be sentenced to a maximum imprisonment of 3 (three) years. Then in paragraph (2), namely If the gross negligence as referred to in paragraph (1) results in death, each Health Worker shall be sentenced to a maximum imprisonment of 5 (five) years.

Keywords: *Legal aspects; Nurse; Authority; Breach delivery.*

Introduction

Health is a very basic and important thing to strive for. Health is very meaningful and priceless, especially the health of mothers and children. Human life requires health aspects that are taken care of. Therefore, health is one form of human right, in this case, the right to have optimal health, and the state is obliged to fulfill this right.

The right to obtain services and protection for maternal and child health is a form of basic right that has been stated in the 1945 Constitution of the Republic of Indonesia. According to Article 23 of Law Number 36 Years 2009 concerning Health, it is stated that health workers are authorized to provide health services.

Article 28 H, Paragraph (1) "everyone has the right to live in physical and spiritual prosperity, to have a place to live, and to have a good and healthy environment and have the right to obtain health services". Paragraph 3 "Everyone has the right to social security that allows his full development as a dignified human being". Article 34: "The poor and neglected children are cared for by the state".

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In line with the mandate of Article 28 H paragraph (1) of the 1945 Constitution of the Republic of Indonesia, it has been emphasized that everyone has the right to obtain health services, then in Article 34 paragraph (3) it is stated that the State is responsible for the provision of adequate public service facilities. And also in Article 9 paragraph 3, Law of the Republic of Indonesia Number 39 of 1999, concerning Human Rights, as follows: "Everyone has the right to a good and healthy environment".

The form of activities and efforts to improve the degree of public health must be carried out based on professional, non-discriminatory, participatory, protective, and sustainable principles which are very important for the formation of Indonesian human resources, increasing the nation's resilience and competitiveness, as well as national development. Improving human resources in medical matters is one form of effort to improve the health of citizens.

According to Article 23 of Law Number 36 of 2009 concerning Health, it is stated that health workers are authorized to provide health services. However, in this paragraph, it is explained that the authority referred to in this paragraph is the authority granted based on education after going through the registration process and granting a permit from the government following the legislation.¹

Every health worker must pay attention to the standards that apply in his profession. As one of the health workers, maternity nurses in carrying out their practice must be following the authority based on their competence. In carrying out their duties, maternity nurses must also comply with the Nursing Code of Ethics. The Nursing Code of Ethics is a comprehensive statement in the profession that guides nurses to carry out nursing practice properly, especially those related to the welfare of their family, community, colleagues, profession, and themselves.

One of the health indicators of a country is the Maternal and Child Mortality Rate. Every day in 2017 around 810,000 mothers died due to childbirth. 94 percent of all maternal deaths occur in low- and middle-income countries. Currently in Indonesia, the Maternal Mortality Rate is still quite high, Indonesia occupies the third position of the highest Maternal Mortality Rate in 2017 with 177 deaths per 100 thousand births.

Breach position is one of the causes of maternal death, where the position of the fetus is elongated with the head located in the uterine fundus and the buttocks occupy the lower part of the uterine cavity. Breach position occurs in 3-4% of deliveries in Indonesia. Perinatal mortality in Breach position is 13 times higher than perinatal mortality in cephalic presentation.²

The government has established a National Health System, one of the goals is to reduce maternal mortality, including implementing the Making Pregnancy safer (MPS) strategy or "making pregnancy safer", Placement of village maternity nurses (Contract maternity nurses), Establishment of standby villages, the establishment of village health posts and the *Jamkesmas* program.³

Based on the background that has been described, the problem in this study is how to regulate the law for maternity nurses in childbirth services. And the purpose of this study is to enforce law enforcement against violations of maternity nurses and their legal consequences because maternity nurses already have the authority and standards of nursing practice. This is done to limit the authority following applicable regulations. Maternity nurses know and can implement their responsibilities following existing regulations without exceeding authority following their competence.

Discussion

Obligations of Maternity Nurses in Carrying Out Labor Actions

As a part of professional health workers, maternity nurses carrying out their practice must be carried out following guidelines and authorities based on their competence. The moral responsibility of the maternity nurse is to carry out the practice following the norms contained in the maternity nursesociety so that it does not conflict with the oath that has been uttered when the maternity nurse graduates from education to be able to provide nursing care to the community. A review in terms of ethical responsibility from the duties of a maternity nurse is a form of responsibility that applies in carrying out their duties in the form of carrying out nursing activities given to provide services to the community following the code of ethics. Nurses are obliged to provide nursing services following professional standards, standards of nursing practice, code of ethics, and SOPs as well as the needs of clients or patients where professional standards, practice standards, and codes of ethics set by professional organizations are guidelines that must be followed by every nursing staff to implement the nursing practice.

Nurses who carry out their duties are required to refer clients and or patients to health care facilities that have better skills or abilities if they are unable to carry out an examination or action. This also depends on the situation, if our environment also does not allow then we as nurses can explain the right reasons. Nurses are obliged to keep everything they know about clients and/or patients confidential, except for legal purposes. This concerns the privacy of clients who are in nursing care because on the other hand nurses are also obliged to respect the rights of clients and/or patients and other professions following applicable rules and regulations. The obligations of maternity nurses themselves refer to the implementation of nursing on

the standards of nursing care for mothers and children as stated in the Regulation of the Minister of Health Number 10 of 2015 concerning Nursing Service Standards in Special Hospitals.

Article 37 of the Nursing Law states that nurses must complete nursing service facilities and infrastructure following nursing service standards, provide services, refer clients to other nurses who are appropriate according to their expertise, make documentation of nursing care, provide complete, honest, correct, clear, and easy to understand information regarding nursing actions to clients or their families following the limits of their authority, carry out acts of delegation of authority from other health workers and or medical personnel following the competence of nurses, and carry out special assignments determined by the government. With this explanation, nurses can carry out nursing service standards, which if an error occurs, can be held accountable. For this reason, nurses have rights and obligations to have a relationship with the community and are protected by law, and nurses are obliged to obey this relationship. Actually, in the obligations of the maternity nurse, it is not stated that the maternity nurse may provide delivery assistance. However, in reality, maternity nurses have competence in providing delivery assistance. Competency in delivery assistance is given to maternity clinic nurses II if there are no health workers authorized to provide delivery assistance. Health services for the community are carried out by health workers. Article 1 point 6 of Law Number 36 Years 2009 states that a health worker is every person who devotes himself to the health sector and has the knowledge and/or skills through education in the health sector which for certain types requires the authority to carry out health efforts.

The Legal Responsibility of The Maternity Nurse in Carrying Out the Act of Breach Delivery

Criminal law provides an understanding that accountability means that every individual who violates the provisions of criminal law is legally obligated to account for the actions he has committed following statutory regulations. So that in another sense every mistake will be criminally responsible following the portion of the error committed. The error must meet three elements, namely:

1. The ability to be responsible means being in good physical health.
2. Is the act a form of intentional (*dolus*) or negligence (*culpa*).
3. There are no excuses or erasers of guilt.⁴

Maternity nurses must carry out their responsibilities following ethics and morals. Maternity nurses in carrying out their practice must be carried out following their authority based on their competence, namely to assist in childbirth but do not have the authority to provide delivery assistance. Article 30 paragraph 1 letter g of the Nursing Law states that nurses are authorized to take action in emergency situations according to their competence. So that when in an emergency situation, maternity nurses are allowed to assist in childbirth. Directly, criminal legal responsibility will be given to the maternity nurse if the action taken is not in an emergency condition and there is a *culpa lata* or a conspicuous error and can result in permanent or non-permanent disability and also death in carrying out the delivery assistance. If during delivery assistance by the maternity nurse is not in an emergency and *culpa lata* occurs, then the criminal responsibility that can be imposed on the maternity nurse refers to the criminal provisions of the Health Personnel Law Article 84(1) Every Health Worker who commits gross negligence resulting in serious injury to the Health Service Recipient shall be sentenced to a maximum imprisonment of 3 (three) years. (2) If the gross negligence as referred to in

paragraph (1) results in death, each Health Worker shall be sentenced to a maximum imprisonment of 5 (five) years. The regulation of medical actions in general in Law No.23/1992 on Health can be seen in article 32 paragraph (4) which states that “the implementation of treatment and or care based on medical science and nursing science can only be carried out by health workers who have the expertise and authority to administer medical care and treatment. that”. These provisions are intended to protect the public from the actions of someone who does not have the expertise and authority to carry out treatment/care so that the consequences that can be detrimental or harmful to the patient’s health can be avoided, especially medical actions that contain risks. The regulation of the authority of health workers in carrying out medical actions is regulated in article 50 of Law No.23/1992 on Health which formulates that “health workers are tasked with organizing or carrying out health activities following the fields of expertise and or authority of the health workers concerned”.

Criminal acts by health workers can occur if, in the practice of health services, every person who is not a health worker or a health worker himself does things as regulated in Article 83 to Article 86 of Law Number 36 of 2014 concerning Health Workers and the types of acts that carried out as follows: 1. Everyone who is not a health worker practices as if he were a licensed health worker; 2. The health worker commits serious negligence which results in the recipient of the health service is seriously injured or dead; 3. Health workers practice without having STR; 4. Health workers of foreign nationals who intentionally provide health services without having a temporary STR; 5. Every health worker who practices without having a permit; 6. Every foreign health worker intentionally provides health services without having a SIP.⁵

Nurses are included in the definition of health workers as referred to in Article 1 point 6 of Law

Number 36 of 2009.⁶The legal review in terms of civil law, in this case, there is an agreement between the maternity nurse and the patient to assist in childbirth so that the legal relationship that arises in the therapeutic transaction between the maternity nurse and the patient can be categorized into a contractual relationship. The contractual relationship arises because there is a meeting of wills between the two. The wills between the two are not the same but are related. According to J. Guwandi, the relationship between health workers and recipients of health services is based on two characteristics, namely the existence of the agreement on the provision of services (consensual) and the existence of trust between service providers and recipients of health services.⁷

The patient expects that the contractual relationship between the pregnant mother and the maternity nurse will go well, but if in the state of the pregnancy process it is known that the presentation of the baby is Breach, the delivery is in a Breach location which is very risky to the health of the mother and fetus so that unexpected events can occur such as the death of the mother and fetus, in this case, the material nurse does not fulfill the agreement.

With the occurrence of default, of course, it will cause harm to the patient, therefore the patient has the right to claim compensation. The patient's right to obtain compensation for the default, in addition to being based on the provisions of the law of engagement, is also based on the provisions of the health law as regulated in Article 58 of Law no. 36 of 2009 concerning Health which stipulates that: "Everyone has the right to claim compensation for someone, a health worker who causes a loss due to an error or negligence in the health service he receives".⁸

The position of the fetus in the uterus depends on the process of adaptation of the fetus to space in the uterus. At approximately 32 weeks of gestation, the amount of amniotic fluid is relatively more, allowing

the fetus to move freely. Thus the fetus can place itself in a head presentation, Breach position, or transverse position. In the last trimester of pregnancy, the fetus grows rapidly and the amount of amniotic fluid is relatively reduced. Because the buttocks with both legs folded are larger than the head, the buttocks are forced to occupy a larger space in the uterine fundus, while the head is in a smaller space in the lower uterine segment.

Thus, it can be understood why in preterm pregnancies, the frequency of breach placement is higher, whereas, in term pregnancies, the fetus is mostly found in cephalic presentation. Other factors that play a role in the occurrence of breach location include multiparity, twin pregnancy, hydramnios, hydrocephalus, placenta previa, and a narrow pelvis. Sometimes the Breach position is caused by uterine abnormalities and uterine deformities. The placenta is located in the area of the uterine fundus cornu can also cause a Breach position because the placenta reduces the space in the fundus area.⁹

Pregnancy in a Breach position will give a poor prognosis in labor because it will increase complications for the mother and fetus. Complications that occur in the fetus cause after coming head, suffocation/aspiration, asphyxia, intracranial trauma, fracture/dislocation, brachial nerve paralysis. Meanwhile, complications that will occur in the mother are bleeding, birth canal trauma, and infection.

In pregnancy and childbirth, the Breach position often occurs with a fatal prognosis, based on the Indonesian doctor's competency standard in 2012, the incidence of Breach presentation is included in level 3 competence, which means level 3 ability is to diagnose, perform early management, and refer to a gynecologist.

If it is associated with the maternity nurse, the nurse can recommend preventing the occurrence of Breach

delivery by providing nursing care during antenatal care before 28 weeks of gestation, nurses can provide counseling to pregnant women that mothers can help change the position of the fetus by doing pregnancy exercises with routine. Pregnancy exercise is effective if it is carried out until 34 weeks of gestation (in the first pregnancy) to 36 weeks (second pregnancy and so on), if at the health service the doctor has given a diagnosis of the location of the breach, the patient must give birth at a health service place that has an obstetrician and has a more adequate equipment.

Article 30 paragraph 1 letter G of the Law of the Republic of Indonesia Number 38 of 2014 states that nurses are authorized to take action in emergency situations according to their competence. Look in the above provisions, concerning delivery assistance by maternity nurses, it can be seen that criminal legal responsibility will be given if the maternity nurse is not in an emergency and there is a culpa lata, criminal responsibility can be imposed on the maternity nurse.¹⁰

Handling the labor process in women who give birth in a Breach location is to provide delivery assistance using the Brach, classical, Loevset, Muller, and Mauriceau methods. In addition, SectioCaesaria (SC) can also be performed. During pregnancy, you should do routine prenatal care or antenatal care at least 4 times and do ultrasound to determine the state of the fetus.¹¹

In the standard nursing code of ethics, several types of nursing ethical violations are described. One form of serious violation which includes taking nursing actions without following procedures so that the patient's suffering gets worse and even dies, giving the wrong medicine so that it is fatal to the patient, leaving the patient in a serious condition or dying without giving help, gambling or drinking alcoholic beverages until he is drunk. in the treatment room, tarnishing the honor of the patient, hitting or

violence on the patient intentionally until physical disability occurs, abusing the patient's medication for personal or group interests, and vilifying or making hoax stories about the nursing profession in other professions in forums, print media, and online media. resulting in lawsuits.¹²

As the competence of maternity nurses, namely to assist in childbirth but do not have the authority to provide delivery assistance, especially in cases with fatal risks that can cause death to the mother and fetus. When referring to the criminal provisions of the Health Personnel Law Article 84 paragraph (1), any Health Personnel who commits gross negligence resulting in serious injury to the Health Service Recipient shall be sentenced to a maximum imprisonment of 3 (three) years. Then in paragraph (2), namely If the gross negligence as referred to in paragraph (1) results in death, each Health Worker shall be sentenced to a maximum imprisonment of 5 (five) years.

Therefore, as regulated in Article 1 number 4 of the Law of the Republic of Indonesia Number 38 of 2014 concerning Nursing, nursing practice can only be authorized to carry out services provided by nurses in the form of nursing care, in the case of a Breach position diagnosed by a doctor, the maternity nurse can only provide nursing care then for the process of continued delivery then through a general practitioner and referred to a gynecologist in order to reduce the risk of maternal and child mortality.

Health workers have an important role in efforts to improve the quality of health services, according to the existing laws and regulations that the health workers in question are doctors, nurses, midwives, pharmacists and others, nurses in carrying out nursing practices in accordance with article 29 number (1) Law of the Republic of Indonesia Number 38 of 2014 concerning nursing, nurses have duties as implementing nursing care, providing counseling and counseling to patients, conducting research in

the field of nursing, and carrying out tasks based on delegation of authority and as executor of tasks in certain circumstances.

Then besides that nurses also have 3 functions, namely, an independent function, namely nurses take actions that are independent, which means that nurses have obtained the authority obtained through law to provide health services in terms of nursing practice, an independent function, namely nurses take collaborative actions with staff. other health services where in this case nurses together with other health workers are jointly responsible for the actions of health services provided to patients, and a dependent function, namely nurses take actions to assist doctors in providing health services in the form of medical actions that should be the authority of doctors, forms of authority This function is obtained through the delegation of authority by the doctor.¹³

Conclusion

As the competence of maternity nurses, namely to assist in childbirth but do not have the authority to provide delivery assistance, especially in cases with fatal risks that can cause death to the mother and fetus. When referring to the criminal provisions of the Health Personnel Law Article 84 paragraph (1), any Health Personnel who commits gross negligence resulting in serious injury to the Health Service Recipient shall be sentenced to a maximum imprisonment of 3 (three) years. Then in paragraph (2), namely If the gross negligence as referred to in paragraph (1) results in death, each Health Worker shall be sentenced to a maximum imprisonment of 5 (five) years.

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Conflict of Interest: Nil

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Isolation and Identification of *Salmonella typhimurium* in an Umbilical Area from Chicks in Kerbala City

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Abstract

One hundred samples of umbilicus were taken from chicks. Out of these, 27 (27%) were positive. The most common of bacterial isolates were *Salmonella typhimurium*. The biochemical tests for all isolates, whether from chicks, showed a positive test to check the production of hydrogen sulfide gas, the fermentation of glucose sugar, the use of citrate as a source of carbon, non-lactose fermented and negative for urease enzyme, the result found the most common infection in the cold weather February, they were found nine isolates (33.33%) more than other groups as 29.63%, 14.82% and 11.11%, for January, November, December and October, respectively. The results were found a total of 27 isolates were *Salmonella spp.*, on the other hand 11 isolates were identified as *Salmonella typhimurium* (40.74%) by Vitek 2 compact system, All *Salmonella spp.* showed high susceptibility against Nitrofurantoin, 27 (100%) and ciprofloxacin 24 (88.8%), whereas they were highly resistance against Amoxicillin, Cefotaxime, and Vancomycin 24 (88.8%). Chicks with umbilical area were found to harbor different pathogens and they are considered a source of infection during successive days in the life of broilers chickens.

Keywords: *Salmonella Typhimurium*, Chicks, Vitek 2 identification

Introduction

Salmonella belong to family Enterobacteriaceae¹, these are 3 µm long and 0.6-0.7 µm in diameter, often motile, growing on ordinary media, Facultative anaerobic², the peptidoglycan surrounded by a periplasmic membrane and an outer membrane, the

outer membrane is in direct contact with the external environment and bears part of the antigenic characteristics of the bacteria. It is also involved in the phenomena of virulence. This membrane contains in particular the lipopolysaccharide (LPS) whose region hydrophilic, in contact with the external medium, corresponds to the "O" antigenic region. The flagella found in most salmonellae, except *S. gallinarum*, consist of a quaternary assembly of several thousand copies of a single protein, "flagellin" and correspond to the "H" antigenic region³.

In addition to the large number of serotypes, the genus *Salmonella* presents a great contrast between the serotypes, some are more adapted to the intestine and do not bypass the gut, others can reach the

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bloodstream and have the ability to colonize the liver and spleen. Some live longer in the environment, others do not, most species of animals can be infected with salmonella, so transient infections are very common among birds⁴.

Omphalitis is a major cause of increased mortality in chicks in the first week, yolk sac infection, a hatchery-born disease, also known as “soft chick disease” or “chicks navel”, it is a common disease⁵, often caused by artificial hatching of chicks significant losses are in the brooding period, as bacteria penetrate into the porous shell of the egg, such as incubation conditions are suitable for bacterial growth and egg incubation as well, variety bacteria, such as *Escherichia coli*, *Staphylococcus*, *Proteus*, *Clostridium faecalis*, and *Pseudomonas* may involve in yolk sac infection⁶. The completely eliminating salmonella from poultry production is a very ambitious goal, the need to combine proper management, biosecurity and proper vaccination protocols along with many other aspects can help take the first steps in the right direction, the use of food additives can be a useful tool in preventing outbreaks by ensuring a healthy gut and good levels of performance⁷. These and other general features of salmonella make it difficult to control, it requires a lot of knowledge and investments. Salmonellosis is not considered the most devastating poultry disease, but it is one of the most difficult (agent) diseases to control, the main reason is the great diversity of serotypes and the highly complex epidemiology of these microorganisms⁸.

The present study aimed to determine bacterial causes of umbilical area through isolation and identification of some *Salmonella* spp and determine the most common serotype.

Materials and Methods

A total of 100 umbilical samples from the chicks layer and broiler were collected from unorganized chicken farms^{9,10} in Kebala city. All samples were

placed in sterile plastic bags (transport medium), labeled, and transported to the Veterinary medical laboratory in Kerbala university by portable coolers at 4 C°, to be processed within 3 – 4 hours of the collection this study was conducted through a period extended from October 2020 to April 2021.

According to modified FDA¹¹, A swab of umbilical was added into a sterile tube containing 5ml of peptone water broth. The mixture was incubated at 37 C° overnight. Aseptically 1 ml peptone water culture added to a sterilized tube containing Selenite broth, then incubated at 37 C° for 24 hrs. after that, a loopful of each broth was streaked on the surface of S.S, XLD, and H.G agar plates and then incubated at 37C° for 24 hrs.

The biochemical characters of non – lactose fermenting bacteria were determined using the TSI agar and Urease test. Colonies that show biochemical characteristics similar to that of *Salmonella* spp were diagnosed by VITEK® 2 Compact.

The antimicrobial susceptibility testing was done by the agar discs diffusion method as that described by Bioanalyse® sensitivity discs Co. At least 3-5 well-isolated colonies were suspended in 4-5 ml Brain heart infusion, the broth culture was incubated at 37 C° for 8 hrs, the turbidity of the actively growing broth culture was adjusted with sterile broth to obtain turbidity optically comparable to the 0.5 McFarland standard.

Results and Discussion

The results of bacterial isolation confirmed the obtaining of (27) bacterial isolates related to the genus *Salmonella typhimurium* out of (100) exit forms that were examined from chicks umbilical, during the period from October 2020 until the end of February 2021, the rate of infection was 27% Also, (9) isolates of *S.typhimurium* were confirmed out of (27). Figure (2) ,

Table (1): showed number and percentage of Salmonella with seasonal study.

Study period	Number of isolates	Number of positive isolates	Percentage %
October 2020	20	3	11.11
November 2020	20	4	14.82
December 2020	20	3	11.11
January 2021	20	8	29.63
February 2021	20	9	33.33
Total	100	27	100

Chi-square = 8.422 , DF= 4 , P value = 0.077

The result found the most common infection in the cold weather February, it was found none 9 isolates (33.33%) more than other groups as 29.63%, 14.82 %, and 11.11%, for January, November, December, and October, respectively. on the other hand, it was not a significant association between Salmonella infection and seasonal studies ($X^2 = 8.422$, DF= 4, P-value = 0.077). Seasonal shifts in immunity and host susceptibility, exacerbated by increased exposure through crowds during the colder months, will also increase patterns of infectious disease spread¹², it was different from Plancha and colleagues who were noticed the most common infection of poultry salmonellosis in summer rather than winter¹³. Single

pure colonies were obtained through microbial cultivation on trithionite medium and through transferring them to differential culture media. In both cases, the non-fermented colonies of sugar lactose were distinguished through:

The shape of the colonies: The colonies growing on the middle of Salmonella- Shigella agar (SS agar) were small, round, smooth convex¹⁴, pale yellow with a black center figure(1)

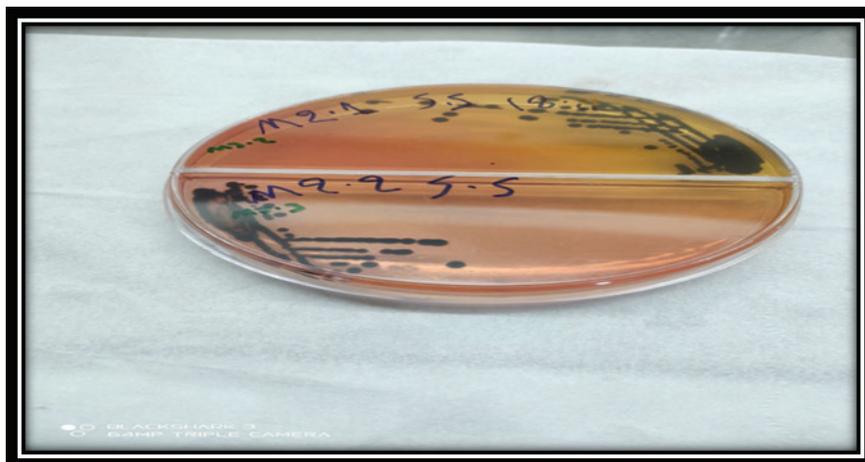


Figure (1): colonies of *Salmonella typhimurium* in the S S agar.

These results were in agreement with ¹⁵ who have detected the *Salmonella* colonies on SS agar.

Salmonella spp. can decarboxylate lysine, which increases the pH and the colonies remain red, furthermore, *Salmonella* spp. produce hydrogen sulfide (H₂S) from thiosulfate, which results in a black precipitate with ferric salts, this result was recorded by ¹⁶.

Biochemical testes of *Salmonella typhimurium*

The biochemical tests for all isolates, whether from chicks, showed a positive test to check the production of hydrogen sulfide gas, the fermentation of glucose sugar, the use of citrate as a source of carbon, and negative readings for the indole test, lactose fermentation and urease enzyme. ¹⁷, *Salmonella* reactions on TSI tubes. Isolates giving any of the following reactions may be considered negative for *Salmonella*: 1. Isolates producing a “no change”, or alkaline slant and butt (K/K with H₂S) in TSI (red)¹⁸.

Table (2) biochemical tests of *Salmonella typhimurium*

Biochemical test	Results
H ₂ S production	+
Citrate utilization	+
Glucose fermentation	+
Urease production	-
Lactate fermentation	-
Indole production	-
Triple sugar iron agar	Alkaline/Alkaline with H ₂ S

Vitek 2 identification system

All salmonella culturing isolates were diagnosed in this study by using the Vitek2 compact system and diagnostic GP/ ID card. The result was found a total of 27 bacterial isolates, ¹⁸ was recorded as *Salmonella typhimurium* (40.74%) by Vitek 2 compact system. This technique showed speed and accuracy in diagnosing isolates with a probability ratio (97%) based on the results of 64 biochemical tests as shown insensitivity test was performed to *Salmonella typhimurium* isolated from poultry' field

By using the disc diffusion method, 10 isolates of *Salmonella typhimurium* were tested for their antimicrobial susceptibility toward 8 antibiotics disks (figure 2).

All tested isolates showed high susceptibility (90 %) toward ciprofloxacin and (100%) against Nitrofurantoin, whereas it was highly resistant (80%) against Cefotaxime, amoxicillin, Vancomycin and. On the other hand, these isolates revealed varying percentages of susceptibility and resistance toward other antibiotics table (3).

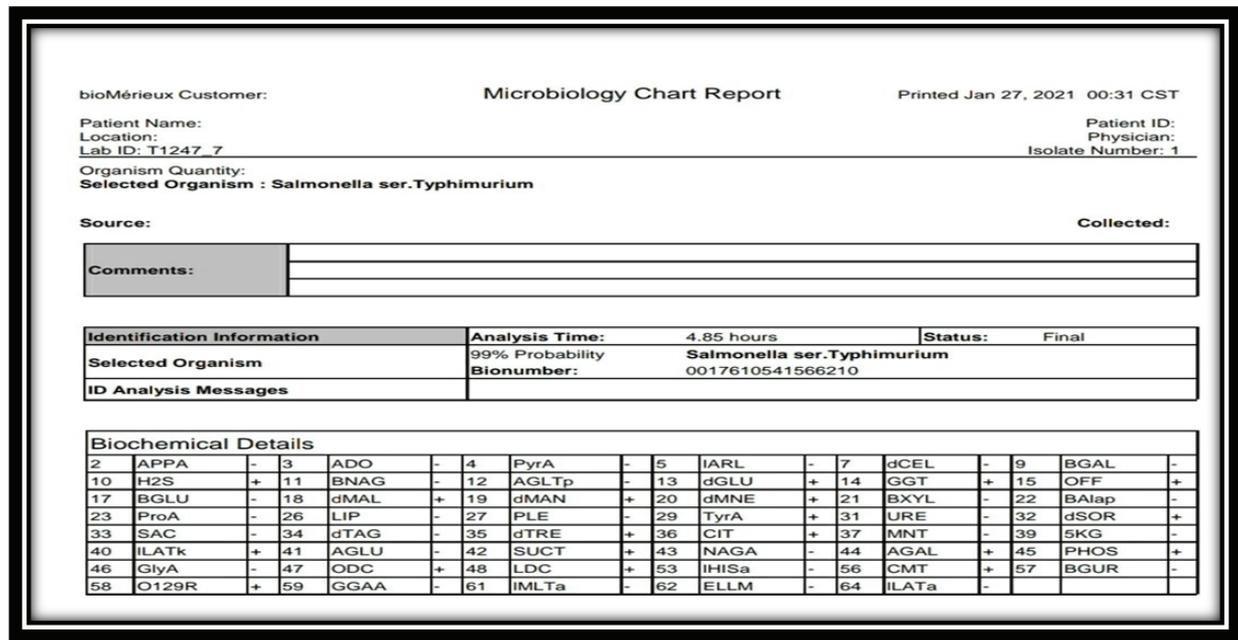


Figure (3) Vitek 2 system results confirmed *Salmonella typhimurium*

Table (3) : The susceptibility tests of antibiotic discs by diffusion method against (27) *Salmonella spp* isolates

Antibiotic discs µg/ml	Sensitive No. (%)	Intermediate No. (%)	Resistant No. (%)
Nitrofurantion	27 (100)	0	0
Ciprofloxacin	24(88.8)	0	3(11.11)
Chloramphenicol	20 (74.0)	0	7(25.9)
Doxycycline	15 (55.55)	5 (18.5)	7(25.9)
Gentamycin	10(37.0)	7 (25.9)	10 (37.0)
Sulfa-trimethoprim	5 (18.5)	18(66.6)	4 (14.8)
amoxicillin	3(3.33)	0	24 (88.8)
Cefotaxime.	2 (7.4)	1 (3.7)	24 (88.8)
Vancomycin	2 (7.4)	1 (3.7)	24 (88.8)

The most important results, the sensitivity test for *Salmonella* spp isolates showed, they were sensitive to the Nitrofurantoin 27 (100%), Ciprofloxacin 24 (88.8%), Chloramphenicol 20 (74%), Doxycycline 15 (55.5%), Gentamycin 10 (37%), Sulfa-trimethoprim 5 (18.5%), amoxicillin 3 (3.3%), and Cefotaxime, Vancomycin 2 (7.4%). Most of them are resistant to Amoxicillin, Cefotaxime, and Vancomycin 24(88.8%).

The production of β -lactamases by *Salmonella* spp has become an important and common mechanism for β -lactam resistance^{19,20}, and some chloramphenicol resistance in *Salmonella* is associated with drug target modification by chloramphenicol acetyltransferases encoded by the genes²¹.

Conflict of Interest: None declared.

Ethical Clearance: Taken from institutional ethical committee.

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Original Article

The Effect of Hypertension as a Comorbid Factor on the Length of Stay in Patients Undergoing Cholecystectomy

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Abstract

Background: One of the gold standard procedures for cholelithiasis is cholecystectomy. Cholecystectomy has numerous varied Length of stay results relies upon every nation and medical clinic. Hypertension is perhaps the most widely recognized comorbid factors and are thought to impact prolonged length of stay.

Aim of Research: To study the effect of hypertension as a comorbid factor on the length of stay in patients undergoing cholecystectomy in Dr. Ramelan Naval Hospital Surabaya from June 2019-September 2020.

Method of Research: This is an analytic descriptive research with retrospective study. The sampling technique used in this research is total sampling. The data were collected from medical record data of patients at the Internal Medicine and Digestive Surgery Polyclinic.

Result of Research: The number of patients who underwent cholecystectomy with hypertension who underwent a length of stay > 5 days were 36 people (83.7%). The result from the Coefficient Contingency test shows that there is a correlation between hypertension as a comorbid factor and the length of stay ($p < 0,0001$).

Conclusion: This research shows that there is comorbidity such as hypertension affects the length of stay in cholecystectomy.

Keyword: *Cholelithiasis, Cholecystectomy, Hypertension, Length of Stay*

Introduction

One of the gold standard procedures for cholelithiasis is cholecystectomy^{1,2}. Cholecystectomy

surgery can be assessed from the rate of morbidity, mortality and length of stay as the outcomes.

Length of stay is an estimation of the absolute number of days a patient should be hospitalized. The way to decide the length of stay is to deduct the date of release from the hospital (regardless of whether alive or dead) from the date of first hospitalization^{3,4}. The normal length of stay for patients going through cholecystectomy medical procedure is 5 days, the

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length of stay is drawn out on the off chance that it requires over 5 days⁵.

Hypertension is some of the most common comorbid factors and are thought to influence prolonged length of stay, 22% of the world's population suffers from hypertension⁶, a study conducted by Nera Agabiti in 2013 said that there were 1089 (8.0%) patients with hypertension as a comorbid factor⁷. Srinivas J. Ivatury also reported that there were 92 (39.7%) patients with hypertension as preoperative factor⁸.

This examination was led to decide if there is an impact of hypertension, on the event of the prolonged length of stay after cholecystectomy medical procedure. Counteraction of delayed length of stay after cholecystectomy will decrease the expense of care^{5,9}.

Method

Research Design

The design of this research is descriptive analytic. This is a qualitative research.

Research Method

This research uses secondary data. The data were gathered from medical record information of patients at the Internal Medicine and Digestive Surgery Polyclinic Dr. Ramelan Naval Hospital Surabaya from June 2019 - September 2020.

Population and Sample

The sample used in this study was obtained using non-probability sampling with the sampling technique carried out by total sampling where the number of samples was the same as the population¹⁰. The sample used in this study were patients who were treated in the operating room at Dr. Ramelan Naval Hospital Surabaya.

Inclusion Criteria

All cholelithiasis patients underwent cholecystectomy and were treated in the operating room at Dr. Ramelan Naval Hospital Surabaya from June 2019 - September 2020.

Exclusion Criteria

Patients with fragmented/missing medical records, a history of abdominal trauma, abdominal surgery, and biliary system malignancies at Dr. Ramelan Naval Hospital Surabaya.

Sample Size

The sample size in this study was all the medical record data of patients with cholelithiasis that met the inclusion and exclusion criteria for the study at Dr. Ramelan Naval Hospital Surabaya for the period June 2019 - September 2020.

Data Analysis

To see the significant difference in the length of days of treatment in cholecystectomy patients with hypertension, the Contingency Coefficient statistical test was used. Furthermore, the test for differences in the length of day of treatment between groups with hypertension and without hypertension was carried out using the Contingency Correlation test. The degree of significance used was $\alpha = 0.05$.

Result

From the results of medical record data research at the Internal Medicine and Digestive Surgery Polyclinic, Dr. Ramelan Naval Hospital Surabaya June 2019-September 2020, the total number of samples is 201 patients, yet the number of samples that qualified inclusion and exclusion criteria was 146 patients. Furthermore, the data obtained will be analyzed descriptively and analytically.

Table 1. Distribution of Diagnose and Length of stay

Diagnose	Hypertension	Length of stay ≤ 5 days	Length of stay > 5 days	P value
Cholelithiasis	With	5	16	0.002
		6.4%	20.5%	
	Without/Others Comorbid	36	21	
		46.2%	26.9%	
Cholelithiasis with choolangitis	With	0	3	0.546
		0.0%	25.0%	
	Without/Others Comorbid	1	8	
		8.3%	66.7%	
Cholelithiasis with cholecystitis	With	2	10	0.030
		5.0%	25.0%	
	Without/Others Comorbid	15	13	
		37.5%	32.5%	
Cholelithiasis with cholecystitis with choolangitis	With	0	1	
		0.00%	100.0%	
Cholelithiasis with cholecystitis with obstruction	With	0	6	
		0.00%	54.5%	
	Without/Others Comorbid	0	5	
		0.00%	45.5%	
Cholelithiasis with obstruction	With	0	1	0.248
		0.0%	25.0%	
	Without/Others Comorbid	2	1	
		50.0%	25.0%	

Based on Table 2. It can be seen that there were 2 procedures were performed in this study, the number of patients who underwent laparoscopic cholecystectomy were 90 (61.6%), and 56 (38.4%) patients underwent open cholecystectomy. it was found that a significant difference was due to the value of <0.0001 ($p < 0.050$).

Table 2. Distribution of Procedures and Length of stay

Procedures	Length of Stay \leq 5 days	Length of stay > 5 days	P Value
Laparoscopic cholecystectomy	53	37	<0.0001
	36.3%	25.3%	
Open Cholecystectomy	8	48	
	5.5%	32.9%	

Based on Table 3. It can be seen that the number of patients who underwent cholecystectomy with hypertension who underwent a length of stay > 5 days were 36 people (24.7%), and 7 people who underwent \leq 5-day length of stay (4.8%).

Table 3. Distribution of Hypertension and Length of stay

Comorbid Factor	Length of Stay > 5 days	Length of Stay \leq 5 days	P Value
Hypertension	36 (24.7%)	7 (4.8%)	<0.0001
Non-Hypertension	49 (33.6%)	54 (37.0%)	

The number of patients who underwent cholecystectomy without hypertension who underwent treatment days > 5 days was 49 (33.6%), and 54 people (37.0%) had \leq 5 days of treatment. From the statistical analysis of the Contingency Coefficient Correlation, it was found that a significant difference was due to the value of <0.0001 ($p < 0.050$).

Based on Table 4. The number of geriatric patients who underwent a length of stay > 5 days were 12 (8.2%), and 9 (6.2%) patients who underwent \leq 5-day length of stay, without a significant difference due to the value of $p > 0.050$ (0.914).

Table 4. Distribution of Geriatric status and Length of stay

Status	Length of Stay \leq 5 days	Length of Stay > 5 days	P value
Non-Geriatric	52	73	0.914
	35.6%	50.0%	
Geriatric (>65 years old)	9	12	
	6.2%	8.2%	

Discussion

This study is a descriptive analytical study using secondary data from the medical records of patients from internal medicine and digestive surgery polyclinic at Dr. Ramelan Naval Hospital Surabaya. In this study, 6 (26.1%) cases with diabetes mellitus experienced morbidity. The results showed that diabetes mellitus influenced the occurrence of morbidity. This study has the same result with other studies, including one conducted by Gelbard in 2010, which stated that diabetes mellitus is an independent risk factor for morbidity such as death, infectious complications, cardiovascular disease and post-cholecystectomy renal failure with a p value of 0.034 [5].

According to Abdulkadir's 2001 study, it was reported that the operative complication ratio was 9 cases (4.9%) and postoperative complications amounted to 12 cases (6.5%) higher in patients with diabetes with p values of 0.026 and 0.0061 respectively [2]. It is possible that according to Alves, in 2012 diabetes mellitus was associated with decreased T cell responses, neutrophil function and impaired humoral immunity. Sequentially, diabetes mellitus increases the likelihood of infection. Infection that is exacerbated by diabetes in the patient can cause morbidity such as In this study, there were 36 (24.7%) cases with comorbid hypertension who experienced a prolonged length of stay (length of stay). The results showed that hypertension comorbid factors affected the length of hospitalization days. Other studies that are in line with the results of this study, among others, a study conducted by Shih-Ping Cheng in 2007 said that hypertension is one of the comorbid factors that cause an extension of the length of hospitalization days more than 5 days, whereas many as 28 (60.9%) patients hypertension experienced an extended length of stay ($p < 0.001$)⁵.

Possibly according to Kiefer, the Renin-Angiotensin-Aldosterone System (RAAS) plays an

important role in controlling blood pressure^{11,12}. This system is responsible for the pathophysiology of hypertension and target organ damage¹³. Target organ damage includes vascular remodeling, resulting in inhibition of angiogenesis in wound healing through activation of AT2 receptors, which will prolong wound healing time so that the length of hospitalization day will be prolonged¹¹.

According to Varon and Manik during surgical procedures, patients with or without previous hypertension tend to experience increased blood pressure and tachycardia during induction of anesthesia. So that when going to carry out surgery, the increase in excessive blood pressure must be controlled to normal limits, because it is necessary to treat hypertensive patients first before going to a surgical procedure, so hypertension can affect the length of hospitalization days¹⁴.

The type of surgical procedure also affects the length of treatment days, according to Steven L. Zacks, patients who underwent open cholecystectomy were hospitalized longer than patients who underwent laparoscopic cholecystectomy¹⁵, Hospitalization for more than five days was 48 (32.9%) and only 8 (5.5%) patients underwent hospitalization for less than 5 days. Meanwhile, 53 (36.3%) patients who underwent laparoscopic cholecystectomy were hospitalized within 5 days more than patients who required hospitalization for more than 5 days.

In addition to hypertension, age also plays a role in lengthening the length of stay, according to a study conducted by Sivesh K. Kamarajah, increasing age increases the risk of complications, conversions, postoperative mortality, and lengthening the length of stay¹⁶, but in this study, there was no difference. significant in patients with geriatric status and non-geriatric patients, This is because the sample size of geriatric cases undergoing cholecystectomy is too small, the number of cases is 21 (14.4%).

Research Limitations

This study tracked down various shortcomings and limits, including the low number of samples used in this study, the sample used was 146 cases out of 201 cases of cholelithiasis that qualified inclusion and exclusion criteria of this study, this number is exceptionally little when contrasted with research and existing journal.

Notwithstanding the low example size, this study didn't order patients dependent on the kind of cholecystectomy performed, which may prompt a bias of the outcomes in patients undergoing open cholecystectomy and laparoscopic cholecystectomy.

Conclusion

This study shows that hypertension affects the prolonged length of stay in patients undergoing cholecystectomy.

Conflict of Interest – Nil.

Source of Funding – Self.

Ethical Clearance – Taken from Sub Komite Etik Penelitian Kesehatan Rumkital Dr. Ramelan Surabaya.

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Prostate Specific Antigen as Predictive Factor for Androgenemia in Women

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Abstract

Background: Sex hormones of steroid origin - androgens, play an important role in the life of the body. They are involved in the regulation of bone maturation, gonadotropin secretion and the synthesis of high-density lipids, the production of β -endorphins. Along with the anabolic effect, androgens regulate libido and sexual potency, stimulate the function of the sebaceous glands and hair follicles. At physiological concentrations, androgens participate in the regression mechanism of the follicle in the ovaries and provide pubic hair and inguinal hair growth. The present study was conducted with the aim the possibility of using PSA for the diagnosis of hyper androgenic conditions in women. The study included 105 girls aged 17–26 were examined. The concentration in the peripheral blood of PSA and a androgens, testosterone and DHEA-S, were determined. In addition, the concentration of the transport proteins of androgens SHBG and albumin was investigated. The calculation of (FT) and (BT) was carried out according to special computer programs. The aim of this work was diagnostics of hyperandrogenic conditions in women. The result shows that the concentration of PSA increases with an increase in T peripheral blood, the concentration of PSA increases with an increase in DHEA-S in peripheral blood and the concentration of PSA increases with an decrease in SHBG in peripheral blood. In conclusion, we found that there is a relationship between androgen levels and PSA concentration in peripheral blood, and PSA is a valuable marker in the diagnosis of hyperandrogenism in women.

Keywords: PSA, androgen, hyperandrogenism.

Introduction

In a woman's body, androgen production is carried out by the ovaries, adrenal glands and in peripheral organs (liver, skin) and tissues (fatty, muscular) ^(1,2,3). The substrate for the synthesis of androgens is cholesterol, which enters the adrenal glands and ovaries in the composition of LDL, or is

formed locally from acetate. Androgens are formed from cholesterol under the influence of enzymes (17, 20-lyase, 17 α -hydroxylase, 3 β -hydroxysteroid dehydrogenase). The chain of transformations of prohormones into androgens is carried out in four stages with the formation of the following metabolites: dehydroepiandrosterone (DEA), androstenedione (An), testosterone (T) and dihydrotestosterone (DHT) (listed in order of increasing androgenic activity) ^(4,5). It is shown that the adrenal glands are the main structure synthesizing DHEA (70%) and its less active metabolite, dehydroepiandrosterone sulfate (DHEA-S

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(85%). DHEA-Sis subject to continuous hydrolysis, thereby maintaining a constant level of DHEA in the blood plasma. The contribution of the adrenal glands to the synthesis of An approaches 40-45%, the other part of which is synthesized by the ovaries ^(6,7). It is also important to note that only 15-25% of the total T are synthesized by the adrenal glands. Excessive production of androgens is observed when there are tumor cells in the glands (Cushing's syndrome), or when certain enzyme systems are deficient, often 21-hydroxylase, this leads to a deficiency of cortisol and the accumulation of DHEA and An ^(8,9). In the ovaries, androgens are secreted mainly by the theca cells of the inner maturation membrane of the follicles and interstitial stromal cells. About 25% of T is synthesized here. The main product of the biosynthesis of androgenic steroids in the ovaries is An (50%). DHEA is limited in the ovaries, only 15% of the total ^(10,11). Aromatization of An and T occurs in the cells of the dominant follicle granulosa. The synthesis of androgenic steroids is under the regulatory influence of such tropic hormones of the pituitary gland, such as LH, the receptors for which are on the surface of both theca-cells and pre-antral and antral follicle granulosa cells, as well as FSH, to which only granulosa cells have receptors. Tropic hormones act via membrane receptors through a classic mechanism involving cyclic adenosine monophosphate (C-AMP). In response to the action of LH, the tech-cells produce androgens, which in the dominant follicle are subject to FSH-stimulated aromatization into estrogens ^(12,13,14). Androgens also begin to be secreted in significant quantities in the presence of androgen-producing ovarian tumors (epithelial, stromal tumors of the genital strand, lipid cell and germ cell tumors). Sometimes a hormonally inactive tumor can cause stromal proliferation and increase the production of androgens ^(15,16). The T content in the blood may not display the actual level of androgenization since the bulk of androgens are

in the bound state of the blood plasma, which makes them inactive. So, about 20% of them are associated with albumin and 80% - with globulins. The most stable connection is provided by sex steroid-binding globulins, which are synthesized in the liver. Only a small amount of testosterone remains free and active. In the idiopathic form of hyperandrogenism, when the content of free testosterone is elevated, and there are no other signs of virilization, the concentration of SHBG may be low. The main pathogenetic link in the development of this form is considered to be a violation of the processes of peripheral androgen transformation ^(17,18). It is known that PSA in men is produced by the prostate gland and is present in the tissues of the prostate, seminal fluid and serum. A small amount of PSA can also produce paraurethral glands, and therefore, a certain amount of this fluid is also found in the urine. There is no doubt that the serum PSA concentration in men is an important marker for the diagnosis of prostate cancer ^(21,22,23). It should be noted that in the laboratory diagnosis of the peripheral form of the hyperandrogenic status in women, there are several difficulties associated with the high cost of methods and the need to determine a large number of metabolites. In this regard, we are interested in the research of Canadian scientists Diamandis. Negri et al. (1998, 2000), who demonstrated the presence of PSA in some female tissues and biological fluids (mammary gland, ovaries, endometrial tissue, amniotic fluid, milk) ^(19,20). The presence of PSA in them is closely related to the regulatory action of steroid hormones, especially androgens, glucocorticoids and progestins ^(24,25).

Result and Discussions

From the presented data, it follows that there is a relationship between certain androgens and the level of PSA in the peripheral blood.

In results are presented in (Figure 1) we see that the PSA concentration increases with an increase in T

blood.

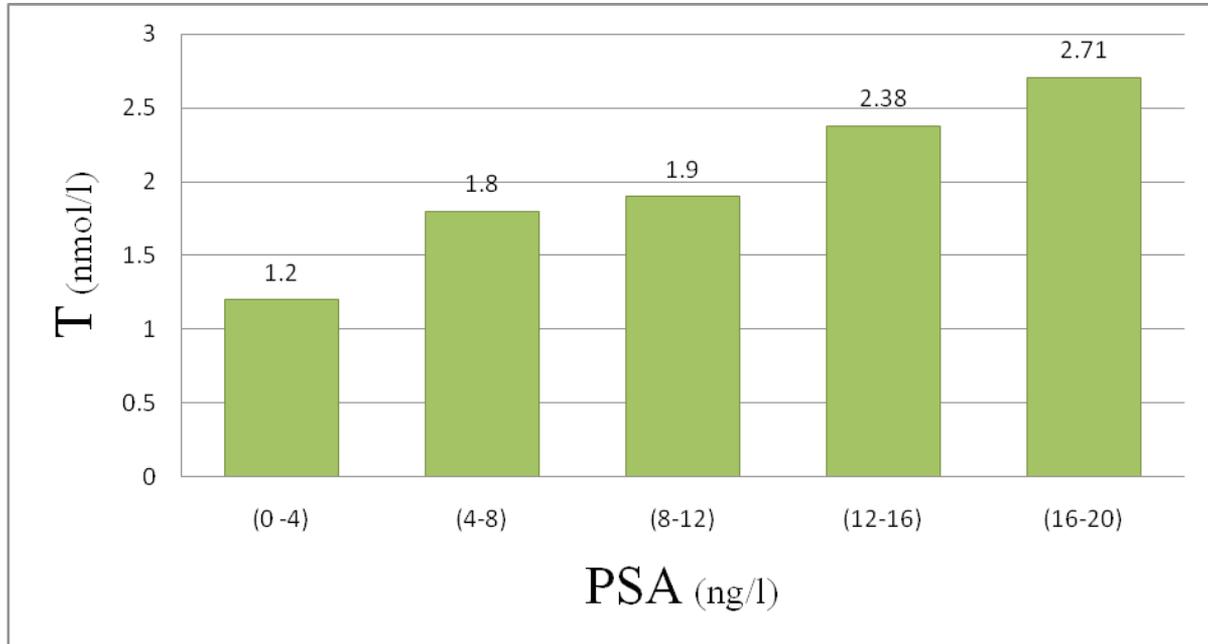


Figure 1. The content of T in the blood of women depending on the level of PSA.

The most obvious effect on PSA concentration is the effect of FT and BT: with increasing concentration of these androgens, the level of PSA in peripheral blood increases as in (Figure 2) and (Figure 3)

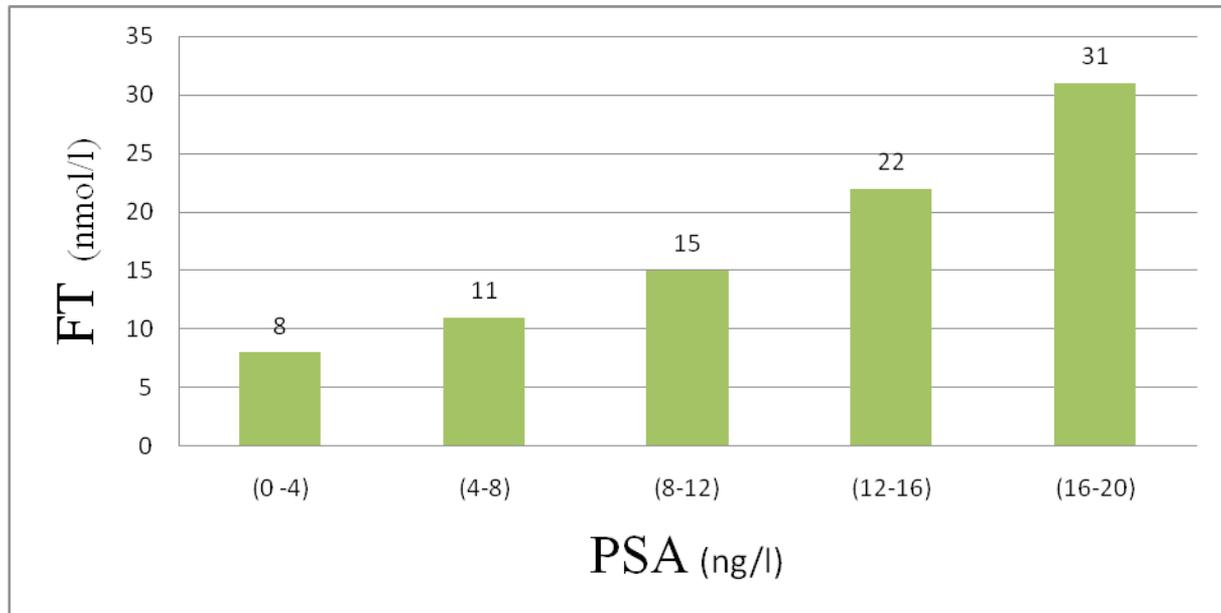


Figure 2. The content of FT in the blood of women depending on the level of PSA

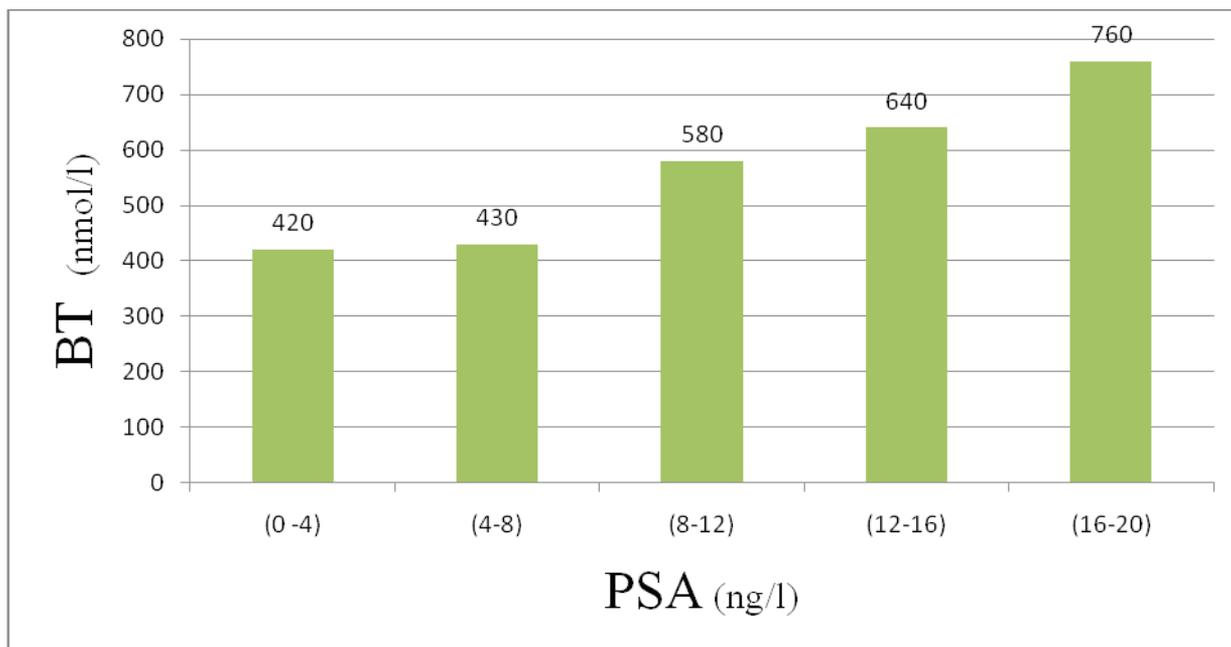


Figure 3. The content of BT in the blood of women, depending on the level of PSA

according to the results obtained in (Figure 4), we found that an increase in the value of PSA in women increases the concentration of DHEA-S in women.

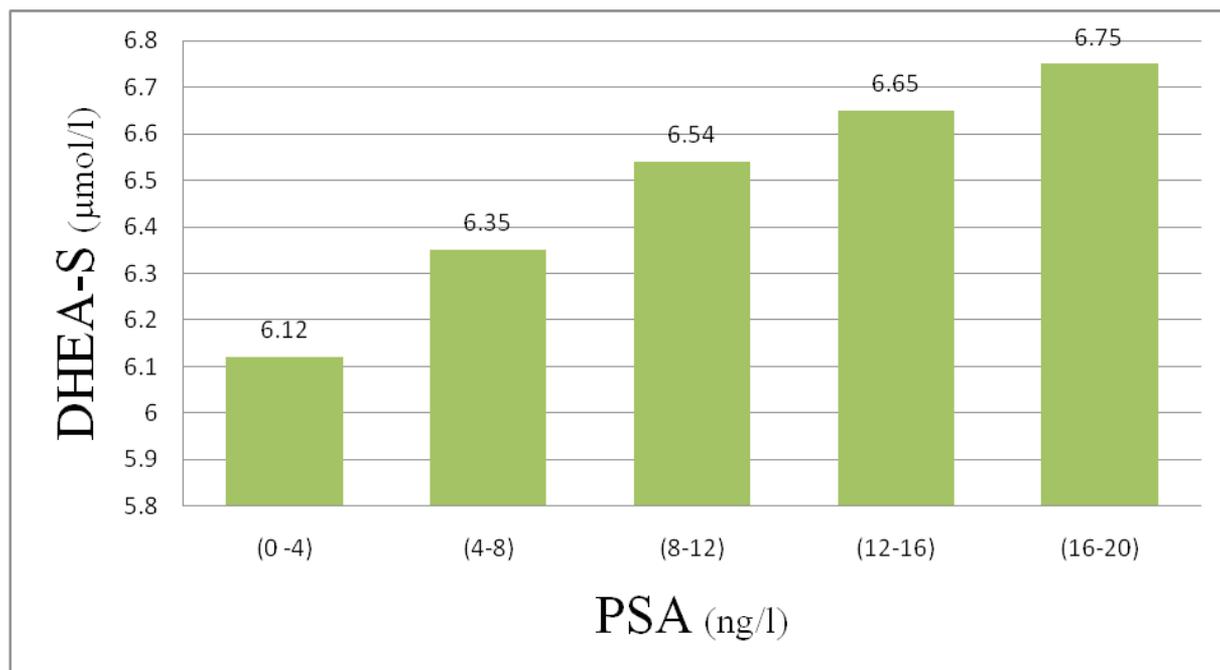


Figure 4. The content of DHEA-S in the blood of women, depending on the level of PSA .

while in results are presented in (Figure 5) that when the concentration of PSA increases, be decrease in SHBG in peripheral blood .

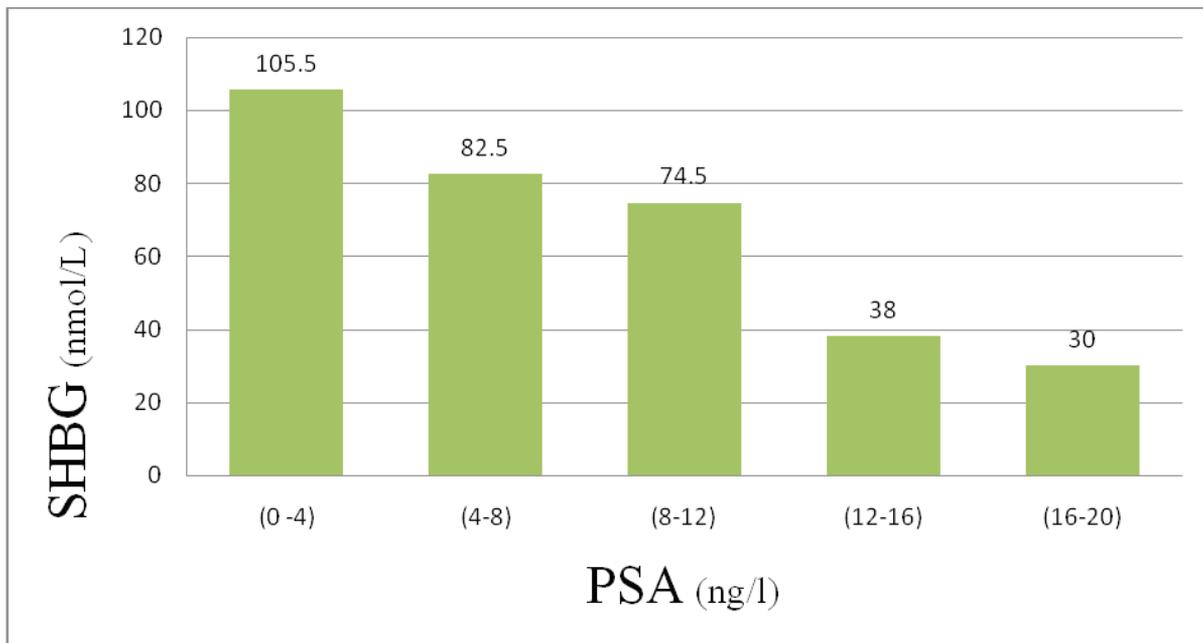


Figure 5. The content of SHBG in the blood of women depending on the PSA level

The research results are summarized in (Figure 6).

From the presented data, it follows that there is a relationship between androgens and the level of PSA in the peripheral blood.

The most pronounced effect on the concentration of PSA is exhibited by FT and BT: with an increase in the concentration of these androgens, the PSA level in peripheral blood increases.

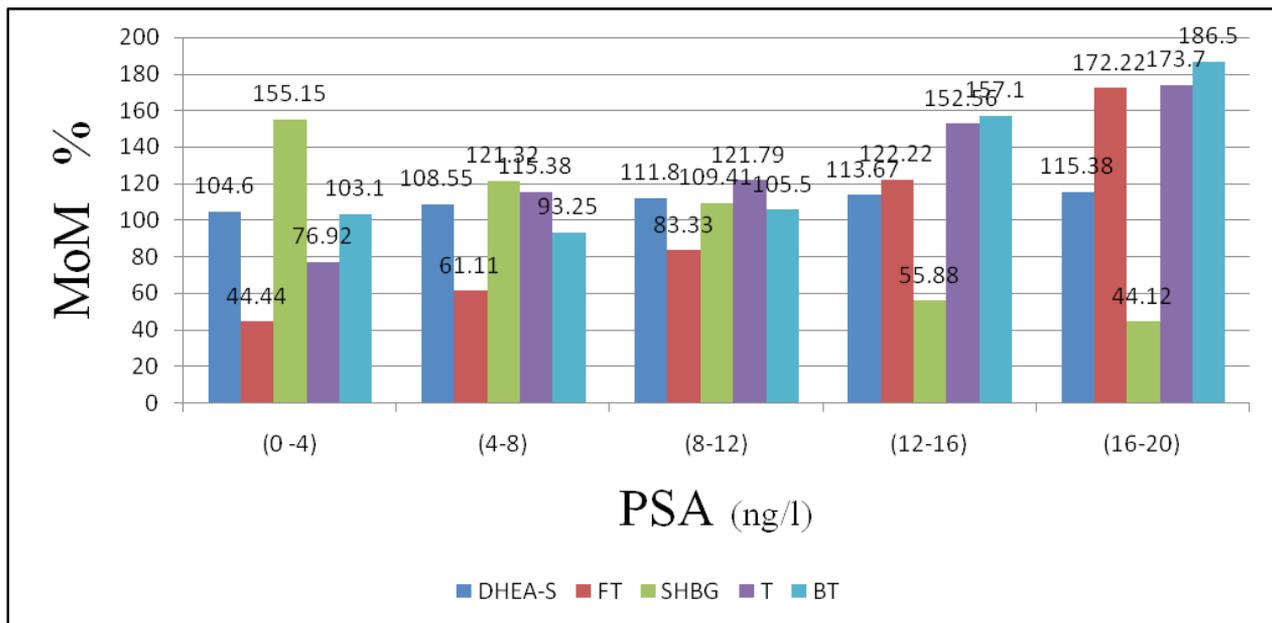


Figure 6. Androgen content in women’s blood (in MoM) depending on PSA level

Androgen level DHEA-S and T affect the level of PSA in peripheral blood.

We also noted an inverse relationship between the level of SHBG and PSA: with an increase in the

concentration of PSA, there is a decrease in the level of SHBG. Such dependence, apparently, is determined by the modulating effect of estrogens and androgens on the level of SHBG.

Conclusion:

There is a relationship between the levels of androgens and the concentration of PSA in peripheral blood, The concentration of PSA increases with an increase in T, FT and BT in peripheral blood and the concentration of PSA increases with decrease in SHBG in peripheral blood, therefore PSA is a valuable marker in the diagnosis of hyperandrogenism women.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

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The Effect of ARV on Rate of HIV Vertical Transmission from Exclusive Breastfeeding Mothers: A Systematic Review

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Abstract

Objective: To give a visualization and explanation whether the use of ARV during exclusive breastfeeding period is able to reduce the rate of transmission during exclusive breastfeeding.

Methods: Systematic review of cross-sectional studies, case control, cohort studies, and prospective studies using Pubmed, LWW Journals, and Science Direct for literature search. Literature published between 2010 and 2020 are researched. Results are recorded using PRISMA, MMAT is used for bias-risk evaluation.

Results: A total of 2090 breastfed infants were included among the 2 studies. The first journal included was by Coovadia *et al.* 2012 was a 3rd phase clinical regarding the use of extended NVP on HIV exposed infants were exclusive breastfed, concluded that the transmission rate for the group that received extended NVP was only 1.1%. Rutagwera *et al.* 2019, focused on the shedding of HIV virus into the breastmilk which is why this study used breastmilk as their sample^{4,6}. Rutagwera *et al.* 2019. found almost 80% of their subjects shed HIV virus into their breastmilk⁶. This number is correlated to the transmission of HIV vertically in breastfeeding mother.

Conclusion: To conclude, the use of ARV does affect the rate of transmission of HIV from mother to child during breastfeeding.

Keywords: HIV, exclusive breastfeeding, mother-to-child transmission

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Introduction

HIV or Human Immunodeficiency Virus is a virus that attacks a person's immune system, especially CD4 cells⁴. The targeted cells are none other than CD4 cells³. According to WHO (World Health Organization), the number of people living with HIV reached 37.9 million at the end of 2018 and 62% of them had access to antiretroviral therapy¹⁵. UNICEF says that at least 1.1 million people living with HIV

are children aged 0-9 years². Meanwhile in Indonesia, data from UNAIDS 2018 said that around 18,000 children aged 0.14 years had HIV¹¹.

The majority of children living with HIV are caused by vertical transmission, also known as mother-to-child transmission. Mother-to-child transmission can occur during pregnancy, childbirth or while breastfeeding¹⁴. This raises questions about the safety of a mother living with HIV to breastfeed her baby. Although it has been made clear that breastfeeding benefits both mother and child, some conditions can interfere with this fact, for example mothers living with HIV. In the United States, the American Academy of Paediatrics does not recommend that mothers living with HIV breastfeed their babies¹. However, it may be different in other countries as not everyone has good access to infant formula¹⁴. According to data from aidsdatahub.org in 2018, the number of pregnant women suffering from HIV in Indonesia reached 12,000, while only 15% of them received ARV⁷. This figure is very concerning because it means that the possibility of transmitting HIV from mother to child is still high. In addition, Indonesia is a developing country where WHO recommends mothers living with HIV to exclusively breastfeed their children. Therefore, this study will focus on the rate of HIV transmission from mother to child in breastfeeding mothers by looking at the mother's use of ARV. The research question that will guide this systematic review is "In HIV mothers who are exclusively breastfeeding, can the use of ARV reduce the rate of transmission to infants during exclusive breastfeeding when compared to those who do not use ARV?" in accordance to PICO.

There are 2 objectives in this systematic review and will be divided into general objective and specific objective. The general objective of this systematic review is to give a visualization whether the use of ARV on exclusively breastfeeding mothers and infants who are exclusively breastfed is able to reduce the

rate of transmission during exclusive breastfeeding. Whereas the specific objective is to give explanation about the impact of the use of ARV on exclusively breastfeeding mothers and infants who are exclusively breastfed to the rate of transmission during exclusive breastfeeding.

Materials and Methods

Research Type and Design

The methodology of this systematic review follows the guideline of Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA). Literature search will be done by using online databases which are LWW Journals, PubMed, and Science Direct. Literature researched are published between the year 2010 and 2020. This research will use several keywords that are related to the PICO characteristics that has been determined with the help of Boolean operators (OR / AND / NOT). The characteristics of PICO are, breastfeeding HIV mothers as population, the intervention is the use of ARV, the comparison is not using ARV, and the outcome is a decrease in the rate of transmission to infants during the breastfeeding period. Search terms that will be used are ("Exclusive breastfeeding" OR EBF) AND (Antiretroviral OR ARV) AND (transmission rate).

Inclusion Criteria

This systematic review will research literatures with designs such as cross-sectional study, case control, cohort study, and prospective study. Publication dates are limited to between 2010 and 2020.

Exclusion Criteria

Literatures will not be researched if full-article is not accessible for public. Moreover, if literature is not in English it will not be researched as well.

Research Variables and Operational Definition

Dependent variable of this research is the

decrease in the rate of transmission to infants during the breastfeeding period. In this study, rate of transmission can be defined as how many infants are infected with HIV from their mothers. Meanwhile, independent variable is the use of ARV and exclusive breastfeeding period. Exclusive breastfeeding period can be defined as the first 6 months of life where the mother will give their infants breast-milk only without being interspersed with any complementary food other than vitamin. Lastly, the use of ARV is defined as whether mother-and-infant pair takes ARV as recommended.

Research Material

This study will use the literature obtained from the search for secondary data. The literature that will be used is published between 2010 and 2020 regarding the use of ARV to see differences in the rate of transmission of HIV transmission from mother to baby during the breastfeeding period.

Research Instrument

The instruments that will be used in this study are journal databases such as LWW Journals, PubMed, and Science Direct.

Collecting Data Procedure

This study will be using secondary data in the form of published literature which will be searched by browsing online databases and using keywords and Boolean operators (OR / AND / NOT) to make the search more relevant. The search terms used

are (“Exclusive breastfeeding” OR EBF) AND (Antiretroviral OR ARV) AND (transmission rate).

Data Management

To determine the literature that will be included in the discussion later, the researcher first sorts out the relevant journals that have been downloaded using Mendeley by reading the abstract. The selected journals will be downloaded and sorted based on the inclusion criteria. This process will be recorded using the PRISMA diagram and will be presented in the discussion. The literature that has been selected will be subjected to a bias risk evaluation to determine whether the literature obtained is biased or not by using the MMAT (Mixed Method Appraisal Tool).

Data Processing and Analysis

The researcher will use a spreadsheet to transfer information from the journal that has been selected previously. This method will help the researcher to break down and sort information based on the relevance which will make it easier to answer research questions.

Results

The literature search started on February 13, 2021. Below are the results of the literature review. The researcher used PubMed, LWW Journals, and Science Direct as databases to search for literatures. In this section, the number of articles that were identified, screened, evaluated for eligibility, and included is shown.

The data included in the extraction are title, author, year, sample, intervention, and results.

Tabel 1. Data Presentation

No	Author(s), Year of Publication, Research Design	Sample	Intervention	Results	P Value
1	Hoosen M Coovadia, et al. 2012. Phase 3 of randomized, double-blind, placebo-controlled trial.	HIV positive women aged 18 years and over, whose infants had negative HIV-1 DNA PCR results at 7 days, weighing at least 2000g, performs exclusive breastfeeding.	Control group: infants are given placebo after being given NVP from 6 weeks of life at 10g/mL orally.	Mothers on HAART: 1/210	Mothers on HAART on both groups: N/A
				Mothers not on HAART: 7/490	
			Treatment group: NVP treatment is extended for infants at 20-28mg/day for 6 months or until breastfeeding stops.	Mothers on HAART: 0/203	Mothers not on HAART on both groups: 0.027
				Mothers not on HAART: 18/492	
2	David GatsinziRutagwera, et al. 2019. Cross-sectional study.	HIV positive mothers who are not eligible for ART and their infants, still exclusively breastfeeding at 38 weeks, and had no missing data.	Infants were given Lopinavir/Ritonavir or 3TC daily up to 50 weeks or 1 week after cessation of breastfeeding in random.	Neither HIV cells detected: 51/248	CD4 count: 0.19
				Only CAV detected: 34/248	
				Only CFV detected: 43/248	Plasma viral load: <0.001
				Both HIV cells detected: 120/248	

Discussion

After searching for journals that met the inclusion criteria, this study included 2 journals from different databases. The two journals included in this systematic review discuss the transmission of HIV from mother to baby in breastfeeding mothers. The number of subjects from the combination of these 2 journals

reached 2090 infants.

Impact of ARV Use on Mother-to-Infant HIV Transmission Rates in Breastfeeding Mothers

The first journal included was written by Coovadia *et al.* 2012. which took place in Tanzania, Uganda, South Africa and Zimbabwe⁶. This study is

a phase 3 clinical trial to determine the efficacy and safety of extended use of NVP in HIV-exposed and exclusively breastfed infants. Study subjects were divided into 2 groups, namely the placebo group and the follow-up NVP group. The two groups were compared in their transmission rate at the end of the study. The results of the study conducted by Coovadia *et al.* 2012. showed that the majority of the study subjects had high adherence to the intervention, which was around 88%-96%. NVP or nevirapine is categorized as NRTI or else called Nucleoside Reverse Transcriptase Inhibitors. This group of ARV works by inhibiting reverse transcriptase enzyme of HIV in order to hinder the virus to copy themselves⁹.

The results of the study from each group (placebo and extended NVP) were divided and compared with mothers who took HAART and mothers who did not take HAART (will not be seen from CD4 + count). In the group with infants who had taken extended NVP and mothers that had taken HAART, it was found that 1 in 210 infants was infected with HIV at 6 months, while in the extended NVP group in which mothers did not take HAART, the transmission rate increased up to 7 out of 490 infants.

The next category, which is infants who were given placebo, was also divided into two, namely mothers who took HAART and mothers who did not take HAART. The results showed that 0 out of 203 infants given placebo with mothers taking HAART were infected with HIV at month 6. In the group of infants given placebo with mothers not taking HAART, the number of infected rose up to 18 out of 492 infants.

The results of the study conducted by Coovadia *et al.* 2012. showed that the infection rate in infants given extended NVP from mothers with high CD4 cells without taking HAART was almost the same as in infants without NVP prolongation with mothers not taking HAART⁶. The infection rates were 0.7%

and 0.5% respectively. These results suggest that extended use of NVP can compensate for the absence of HAART use in mothers with high CD4 cell counts.

Viewing from the study of Coovadia *et al.* 2012., it can be concluded that the extended use of NVP in infants does not significantly affect the transmission of HIV from mother to baby, but when looking at the results of when mothers are not taking HAART, there is a drastic increase in HIV transmission from mother to baby⁶. This is especially helpful for mothers with CD4+ 350 cells/mm³, who are not eligible to take HAART. Overall, the rate of mother-to-child HIV transmission calculated at month 6 of birth in the extended NVP group was only 1.1% (95% CI 0.3-1.8), whereas in the placebo group it was 2.4% (95% CI 1.3-3.6).

The journal, written by Coovadia *et al.* 2012., stated that there is no additional benefit in infants given extended NVP with mothers who are already taking HAART regularly⁶. This was compared with infants given NVP for 6 weeks according to WHO recommendations. These results are not the same as the results of a study that was conducted before Coovadia *et al.* 2012. A previous study found that extended usage of NVP up to 14 weeks resulted in a 69% reduction in postnatal HIV transmission, or a 53% reduction in transmission when comparing 6 weeks of single-dose NVP, and other findings⁶.

The second journal included in this systematic review was conducted by Rutagwera *et al.* 2019. in Burkina Faso, South Africa, Zambia, and Uganda¹⁰. This study focused more on the shedding of the HIV virus from the mother through breast milk. Therefore, this study used breast milk as a sample to be examined in the laboratory. Mothers with HIV who became subjects of this study were not eligible to take ARV during breastfeeding.

The intervention to prevent mother-to-child transmission of HIV in this study was randomized administration of Kaletra (lopinavir/ritonavir) or Lamivudine (3TC) to infants. The administration of Kaletra (lopinavir/ritonavir) or 3TC is done every day for 50 weeks after birth or until 1 week after breastfeeding is stopped. 3TC is categorized as NRTI, which is the same as NVP. Meanwhile, lopinavir/ritonavir is categorized as protease inhibitor which inhibits the work of protease enzyme so it hinders the maturation of HIV virus⁹.

Results from this study conducted by Rutagweraet *al.* 2019. showed that 197 HIV-infected mothers who breastfed had cell-associated HIV (CAV) or cell-free HIV (CFV) detectable in their breast milk¹⁰. This figure reached 79.4%, which means more than half of the research subjects. 13.7% of the subjects released only CAV while 17.3% and 48.4% released both CAV and CFV in one of the samples taken. The remaining 20% of the subjects found neither CAV nor CFV shedding in breast milk samples.

Rutagweraet *al.* 2019 also discussed the use of antiretroviral therapy which has an influential relationship with the release of CAV and CFV¹⁰. This study suggests that without ART, local replication of HIV in the mammary gland can occur. This study also concluded that more than 70% of mothers who release both CAV and CFV in breast milk can play a huge role in increasing the chances of mother-to-child transmission of HIV.

The line that can be drawn from the two studies conducted by Coovadiaet *al.* 2012. and Rutagweraet *al.* 2019. is that the use of ARV can reduce the rate of mother-to-child transmission of HIV during breastfeeding^{4,6}. The results from both literatures correlates with WHO statement that indicated the chances of HIV being transmitted from mother to infant is around 15% to 45%, nevertheless the risk of transmission can be reduced to <5% when precautions

are taken¹³.

Impact of ARV Use on Viral Load

In the study conducted by Coovadiaet *al.* 2012., the median number of maternal CD4+ counts was determined⁶. In the randomization phase, the median CD4+ cell count in the extendedNVP group was 528 cells/ μ L. In addition, in the placebo group, the median number of maternal CD4+ counts was 557 cells/ μ L. This figure was taken during the randomization phase, namely at the 6th week after birth. The study by Coovadiaet *al.* 2012. also looked at the rate of mother-to-child transmission of HIV by dividing the mother's CD4+ count⁶. Results from the group which mothers with CD4+ 350 cells/ μ L did not take HAART in the randomization phase were 3 of the 418 infants got infected with HIV in the extendedNVP group. The number rose up to 13 of the 434 infants in the placebo group of the same population.

The results for the group in which mothers with CD4+ 350 cells/ μ L who took HAART in randomization phase was that only 4 of 71 infants and 5 of 54 infants got infected with HIV at 6 months from the extendedNVP group and placebo group, respectively. The study conducted by Coovadiaet *al.* 2012. also found that extended use of NVP reduces the rate of HIV transmission from mother to child, especially in mothers with CD4+ 350 cells/ μ L, because this population is not eligible for HAART use⁶. Therefore, the extension of NVP provides protection against mother-to-child transmission of HIV, especially during exclusive breastfeeding in the first 6 months of life.

The second journal, which is a study conducted by Rutagweraet *al.* 2019., found that 79.4% of the study subjects shed the HIV virus through breast milk¹⁰. However, 20% of mothers with HIV who breastfeed do not shed the HIV virus through breast milk regardless of not taking ART. Rutagweraet *al.*

2019. concluded that it is possible that the 20% of HIV-infected mothers who do not shed the HIV virus in breast milk do not shed it naturally¹⁰.

Rutagweraet *al.* 2019. showed that if ART is not taken by the mother, there is a significant association between CAV and CFV suggesting that HIV replication occurs in the mammary gland. This bond will be broken in mothers taking ART¹⁰. The disconnection between CAV and CFV is caused by ART suppressing the CAV. This journal also concluded that the shedding of the HIV virus in breast milk was caused by several factors. Factors that play a role in the shedding of the HIV virus in breast milk are the CD4+ count in the mother, the amount of the HIV virus in the mother, and the presence or absence of mastitis in the mother.

The amount of HIV virus affecting the shedding of the HIV virus in breast milk is evidenced by the data obtained by Rutagweraet *al.* 2019., which is a rough estimate of an odds ratio of 1.00 (95% CI) for a viral load of $1000 \leq \text{copies/mL}$ and a probability ratio of 5.37 for those with a viral load $1001 \geq \text{copies/mL}$ ¹⁰. These results prove that people with HIV with a higher amount of virus will tend to shed the virus into breast milk. When compared to the CD4+ count, the odds ratio does not differ much. For patients with $\text{CD4+} \geq 500 \text{ cells}/\mu\text{L}$ the odds ratio is only up to 1.00. Meanwhile, patients with $\text{CD4+} \leq 499 \text{ cells}/\mu\text{L}$ had a higher chance which is 3.75.

A review written by John-Stewart *et al.* 2004 explained a number of factors affecting the increase in rate of vertical transmission of HIV. One of them is viral load count of the mother⁸. On the other hand, a research conducted by Chendiet *al.* 2019 stated that a decrease in viral load was found when patients were given antiretroviral therapy⁵. According to Chendiet *al.* 2019 there is a correlation between ARV adherence with the decrease in viral load count. Chendiet *al.* 2019 found a decrease of the average viral load

count within 0 to 24 weeks. Both of these evidence may support the research conducted by Coovadiaet *al.* 2012. and Rutagweraet *al.* 2019. stated that one of the most important factors in HIV transmission or HIV release in breast milk is the use of ART^{6,10}. Rutagweraet *al.* 2019., in particular, has shown that the use of ART can sever CAV and CFV where it can suppress viral production and decrease viral load¹⁰.

Subsequent Interventions For Patients

The two studies compiled in this systematic review stated that the use of ARV is very important for the prevention of mother-to-child transmission of HIV during breastfeeding. The study conducted by Coovadiaet *al.* 2012. found that extended administration of NVP may not provide any additional benefit in mother-and-child pair where the mother was already taking HAART regularly compared to pair where the mother was not eligible for HAART⁶. These findings meant that extended use of NVP in infants may reduce the rate of mother-to-child transmission of HIV during breastfeeding in mothers who are not eligible for HAART.

Research by Rutagweraet *al.* 2019. found that 79.4% of study subjects shed the HIV virus in breast milk¹⁰. These results were obtained from breastfeeding mothers with HIV who were not eligible to take ARV. The shedding of the HIV virus in breast milk can cause vertical transmission during breastfeeding.

The line that can be drawn from the two studies above is that extending the administration of NVP up until the end of breastfeeding period can reduce the vertical transmission rate from mother to baby during the period of exclusive breastfeeding. Extended administration of NVP should be given to infants whose mothers are not eligible to take ARV because it has been shown to reduce transmission rates during breastfeeding. New research may be needed for deeper understanding the intervention in the form

of extended administration of NVP is performed in infants whose mothers are not eligible to take ARV during breastfeeding.

Conclusion

In conclusion, the summary of this systematic review is that the use of ARV can reduce the rate of transmission from mother to baby during exclusive breastfeeding. Furthermore, the effect of ARV on the rate of HIV vertical transmission during breastfeeding, namely that the use of ARV can suppress viral production in the mammary glands by breaking CAV and CFV. In addition, the extended use of NVP in exclusively breastfed infants with mothers who are not eligible for antiretroviral therapy may decrease the rate of vertical HIV transmission while breastfeeding, although further research regarding this matter will be needed for confirmation.

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ABBREVIATION

3TC: Lamivudine

ART: Antiretroviral Therapy

ARV: Antiretroviral Therapy

CAV: Cell Associated HIV

CFV: Cell-Free HIV

DNA: Deoxyribonucleic Acid

HAART: Highly Active Antiretroviral Therapy

HIV: Human Immunodeficiency Virus

NVP: Nevirapine

PCR: Polymerase Chain Reaction

PreP: Pre-exposure Infant Prophylaxis

PRISMA: Preferred Reporting Items for Systematic Review and Meta-Analysis

UNAIDS: United Nations Programme on HIV/AIDS

UNICEF: United Nations Children's Emergency Fund

WHO: World Health Organization

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Original Research Article

The Possible Effect of Celastrol on Ameliorating Mitochondrial Dysfunction and Neuro-inflammation in Sodium Valproate Induced- Rat Model of Autism

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Abstract

Autism spectrum disorder (ASD) is a neurodevelopmental disease with impairment in social interactions, language and repetitive stereotypical behaviors. Celastrol is a natural safe compound that has anti-inflammatory and as a neuroprotective effects. 48 male offspringswistar rats divided into 6 groups; normal control group, offsprings receive vehicle, autistic offsprings receive vehicle, autistic offsprings receive risperidone, autistic offspringsreceive celastrol, autistic offspringsreceive both risperidone& celastrol. At the end of experiment behavioral tests were performed then neurochemical analysis and histopathological examination. The obtained data showed that celastrol improved social deficits, decreased repetitive/restricted behaviors in T-maze test, significant increase in SIRT-1, GSH level with significant decrease in DRP-1, IL-6, caspase-3 and MDA with amelioration of histopathological findings in VPA-induced ASD in both cerebellum and hippocampus. These findings pave the way for using celastrol as an adjuvant therapy during long-term clinical use of risperidone in ASD.

Keywords: ASD, celastrol and mitochondrial dysfunction.

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that affect 1.1% of children in first 3 years of life with male: female ratio of 5:1⁽¹⁾. It is characterized by impairments in social interaction, deficits in verbal and nonverbal communication along

with stereotyped and repetitive behaviors⁽²⁾.

The exact aetiology of ASD is unknown although many hypothesis suggest several factors including genetic predisposition, mitochondrial dysfunction, oxidative stress, inflammation and environmental toxicant exposure⁽³⁾.

Current available pharmacotherapeutic options of autism are only symptomatic with various side effects⁽⁴⁾. Although, risperidone and aripiprazole are the only two psychotropic drugs that have been approved by the US Food and Drug Administration (FDA) for the treatment of autistic children⁽⁵⁾, However, they fail to improve the core behavioral alterations of autism.

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Furthermore, considerable limitations are associated with their long term therapy⁽⁶⁾. The most common adverse effects occurring with risperidone long term therapy are significant weight gain, somnolence, hyperprolactinemia, diabetes mellitus⁽⁷⁾. So, there is an urgent need for development of new and safe disease modifying therapies that target the underlying pathophysiology of the disease with minimal side effects. The aim of this study was to evaluate the possible effect of celastrol alone and in combination with risperidone on amelioration of mitochondrial dysfunction, its anti-inflammatory, antioxidant effects in VPA induced rat model of ASD.

Celastrol is a natural pentacyclic triterpenoid derived from the root extracts of *Tripterygium wilfordii* of the Celastraceae family⁽⁸⁾. The therapeutic potential of this compound came from its safety⁽⁹⁾ and efficacy as anti-inflammatory⁽¹⁰⁾, anti-oxidant, neuroprotective and amelioration of mitochondrial dysfunction⁽¹¹⁾.

Materials and Methods

Drugs and chemicals

Celastrol dissolved in DMSO, administered orally by oral gavage in a dose of 20mg/kg/day⁽¹²⁾, Risperidone dissolved in DMSO, administered orally by oral gavage in a dose of 2mg/kg/day⁽¹³⁾, both obtained from (AdooQ BioScience, California, USA), valproic acid sodium salt dissolved in saline

at concentration of 250mg/ml was administered by intraperitoneal injection (i.p.) in a dose of 400mg/kg⁽¹⁴⁾ (Sigma, St., Louis, MO, USA).

Animals and study design

60 female wistar rats were mated overnight, two females were allowed to mate with one male in the same cage, in the morning when vaginal plug was found it is defined as the first day of gestation⁽¹⁵⁾. Females injected by 400mg/kg sodium valproate single intraperitoneal injection on the 12.5 day of gestation⁽¹⁶⁾. Each female was individually housed to allow her to put her own litters, after weaning male offsprings were divided into 6 equal groups (**Fig.1**). Group 1: Normal control group, Group 2: Offsprings of female wistar rats (treated with normal saline) received vehicle from post-natal day (PND) 21st -35th, Group 3: Autistic offsprings of female wistar rats (treated by VPA) received vehicle from PND 21st -35th, Group 4: Autistic offsprings of female wistar rats (treated by VPA) received risperidone from PND 21st -35th, Group 5: Autistic offsprings of female wistar rats (treated by VPA) received celastrol from PND 21st -35th, Group 6: Autistic offsprings of female wistar rats (treated by VPA) received both risperidone & celastrol from PND 21st -35th. At the end of experiment behavioral tests including three chamber test and T-maze were performed.

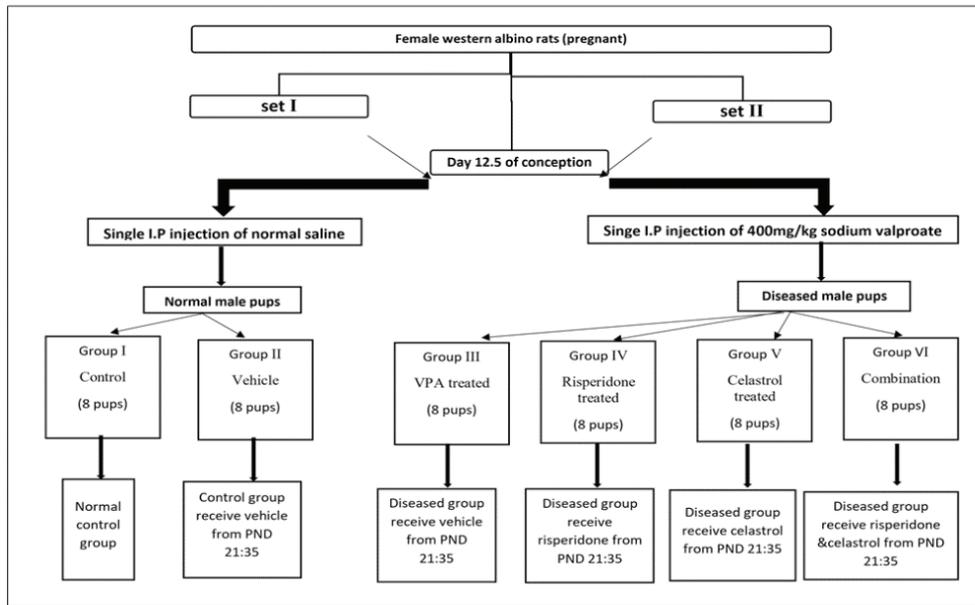


Fig. (1): Experimental design of the study.

Three chamber test is for social interaction and social novelty interest. Sociability is a significant tendency to spend time with stranger rat rather than spending time in empty chamber, while social novelty interest is a significant tendency to spend time with a new rat rather than familiar one. Apparatus is a rectangular box divided by clear Plexiglas walls into equal three chamber, each one is 19 x 45 cm. The dividing walls have an open middle section to allow free movements in between three chambers.

Two large identical wire cups that can hold a single rat were used. Test was started by habituation, subjected rat was placed in the center of middle chamber for five minutes and opening in the dividing walls were closed by plastic box during habituation, After habituation (session I) was started as stranger 1 was placed in one of the side chambers and enclosed in the wire cup which allow contact and prevent fighting between subjected rat and stranger, subjected rat was allowed to explore whole chambers for 10 minutes, length of time in empty chambers and time

spent with stranger 1 were recorded. At the end of this session rat was tested for a second 10 minutes (session II) that test social memory and novelty, stranger 2 was placed inside an identical wire cup in the opposite side chamber that was empty during session I, length of time spent with stranger 1 as well as with stranger 2 were recorded (17).

T-maze testis used to evaluate repetitive/restricted behavior. Normal rats tend to alternate between arms by their memory, this reflecting their motivation for environmental exploration. For each rat five sessions were performed and the first choice was evaluated (18). T maze is a wooden enclosed apparatus in the form of T placed horizontally with the start alley measuring 30 cm length and 10 cm width, the goal arm also measuring 30 cm length and 10 cm width and wall height is 20 cm (19). The rat was placed for 10 minutes in the examination room, then we put it in the start zone and allowed to choose the goal arm. The rat was confined in the chosen arm for 30 seconds then removed and placed in the home cage for 60

seconds. Afterward, it was taken again to the start arm to begin the 2nd trial. This trial was repeated for 5 consecutive times with 1 minute in between and 30 seconds of habituation in the chosen arm. Percentage of alternation (%) between the left and right arms was analyzed.

Biochemical assays

Tissue preparation

The rats were anesthetized by ether and were sacrificed and cerebellum and hippocampus were dissected, washed with phosphate buffered saline (PBS) solution, pH 7.4, to remove any red blood cells and clots. The right half of both cerebellum and hippocampus were fixed in 10 % formalin and processed for examination of histopathological changes by light microscope, while the left half of both cerebellum and hippocampus were stored at (-80°C) until prepared for the assessment of the tissue parameters.

Determination of mitochondrial parameters (SIRT-1&DRP-1):

Isolation of Mitochondria: We used the left half of both cerebellum and hippocampus for mitochondrial extraction. Tissues were sampled, immediately washed with phosphate-buffered saline, and then homogenized with a glass grinding tube on ice for about 20 times in 1 ml mitochondrial isolation buffer (0.01mol/liter Tris-HCl, 0.0001 mol/liter EDTA-2Na, 0.01mol/liter sucrose, 0.8% NaCl, pH 7.4). The homogenate was kept at (4°C) and centrifuged at 1,500 rpm for 10 min. The supernatant was collected and then centrifuged again at 10,000 rpm for 15 min. The precipitate is the mitochondria⁽²⁰⁾

Determination of tissue sirtuin-1 (SIRT1) (ng/ml):

Rat sirtuin-1 was performed using ELISA kit supplied by biodiagnostic; Catalogue No. 201-11-

1498.

Determination of tissue Dynamin related protein 1 (DRP1) (pg/ml):

Rat Dynamin related protein 1 was performed using ELISA kit supplied by Biodiagnostic; Catalogue No. 201-11-3125.

Determination of tissue caspase-3 (ELISA) (ng/ml):

Rat cysteinyl aspartate specific proteinases 3(Caspase-3/ CPP32) was performed using ELISA kit supplied by Sun Red; Catalogue No. 201-11-0281.

Determination of tissue Interleukin-6 (IL-6):

IL-6 was measured in tissue homogenate by kits obtained from Chongqing Biospes Co., Ltd Company, China, catalog No.: BEK1110 according to the method described by⁽²¹⁾.

Determination of tissue reduced glutathione (GSH) (mg/ml)

Reduced glutathione (GSH) level assay was performed using Biodiagnostic supplied Kit (Cat. No TA 2511.), based on the Beutler spectrophotometric process, ⁽²²⁾. **Determination of malondialdehyde (MDA) (nmol / ml)**

Lipid peroxidation was assessed by calculating serum malondialdehyde (MDA) levels according to the Ohkawa et al. method ⁽²³⁾ using Biodiagnostic supplied kit (Cat. No. MD 2529).

Histopathological examinations

The right half of both cerebellum and hippocampus were fixed in 10 % formalin, stained with hematoxylin and eosin (H&E) and processed for examination of histopathological changes by light microscope.

Statistical Analysis

Data were represented as mean ± standard error

of mean (SEM). The significance was considered at values of $P < 0.05$.

Results

Celastrol improved sociability and social affiliation in VPA-induced ASD:

VPA-treated group (group3) revealed a significant decrease in length of time in minutes spent with stranger 1 as compared to the normal control group (group1), indicating impaired sociability. Risperidone treated group (group4) revealed a significant increase in length of time in minutes spent with stranger 1 as compared to the valproate treated group (group3), indicating improved sociability. Celastrol treated group (group5) showed a significant increase in length of time in minutes spent with stranger 1 as compared to the valproate treated group (group3), indicating improved sociability. Combination group (group6) showed a significant increase in length of time in minutes spent with stranger 1 as compared to the valproate treated group (group3), indicating improved sociability, non-significant increase in length of time in minutes spent with stranger 1 as compared to risperidone treated group (group 4), non-significant increase in length of time in minutes spent with stranger 1 as compared to celastrol treated group (group 5).

Celastrol improved social memory & novelty in VPA-induced ASD:

VPA-treated group (group3) revealed a significant decrease in length of time in minutes spent with stranger 2 as compared to the normal control group (group1), indicating decreased social motivation and novelty. Risperidone treated group (group4) revealed a significant increase in length of time in minutes spent with stranger 2 as compared to the valproate treated group (group3), indicating increased social motivation and novelty. Celastrol treated group (group5) showed a significant increase in length of

time in minutes spent with stranger 2 as compared to the valproate treated group (group3), indicating increased social motivation and novelty. Combination group (group6) showed a significant increase in length of time in minutes spent with stranger 2 as compared to the valproate treated group (group3), indicating increased social motivation and novelty, non-significant increase in length of time in minutes spent with stranger 2 as compared to risperidone treated group (group 4), non-significant increase in length of time in minutes spent with stranger 2 as compared to celastrol treated group (group 5).

Celastrol improved repetitive/restricted behaviors in VPA-induced ASD :

VPA-treated group (group3) revealed a significant decrease in percentage of alternation as compared to the normal control group (group1), reflecting repetitive/restricted behaviors. Risperidone treated group (group4) revealed a significant increase in percentage of alternation as compared to the valproate treated group (group3), reflecting improvement of repetitive/restricted behaviors. Celastrol treated group (group5) showed a significant increase in percentage of alternation as compared to the valproate treated group (group3), reflecting improvement of repetitive/restricted behaviors. Combination group (group6) showed a significant increase in percentage of alternation as compared to the valproate treated group (group3), reflecting improvement of repetitive/restricted behaviors, significant increase in percentage of alternation as compared to risperidone treated group (group 4), significant increase in percentage of alternation as compared to celastrol treated group (group 5).

Celastrol ameliorated mitochondrial dysfunction in VPA-induced ASD:

VPA-treated group (group3) revealed a significant decrease in SIRT-1 level, significant

increase in DRP-1 level as compared to the normal control group (group1) in both cerebellum, and hippocampus, indicating mitochondrial dysfunction. Risperidone treated group (group4) revealed non-significant difference in SIRT-1 and DRP-1 level as compared to the valproate treated group (group3) in both cerebellum, and hippocampus, indicating no improvement in mitochondrial dysfunction. Celastrol treated group (group5) showed a significant increase in SIRT-1 level, significant decrease in DRP-1 level as compared to the valproate treated group (group3), indicating amelioration of mitochondrial dysfunction, significant increase in SIRT-1 level, significant decrease in DRP-1 level as compared to risperidone treated group (group 4) in both cerebellum, and hippocampus. Combination group (group6) showed a significant increase in SIRT-1 level, significant decrease in DRP-1 as compared to the valproate treated group (group3), significant increase in SIRT-1, significant decrease in DRP-1 level as compared to risperidone treated group (group 4), and non-significant difference in SIRT-1 and DRP-1 level as compared to celastrol treated group (group 5) in both cerebellum, and hippocampus.

Celastrol ameliorated apoptosis in VPA-induced ASD:

VPA-treated group (group3) revealed a significant increase in caspase level as compared to the normal control group (group1) in both cerebellum, and hippocampus. Risperidone treated group (group4) revealed non-significant difference in caspase level as compared to the valproate treated group (group3) in both cerebellum, and hippocampus. Celastrol treated group (group5) showed a significant decrease in caspase level as compared to the valproate treated group (group3), indicating improved apoptosis, significant decrease in caspase level as compared to risperidone treated group (group 4) in both cerebellum, and hippocampus. Combination group (group6) showed

a significant decrease in caspase level as compared to the valproate treated group (group3), significant decrease in caspase level as compared to risperidone treated group (group 4), and non-significant difference in caspase level as compared to celastrol treated group (group 5) in both cerebellum, and hippocampus.

Celastrol improved inflammation in VPA-induced ASD:

VPA-treated group (group3) revealed a significant increase in IL-6 level as compared to the normal control group (group1) in both cerebellum, and hippocampus. Risperidone treated group (group4) revealed a significant decrease in IL-6 level as compared to the valproate treated group (group3) in both cerebellum, and hippocampus. Celastrol treated group (group5) showed a significant decrease in IL-6 level as compared to the valproate treated group (group3) in both cerebellum, and hippocampus, denoting improved inflammation. Combination group (group6) showed a significant decrease in IL-6 level as compared to the valproate treated group (group3), significant decrease in IL-6 level as compared to risperidone treated group (group 4), and significant decrease in IL-6 level as compared to celastrol treated group (group 5) in both cerebellum, and hippocampus.

Celastrol ameliorated oxidative stress status:

VPA-treated group (group3) revealed a significant decrease in GSH level, a significant increase in MDA level as compared to the normal control group (group1) in both cerebellum, and hippocampus. Risperidone treated group (group4) revealed non-significant difference in GSH and MDA level as compared to the valproate group (group3) in both cerebellum, and in hippocampus. Celastrol treated group (group5) showed a significant increase in GSH level, a significant decrease in MDA level as compared to the valproate group (group3), a significant increase in GSH level, significant decrease in MDA level as

compared to risperidone treated group (group 4) in both cerebellum, and hippocampus. Combination group (group6) showed a significant increase in GSH level, a significant decrease in MDA level as compared to the valproate group (group3), a significant increase

in GSH level, a significant decrease in MDA level as compared to risperidone treated group (group 4) and non-significant difference in GSH and MDA level as compared to celastrol treated group (group 5) in both cerebellum, and hippocampus.

Table-I:Effect of risperidone, celastrol and the combination of both on different measured parameters in VPA-rat animal model of autism. Results expressed as mean± SEM of 6 groups (8 rats each).

Groups Parameter		Group1 Control	Group 2 Vehicle	Group 3 VPA	Group 4 Risperidone	Group 5 Celastrol	Group 6 Combination
Three chamber test (session-I)	Length of time in empty chamber	2.3 ± 0.4	3.0 ± 0.7	8.0 ± 0.5	3.9 ± 0.5	4.2 ± 0.5	2.2 ± 0.2
	Length of time spent with stranger-1	7.7 ± 0.4 +	7.0 ± 0.7 +	2.0 ± 0.5 #+	6.1 ± 0.5 * +	5.8 ± 0.5 *+	7.8 ± 0.2 *+
Three chamber test (session-II)	Length of time spent with stranger-1	2.4 ± 0.3	2.9 ± 0.2	7.5 ± 0.4	3.9 ± 0.2	3.9 ± 0.4	3.5 ± 0.4
	Length of time spent with stranger-2	7.6 ± 0.3 +	7.1 ± 0.2 +	2.5 ± 0.4 #+	6.1 ± 0.2 *+	6.1 ± 0.4 *+	6.5 ± 0.4 *+
T maze test (percentage of alternation %)		95.0 ± 3.3	90.0 ± 3.8	27.5 ± 3.7 #	57.5 ± 4.5 *&	65.0 ± 5.0 *&	87.5 ± 5.3*
Tissue SIRT-1	Cerebellum	10.5 ± 0.6	9.9 ± 0.8	1.1 ± 0.1#	3.5 ± 0.8 &	8.1 ± 0.7 *	10.1 ± 0.4 *
	Hippocampus	9.9 ± 0.3	9.3 ± 0.4	1.4 ± 0.2 #	3.1 ± 0.5 &	7.5 ± 0.5 *	8.8 ± 0.4 *
Tissue DRP-1	Cerebellum	176.0 ± 10.8	183.7 ± 10.2	410.1 ± 8.8 #	357.0 ± 20.4 &	235.1 ± 12.7 *	181.9 ± 10.1 *
	Hippocampus	164.9 ± 8.4	168.3 ± 10.1	388.8 ± 16.4 #	342.5 ± 21.7 &	230.0 ± 18.5 *	184.5 ± 2.7 *
Tissue caspase-3	Cerebellum	0.18 ± 0.08	0.36 ± 0.09	1.7 ± 0.09 #	1.4 ± 0.2 &	0.75 ± 0.08 *	0.32 ± 0.13 *
	Hippocampus	0.11 ± 0.04	0.32 ± 0.09	1.5 ± 0.05 #	1.1 ± 0.14 &	0.44 ± 0.13 *	0.17 ± 0.05 *
Tissue Interleukin-6	Cerebellum	92.6 ± 4.4	117.0 ± 7.9	257.9 ± 6.1 #	145.1 ± 6.5 *&	128.0 ± 7.6 *&	95.3 ± 6.6 *
	Hippocampus	82.0 ± 3.4	105.5 ± 7.9	246.5 ± 5.8 #	138.6 ± 6.1 *&	119.3 ± 7.7 *&	92.8 ± 3.1 *
Reduced glutathione (GSH)	Cerebellum	5.4 ± 0.3	4.7 ± 0.2	2.7 ± 0.2 #	3.0 ± 0.2 &	4.8 ± 0.2 *	5.4 ± 0.1 *
	Hippocampus	4.6 ± 0.2	3.7 ± 0.2	2.1 ± 0.2 #	2.6 ± 0.2 &	3.9 ± 0.3 *	4.3 ± 0.2 *
Malondialdehyde (MDA)	Cerebellum	15.5 ± 0.4	24.4 ± 1.1	61.3 ± 5.2 #	48.1 ± 8.3 &	28.3 ± 2.7 *	16.6 ± 0.9 *
	Hippocampus	13.9 ± 0.8	21.2 ± 0.7	53.5 ± 5.4 #	43.1 ± 7.4 &	25.9 ± 1.5 *	15.6 ± 1.1 *

Celastrol improved histopathological findings in VPA-induced ASD in both cerebellum and hippocampus (Fig.2):

Histopathology in the VPA-treated group (group 3) Showed diminished number of purkinje cells with altered cerebellar structure (Fig.2C), numerous neuronal degeneration and chromatolysis in hippocampus indicating VPA-induced apoptosis in neurons (Fig.2D). Risperidone treated group (group

4) Showed diminished number of purkinje cells with altered cerebellar structure (Fig.2E), neuronal degeneration and chromatolysis (Fig.2F). celastrol treated group (group 5) Showed apparently normal cerebellum with intact Purkinje cell layer (Fig.2G), normal hippocampal architecture with minimal degeneration (Fig.2H). Combination group (group 6) Showed apparently normal cerebellum with intact Purkinje cell layer (Fig.2I), normal hippocampal architecture with minimal degeneration (Fig.2J).

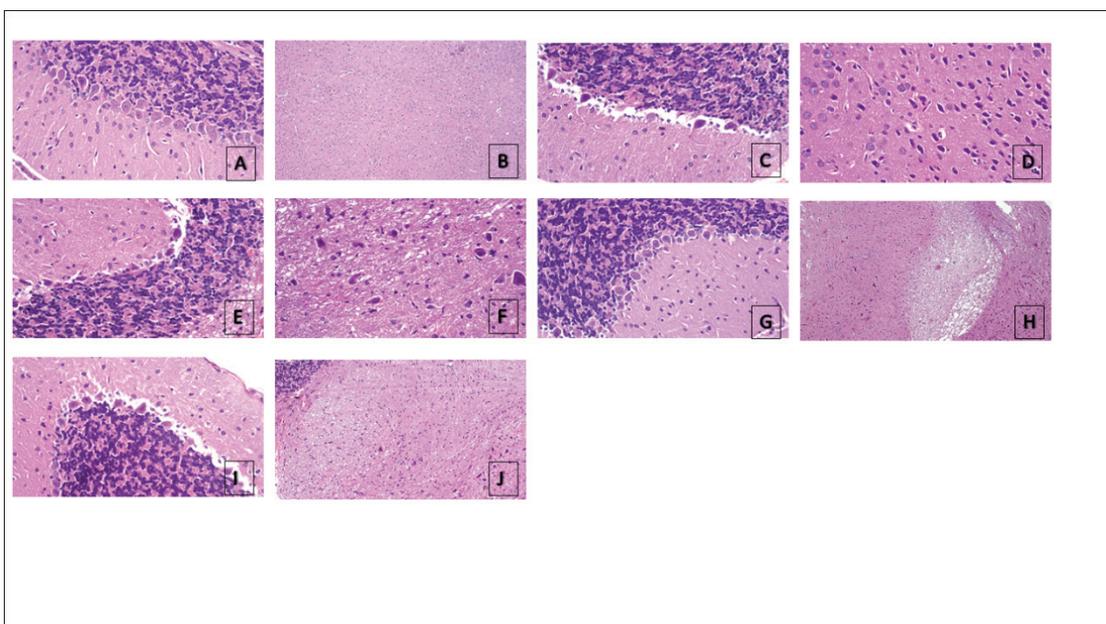


Fig. 2; Histopathology in the control group (group 1) (A) Showing normal cerebellum with intact Purkinje cell layer (H&E X400), (B) Showing normal hippocampal architecture (H&E X100). Histopathology in the VPA-treated group (group 3) (C) Showing diminished number of purkinje cells (arrows) with altered cerebellar structure (H&E X400), (D) Showing numerous neuronal degeneration and chromatolysis in hippocampus indicating VPA-induced apoptosis in neurons (H&E X400). Histopathology in Risperidone treated group (group 4) (E) Showing diminished number of purkinje cells with altered cerebellar structure (H&E X400), (F) Showing neuronal degeneration and chromatolysis (H&E X400). Histopathology in celastrol treated group (group 5) (G) Showing apparently normal cerebellum with intact Purkinje cell layer (H&E X400), (H) Showing normal hippocampal architecture with minimal degeneration (H&E X100). Histopathology in combination Risperidone and Celastrol group (group 6) (I) Showing apparently normal cerebellum with intact Purkinje cell layer (H&E X400), (J) Showing normal hippocampal architecture with minimal degeneration (H&E X100).

Discussion

Recently, many studies confirm that a significant proportion of individuals with autism have mitochondrial disease. The prominent epigenetic regulatory role of SIRT-1 (silent information regulator-1) in controlling mitochondrial function may underlie its recently reported neuroprotective effect in numerous neurological diseases⁽²⁴⁾. SIRT-1 is a histone deacetylase that control PGC1 α (peroxisome-proliferator-activated-receptor c coactivator 1 α) which is the key regulator of mitochondrial biogenesis⁽²⁵⁾, PGC1 α is found to be highly expressed in cells rich in mitochondria as neurons especially newly generated neurons in embryonic as well as early postnatal life. Regarding mitochondrial dynamics, mitochondria undergo continuous remodeling by growth and fission of each mitochondria, two key proteins are responsible for mitochondrial fission dynamin-related protein 1 (DRP1) and fission 1 protein (Fis 1).

The results of the present study substantiate the idea that mitochondrial dysfunction plays an important role in ASD, animals in the valproate treated group presented a state of mitochondrial dysfunction in CNS as represented by significant decrease in SIRT-1 and significant increase in DRP-1 level. Mitochondrial dysfunction results in oxidative stress that further aggravates mitochondrial impairments, as ROS produced inside mitochondria induce DRP-1 causing mitochondrial fission culminating in vicious circle that eventually results in initiating apoptotic cascade leading to neuronal cell death⁽²⁶⁾. VPA induce an imbalance between oxidative stress and antioxidant system, reduced glutathione (GSH) is the main cellular free radical scavenger in the brain and this oxidative stress state leads to neuronal damage, In the present study, valproate treated group presented a state of oxidative stress in both cerebellum and hippocampus presented by significant decrease in reduced glutathione content and marked increase in

lipid peroxidation represented by significant increase in MDA and this result agree with Rossignol et al⁽²⁷⁾.

Regarding caspase-3 level, valproate treated group exhibited a significant increase in caspase-3 in both cerebellum and hippocampus. This result agree with apoptotic results in ASD obtained by El-Ansary et al⁽²⁸⁾.

In the present study, animals in valproate treated group showed a significant increase in IL-6 level, VPA induce microglial activation, active microglia increased production of the pro-inflammatory mediator IL-6 causing neuronal damage and loss, this result is supported by Masi et al⁽²⁹⁾.

In absence of a specific treatment for core symptoms of ASD and the many side effects of risperidone especially with long term use, there is an urgent need to search for and find other safe drugs for long-term periods.

To the best of our knowledge, there is no previous researches investigated the effect of celastrol in VPA-rat animal model of autism. In the current study, regarding behavioral tests celastrol ameliorated social deficits induced by VPA as manifested in three chamber test by significant increase in length of time with stranger-1 compared to time spent at empty chamber at session I, indicating improved sociability as well as it showed preference for the chamber containing a newly introduced animal (Stranger 2) over a chamber containing an already familiar animal (Stranger 1) evidenced by significant increase in length of time in minutes spent with stranger-2 compared to time spent with stranger-1 at session II, indicating increased social motivation and novelty in celastrol treated group as well as there was a significant increase in percentage of alterations(%) in T-maze test indicating decrease in repetitive/ restricted behaviors. Regarding neurochemical results, celastrol treated group showed significant increase in SIRT-1, GSH while it showed

significant decrease in DRP-1, caspase-3, IL-6 and MDA level as compared to valproate treated group. In our study, when celastrol treated group compared to that treated by risperidone, celastrol treated group showed an ameliorating effect on mitochondrial dysfunction as it cause significant increase in SIRT-1 and significant reduction in DRP-1.

These findings suggest that combination of risperidone and celastrol provide an additional amelioration on the disease activity with exhibited additional effects as regard to improvement of behavioral impairment, mitochondrial dysfunction and inflammation. In addition to the additional anti-apoptotic and antioxidant effects of celastrol which provide more neuroprotective influence.

Conclusion

These encouraging results pave the way for using celastrol as an adjuvant therapy during long-term clinical use of risperidone which provide better results and to avoid its neurotoxic impact. This should be verified in further human clinical studies.

Conflict of Interest: None.

Funding: None.

Ethical Clearance: The handling of animals and all experimental procedures were adopted by the institutional "Research Ethics Committee, REC", Faculty of Medicine, Tanta University, Egypt (Approval no. 32548/09/18).

Abbreviations: **ASD:** Autism spectrum disorder; **DRP-1:** Dynamin-related protein 1; **Fis-1:** Fission 1 protein; **GSH:** Reduced glutathione; **IL-6:** Interleukin-6; **IP:** intraperitoneal; **MDA:** Malondialdehyde; **PGC-1 α :** Peroxisome proliferator-activated-receptor coactivator 1 α ; **PND:** Post-natal day; **ROS:** Reactive oxygen species; **SIRT-1:** Sirtuin-1; **TW:** Tripterygium wilfordii; **VPA:** Valproic acid.

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The Effectiveness of Facet Joint Local Corticosteroid Injection in Diagnosis and Treatment of Facet Joint Syndrome

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Abstract

Objectives: To evaluate outcomes of lumbar facet joint injection with local corticosteroid in treatment of chronic low back pain according to Oswestry disability Index(ODI).

Patients and Methods: Interventional prospective study on 23 patients attended the outpatient clinic of Orthopedic Surgery in Cairo University Hospitalscomplaining of chronic low back pain not responsive to medical treatment and physiotherapy from September 2018 to august 2019.

Results: The mean age of the patients was 41.17 ± 9.74 years and 47.8% were males. Facet joint corticosteroid injection resulted in significant reduction of pain severity of patients as it ranged 5:10 (mean 7.3 ± 1.5) pre-injection and improved to 0 :6 (mean 3.6 ± 1.7) 3-month post injection(Pvalue:0.001) while the ODI score ranged 26:80 (mean 47.4 ± 15.7) pre-injection and improved to 4:46 (mean 28.8 ± 10.4) three months post injection(Pvalue 0.001).

Conclusion: intra-articular facet joint injection is crucial in the diagnosis and treatment of facet joint syndrome.

Keywords: back pain, corticosteroids, facet joint, and injection.

Introduction

Low back pain continues to be responsible for more years lived with disability than any other disorder⁽¹⁾. Multipleinternational studies attest to the massive

health care and societal costs of low back pain. Most of those affected have non-specific low back pain⁽²⁾.

Facet joint pain is defined in a functional capacity as pain originating from any structure integral to both the function and configuration of the lumbar facet joints^(3, 4). Facet joint injury can occur from mechanical damage due to compressive forces or extensive stretching; degenerative changes such as osteoarthritis and inflammatory processesincluding rheumatoid arthritis^(5, 6). Facet joints are richly innervated by the medial branches from the dorsal rami above and below each joint⁽⁷⁾.The prevalence of facet joint syndrome was 5:10% of patients with

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chronic LBP and it was determined by pain relieve more than 75% after intra-articular lidocaine and bupivacaine injection⁽⁸⁻¹⁰⁾. The diagnosis is mainly based on history and physical examination. The patient is presented with chronic LBP not responding to medical treatment more than 6 month that increase with rotational and extension maneuver and radiating to the groin region or thigh⁽¹¹⁻¹³⁾.

Corticosteroids are established anti-inflammatory agents with demonstrable, short-term, benefits when injected intra-articularly to treat shoulder impingement syndrome or osteoarthritis of peripheral joints^(14, 15)

Despite the plethora of research and clinical emphasis on this disorder, almost every aspect of facet joint pain, from diagnosis to treatment, remains mired in controversy. Even among pain specialists, lumbar facet joint pain remains a misunderstood, misdiagnosed, and improperly treated medical condition. So in this study we aim to evaluate the outcomes of lumbar facet joint injection with local corticosteroid in chronic low back pain to reach better and less invasive method for treatment of low back pain.

Patients and methods

Study setting and population:

The study was carried out in the Department of Orthopedic Surgery in Cairo University Hospitals.

The study included patients attending the outpatient clinic of Orthopedic Surgery in Cairo University Hospitals complaining of chronic low back pain not responsive to medical treatment and physiotherapy from September 2018 to august 2019.

Study population: patients attending the outpatient clinic of Orthopedic Surgery in Cairo University Hospitals complaining of chronic low back pain not responsive to medical treatment and physiotherapy. The Sample size is 23 patients.

All patients were complying with the following inclusion and exclusion criteria:

· Inclusion criteria:

§ Age 20:60.

§ Both sexes.

§ LBP at least for 6 month duration exaggerated by external rotation and extension.

§ Failure to respond to conservative medical treatment and physiotherapy.

· Exclusion criteria:

§ History of allergy to local anesthetics or steroid

§ Coagulopathy.

§ Severe foraminal stenosis.

§ Progressive neurological disorders.

§ Pregnancy

§ radiculopathy

§ Acute lumbar Fracture.

Evaluation of pain severity & functional outcome:

Precise assessment of the severity of back and leg pain and the functional condition of the patient before the procedure was compared with the post injection results so we can evaluate the effect of the treatment or the procedure performed.

A) The Visual analogue scale:

It is a scale from 0 to 10 where is 0 means no pain at all and 10 means the worst pain you can imagine (Fig.2). Every patient chooses a number on the scale once for his back pain and another for his leg pain and to be repeated within 6 weeks and then within 3 months post injection.

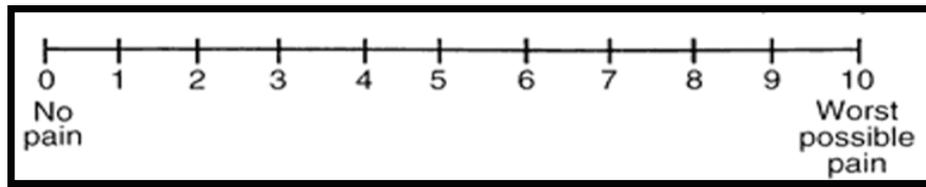


Fig.1: the visual analogue scale

B) The Oswestry Disability index:

It is used to evaluate the functional condition of the patient’s back. Each section is scored on a 0–5 scale, 5 representing the greatest disability (Fig.2).

The index is calculated by dividing the added score by the total possible score, which is then multiplied by 100 and expressed as a percentage. Thus, for every question not answered, the denominator is reduced by 5.

Oswestry Disability Index

Name _____ Date _____

Instructions: Please circle the **ONE NUMBER** in each section which most closely describes your problem.

Section 1 – Pain Intensity

- 0. The pain comes and goes and is very mild.
- 1. The pain is mild and does not vary much.
- 2. The pain comes and goes and is moderate.
- 3. The pain is moderate and does not vary much.
- 4. The pain comes and goes and is severe.
- 5. The pain is severe and does not vary much.

Section 2 – Personal Care (Washing, Dressing, etc.)

- 0. I would not have to change my way of washing or dressing in order to avoid pain.
- 1. I do not normally change my way of washing or dressing even though it causes some pain.
- 2. Washing and dressing increase the pain but I manage not to change my way of doing it.
- 3. Washing and dressing increase the pain and I find it necessary to change my way of doing it.
- 4. Because of the pain I am unable to do some washing and dressing without help.
- 5. Because of the pain I am unable to do any washing and dressing without help.

Section 3 – Lifting

- 0. I can lift heavy weights without extra pain.
- 1. I can lift heavy weights but it gives extra pain.
- 2. Pain prevents me lifting heavy weights off the floor.
- 3. Pain prevents me lifting heavy weights off the floor, but I can manage if they are conveniently positioned, e.g., on a table.
- 4. Pain prevents me lifting heavy weights but I can manage light to medium weights if they are conveniently positioned.
- 5. I can only lift very light weights at most.

Section 4 – Walking

- 0. I have no pain on walking.
- 1. I have some pain on walking but it does not increase with distance.
- 2. I cannot walk more than 1 mile without increasing pain.
- 3. I cannot walk more than ½ mile without increasing pain.
- 4. I cannot walk more than ¼ mile without increasing pain.
- 5. I cannot walk at all without increasing pain.

Section 5 – Sitting

- 0. I can sit in any chair as long as I like.
- 1. I can sit only in my favorite chair as long as I like.
- 2. Pain prevents me from sitting more than 1 hour.
- 3. Pain prevents me from sitting more than ½ hour.
- 4. Pain prevents me from sitting more than 10 minutes.
- 5. I avoid sitting because it increases pain immediately.

Section 6 – Standing

- 0. I can stand as long as I want without pain.
- 1. I have some pain on standing but it does not increase with time.
- 2. I cannot stand for longer than 1 hour without increasing pain.
- 3. I cannot stand for longer than ½ hour without increasing pain.
- 4. I cannot stand for longer than 10 minutes without increasing pain.
- 5. I avoid standing because it increases the pain immediately.

Section 7 – Sleeping

- 0. I get no pain in bed.
- 1. I get pain in bed but it does not prevent me from sleeping well.
- 2. Because of pain my normal nights sleep is reduced by less than one-quarter.
- 3. Because of pain my normal nights sleep is reduced by less than one-half.
- 4. Because of pain my normal nights sleep is reduced by less than three-quarters.
- 5. Pain prevents me from sleeping at all.

Section 8 – Social Life

- 0. My social life is normal and gives me no pain.
- 1. My social life is normal but it increases the degree of pain.
- 2. Pain has no significant effect on my social life apart from limiting my more energetic interests, e.g., dancing, etc.
- 3. Pain has restricted my social life and I do not go out very often.
- 4. Pain has restricted my social life to my home.
- 5. I have hardly any social life because of the pain.

Section 9 – Traveling

- 0. I get no pain when traveling.
- 1. I get some pain when traveling but none of my usual forms of travel make it any worse.
- 2. I get extra pain while traveling but it does not compel me to seek alternate forms of travel.
- 3. I get extra pain while traveling which compels to seek alternative forms of travel.
- 4. Pain restricts me to short necessary journeys under ½ hour.
- 5. Pain restricts all forms of travel.

Section 10 – Changing Degree of Pain

- 0. My pain is rapidly getting better.
- 1. My pain fluctuates but is definitely getting better.
- 2. My pain seems to be getting better but improvement is slow.
- 3. My pain is neither getting better or worse.
- 4. My pain is gradually worsening.
- 5. My pain is rapidly worsening.

TOTAL _____

Fig.2: Oswestry disability index.

Equipment for facet injections:

- Appropriate syringe and needle for local anesthesia.
- Lidocaine 1% without epinephrine.
- Spinal needle, 22 or 25 gauge.
- Bupivacaine 0.25% (1ml).
- Injectable steroid (1ml triamcinolone 40 mg)

Positioning

- The patient lie prone on the table with a pillow placed under the front of the abdomen for comfort.
- Fluoroscopy should be placed in a direct postero-anterior orientation.
- Once the correct level is identified, tilting the X-ray beam caudally 20–30° allowing clearer delineation of the joint, described as a “Scotty-Dog” view.

Technique:

- In operation room at Cairo University Hospitals.
- Standard aseptic technique is mandatory. The skin is prepared above and below the level injected.
- The selected level is identified by palpation of the spinous processes, and confirmed radiographically. The joints lay midway between and lateral to adjacent spinous processes.
- Once the entry point is confirmed, the needle is advanced in the line of fluoroscopy until bone is contacted, and the position again checked radiographically to be directly over the joint.
- Therapeutic injection is then carried out.
- Withdraw the needle and apply a sterile dressing.

Follow up

Patients were followed for a period of 12 weeks. The first visit post-injection was at the second week to assess the immediate effect of facet joint injection, while the second and third visits at the 4th and the 12th weeks respectively to assess the short term effect. The improvement was assessed using the visual analogue scale (VAS) for back pain, and the Oswestry disability index (ODI) for functional outcome.

Results:

Demographic date: The mean age was 41.17 ± 9.74 years ranging from 25 to 60 years and according to gender distribution, they were 11(47.8 %) males and 12 (52.2%) females.

Low back pain characteristics

The low back pain duration among the participants was 22.1 ± 8.9 month ranging from 9- 36 months. The site of pain was felt in the midline by 6 (26.1%), para-median by 3 (13%) and at both sites by 14 (60.9%) patients. It was radiating in the right side in 6 (26.1%), in the left side in 7 (30.4%) and radiating bilaterally in 10 (43.5 %). the pain was aggravated by sitting in 15 (65.2%) patients, standing in 18 (78.3 %) patients, effort in 15 (65.2%) and by walking in 4 (17.4 %) patients. Night sleep was affected in 13 (56.5%) patients. Pain was relieved by rest in 14 (60.9%) patients. daily activities was interrupted mildly in 4 (17.4%) of patients, moderately in 13 (56.5%) and markedly in 6 (26.1%) patients.

Spine examination

During lateral bending , tenderness was felt on the right side in 3 (13%) patients, on the left side in 5 (21.7%) and bilaterally in 15 (65.3) patients. There was tenderness during extension all patients . There was tenderness during flexion in 13 (56.5%) patients. All patients have intact sensory , motor and reflexes .

Facet joint degeneration

Based on Magnetic Resonance Imaging and according to **Weishaupt classification** there were four (17.4%) patients G₀, eight (34.8%) patients G₁, six (26.1%) patients G₂ and five (21.7%) patients G₃.

Evaluation of pain severity and functional prognosis:

Pain severity assessment:

The severity of patient’s pain was assessed using visual analogue scale. The pre-injection VAS ranged from 5:10 with mean 7.3 ± 1.5 . Immediately after injection its range became 0:4 with mean 2.3 ± 1.2 . One month later, the range was 0:4 with mean 3.1 ± 1.7 . After three months, the range was 0 :6 with mean 3.6 ± 1.7 . There was significant decrease in pain severity after injection as P value was 0.001

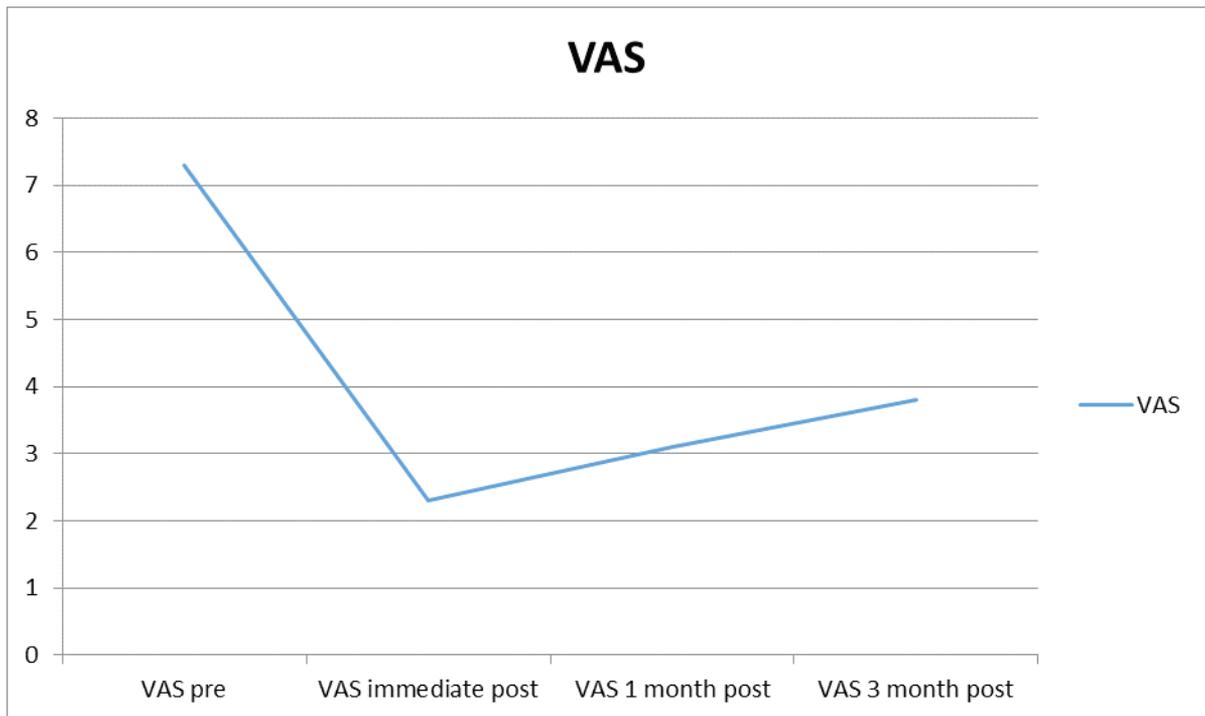


Fig.3: showing the VAS of patients’ pre and post injection.

Functional evaluation:

Functional evaluation was done using ODI. The pre-injection ODI ranged from 26:80 with mean 47.4 ± 15.7 . One month later, the range was 4:40 with mean

21.1 ± 9.6 . At three month, the range was 4:46 with mean 28.8 ± 10.4 . There was significance improvement in functional outcome as P value was 0.001.

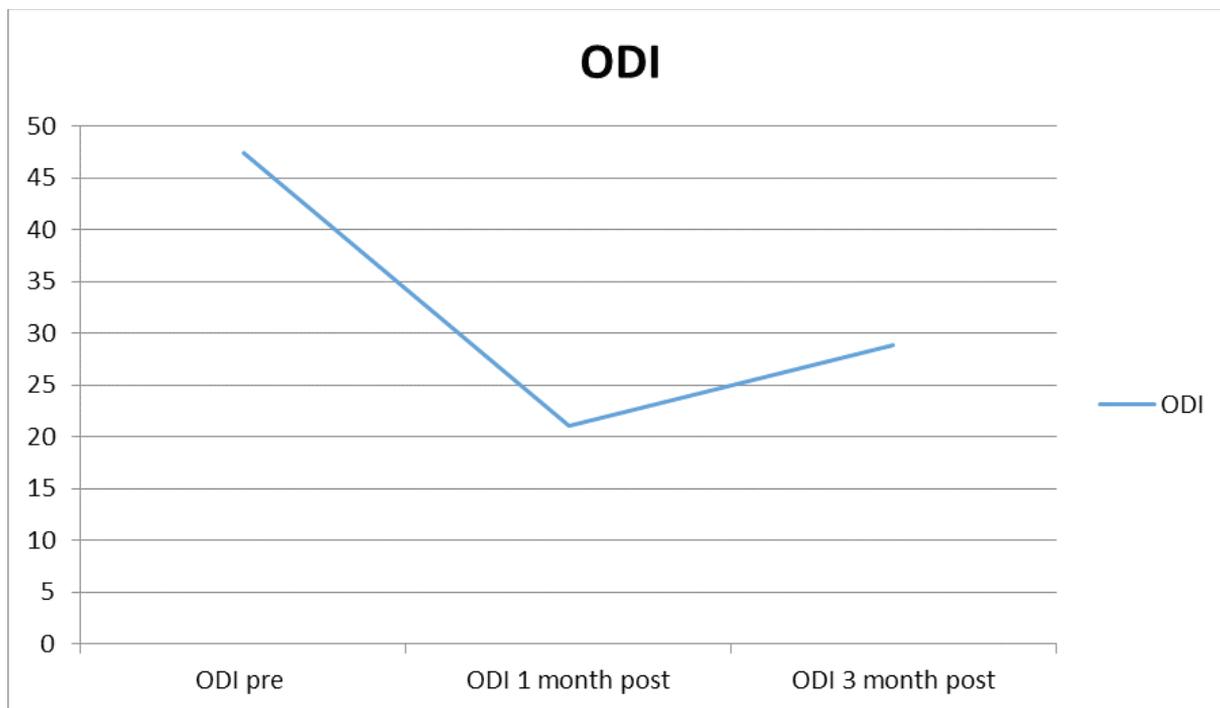


Fig.4: showing the ODI of patients’ pre and post injection.

Relationship between facet joint arthritis and VAS:

Table 1: relationship between grade of facet arthritis and reduction in pain severity after injection

		G0	G1	G2	G3	P-value
VAS	Pre	8.5	7.25	6.83	7.17	0.233
	Immediate post	2.25	1.37	2.67	2.00	0.22
	1 month post	2.75	2.62	3.3	2.8	0.47
	3 months post	3.00	2.5	3.5	5.0	0.044

There was no statically significant difference among the patients before, immediately after injection and 1 month after injection. But three months post injection show statically significant difference as the patients with arthritis grade 2 & 3 start to develop pain.

Discussion

This study was carried out in Cairo University hospitals from august 2018 to august 2019. It included 23 patients with chronic low back pain that were treated with lumbar facet joint injection. The mean age of the patients was 41.7 ±9.74 years. The male

to female ratio was 11:12. The number of patients is nearly comparable with the number of patients presented by **Kawuet al.**,⁽¹⁶⁾**Celik et al.**⁽¹⁷⁾ and **Carette et al.**⁽¹⁸⁾(table 2). The number of patients in our study is limited by rate of cases available in our causality during the time of our work; other studies with higher patient number were retrospective and lasted for longer time.

The inclusion and exclusion criteria of our study are similar to the inclusion criteria of **lilius et al.**⁽¹⁹⁾, **Carette et al.**⁽¹⁸⁾, **Celik et al.**⁽¹⁷⁾, while it differs from the study of **Kim et al.**⁽²⁰⁾; as he accepted patients

with positive straight leg raise test and the study of **Proiettiet al.**⁽²¹⁾who only accepted patients older than 45 years.

The mean age of this study is comparable with the study of **Lilius et al.**,⁽¹⁹⁾**Carette et al.**⁽¹⁸⁾, **Celik et al.**⁽¹⁷⁾, and **Kawu et al.**⁽¹⁶⁾. However, this is less than the age presented with**Proietti et al.**⁽²¹⁾ and **Kim et al.**⁽²⁰⁾.

The sex distribution of this study was comparable with the study of **Lilius et al.**⁽¹⁹⁾, and **Caretteet al.**⁽¹⁸⁾ while it differs from the sex distribution of the study of **Kim et al.**⁽²⁰⁾, **Kawu et al.**⁽¹⁶⁾, and **Proiettiet al.**⁽²¹⁾

Table 2:comparison between current study and other studies according to patient number, age and gender

Study	Number	Age	gender
Current study	23	41.2	47.8% males
Lilius	107	44	44.8 % males
Kim	244	68.2	23.4%males
Karrette	149	42.5	51% males
Celik	40	37.6	32.5% males
Kawu	10	42.3	60% males
Proietti	40	65	37.5% males

The low back pain duration among the participants was 22.1 ± 8.9 month ranging from 9- 36 months.

The pain severity was assessed by VAS and the functional status of the patients were assessed using ODI

We have assessed the pain using visual analogue scale. The pre-injection VAS ranged from 5:10 with mean 7.3 ± 1.5 . Immediately after injection its range

became 0:4 with mean 2.3 ± 1.2 . One month later, the range was 0:4 with mean 3.1 ± 1.7 . After three months, the range was 0 :6 with mean 3.6 ± 1.7 with decrease in pain severity by 3.7. Our results were comparable with the results of **Caretteet al.**⁽¹⁸⁾ and **Celik et al.**⁽¹⁷⁾ and better than the results of **Kawu et al.**⁽¹⁶⁾ and **Proiettiet al.**⁽²¹⁾may be due to the sample small size in**Kawu** and patient’s age inclusion criteria of **Proietti**.

Table 3:comparison between current study and other studies according to VAS

	Pre-VAS	Immediate post VAS	1 month VAS	Three month post VAS
Current study	7.3	2.3	3.1	3.6
Carette	6.3	4.5	3.8	
Celik	8	2	1	3.2
Kawu	7.8		4	4.9
Proietti	8.5	4	5	6
Schulte et al	8	2.5	3.4	4

We have used the ODI to evaluate the Functional status. The pre-injection ODI ranged from 26:80 with mean 47.4 ± 15.7 . One month later, the range was 4:40 with mean 21.1 ± 9.6 . At three month, the range was 4:46 with mean 28.8 ± 10.4 . Our results were better than the results of **Kawu** et al. ⁽¹⁶⁾ as the pre injection ODI was 58.6 ± 6.8 , one month later was 42.3 ± 5.5 and after three months were 39.6 ± 4.9

Our study has points of strength as it is a prospective study and the cases were done by the same team.

On the other hand, the limited number of cases and the short-term follow up are the two points of weakness of this study. The study assessed early outcomes only and there was no long-term follow up.

Conclusion

In conclusion intra-articular facet joint injection is crucial in the diagnosis and treatment of facet joint syndrome. It is an easy to perform and effective treatment for temporary pain relief. Grading lumbar facet joint degeneration by using Weishaupt classification helps to estimate the long term response

to injection.

Ethical Clearance: Taken from kasrAl-Ainy ethical committee.

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Conflict of Interest: None to declare.

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The Management of Giant Cell Arteritis (GCA) Overlapping with Sjogren Syndrome : A Case Report

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Abstract

Giant cell arteritis (GCA) is an autoimmune vasculitis involving large and medium arteries. GCA is rarely associated with autoimmune diseases, such as Sjögren's syndrome. Glucocorticoids (GC) are the cornerstone of the treatment of GCA as they are very effective but are often given for 1 year to avoid relapses. Here we report a rare case of GCA overlapping with Sjögren's syndrome along with the management.

Keywords: Giant Cell Arteritis, Management, Sjogren Syndrome

Introduction

GCA is an immune-mediated systemic vasculitis that mainly involves the large and middle arteries, especially the temporal arteries. GCA mainly occurs in people over the age of 50 years old.¹The lifetime risk of developing GCA is estimated at 1% for women and 0.5% for men.²

The symptoms of giant cell arteritis can overlap with its cousin disease polymyalgia rheumatica (PMR). PMR similarly is an autoimmune disease that affects the elderly. Its hallmark symptoms include muscle pain and weakness affecting the large muscle groups especially in the hips and shoulders. Patients with PMR have trouble getting out of a chair and reaching for objects in cupboards. PMR symptoms also include low-grade fever, malaise, poor appetite, and weight loss. When symptoms affect the neck and higher, giant cell arteritis can be at work. Five

to 15% of PMR patients will be diagnosed with giant cell arteritis, and 50% of giant cell arteritis patients have PMR symptoms.³ GCA is rarely associated with autoimmune diseases, such as Sjögren's syndrome.⁴

Case

A 52 years old woman named Mrs. M was admitted by her family to the outpatient clinic of Dr. Soetomo general hospital, Surabaya, with a chief complaint sudden onset headache.

Current Medical History

She complained about having headache since 2 months ago (pain is worse in 2 weeks ago). The headache was like being pressed and localized to the temple. However, it may be occipital or be less defined and precipitated by brushing the hair. The pain feels heavy everyday so it is difficult to sleep. Vertigo absent.

The patient often has anorexia so that the weight goes down. Sometimes nausea but not vomiting. The left eye feels more blurry than the right eye since 2 months ago. The patient admitted to having both red

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eyes since the headache appeared.

The patient had gone to the neurological clinic for headache since 2 month ago and was given therapy methyprednisolone 3x 16 mg and the last month on prednisone treatment 3x 20 mg but the chief complaint did not improve then the patient was referred to a rheumatology clinic RSUD Dr. Soetomo.

Previous medical history

The patient was diagnosed with hepatitis B 13 years ago, had been prescribed tenofovir 1x 300 mg during initial diagnosis. April 2019 was diagnosed sjogren syndrome, had been prescribed methylprednisolone 1x 4 mg, chloroquine 1x 250 mg. may 2019 peptic ulcer and had been prescribed lansoprazole 1x 30 mg.

Familial medical history

There are no family members that experience similar symptoms.

Socioeconomic history

The patient works as a housewife. The patient is married and has 1 child aged 28 years. Her husband works as a farmer.

Physical examination

From vital signs examination, we obtained a general state of weakness with GCS 456. Blood pressure 120/70 mmHg, pulse 78x/minute, irregular rhythm, normal amplitude. Respiratory rate of 20x/minute with 97% SpO₂ with free oxygen. Axillary temperature of 36.8 C. Pain scale assessment with Visual Analog Scale obtained a score of 5 (moderate pain). The patient's body weight was 50 kg, body height was 150 cm, with a body mass index of 22 kg/m² (normal).

There was a prominence of the temporal artery in the head. On cardiac examination, irregular S1 and S2

were obtained, diastolic murmurs were found in apex. Other examinations were within normal limit.

Additional examinations

From laboratory examinations (13/12/19) we found Hb 13.3 g/dL, Hct 25.7%, Leucocytes 6.760/uL, Neutrophil 71.4%, Lymphocyte 19.2%, Platelets 135.000. random blood sugar 129 mg/dL, BUN 16 mg/dL, creatinine 0.5 mg/dL, SGOT 41 u/L, SGPT 34 u/L, Albumin 3.5 g/dl, Natrium 145 mmol/l, Potassium 4.4 mmol/L, Chloride 104 mmol/L, CRP 12 mg/L, ESR 54 mm/h.

- VODS > 2/ 60. TO DS 14.6/ 14.6 mmHg
- Anterior segment ODS
- Palpebral oedema -/-, spasm -/-, conjungtiva hiperemis -/-, cornea clear +/+
- Posterior segment ODS
- Fundus reflex +/+, papil NVII strict lines/strict lines, bleeding retina -/-. Exudate -/-. macula reflex +/+.

From brain MRI with contrast (6/11/19): wall thickening and mural contrast enhancement a. temporalis superficialis and a. meningea media right left accompanied by vasculitis picture of a. temporalis superficialis and a. meningea media right and left may represent temporal arteritis

Assessment and management

Based on the history, physical and auxiliary examinations, the patient's assessment is giant cell arteritis, sjogren syndrome, chronic hepatitis b.

Patient is planned for hospitalization. Patient is also planned for temporal artery biopsy. Therapy given to the patient is of NaCl 0.9% infusion 500 cc for 24 hours, Inj. Methylprednisolone 62.5 mg for 24 hours during 3 days, Inj. Cyclophosphamide 500

mg for 24 hours.

Disease Course

13rd December 2019

Patients complained of headache disappear and arise with VAS 6, sometimes nausea. The vital signs were within normal limit.

The patient was planned for Inj. Methylprednisolone 62.5 mg for 24 hours in 3 days. Inj. Tramadol 10 mg for 8 hours. We consult the patient to the neuro surgery for TAB and ophthalmology division.

16th December 2019

The headache disappeared improved with VAS 4. The patient was planned for inj. Cyclophosphamide 500 mg for 24 hours.

17th December 2019

The patient was discharged and advised to control regularly in rheumatology. The final diagnosis was giant cell arteritis post cyclophosphamide, sjogren syndrome, chronic hepatitis b.

Discussion

GCA and PMR commonly overlap. PMR is observed in 40- 60% of patients with GCA at diagnosis, and 16- 21% of patients with PMR may develop GCA, particularly if left untreated.⁵

The traditional concept of GCA has focused on cranial symptoms such as headache and visual disturbance, but extra-cranial manifestations such as constitutional symptoms, polymyalgia and limb claudication have also long been recognized. These symptoms may coincide with cranial GCA, occur as an independent clinical subset [large-vessel (LV) GCA] or overlap with PMR. Imaging studies have demonstrated that up to one-third of patients with PMR have subclinical LV inflammation at disease outset. Patients with treatment refractory PMR commonly

have cranial and/or extracranial arteritis on imaging.⁵

In this case, the patient complained about having headache and the left eye feels more blurry than the right eye since 2 months ago. The patient often has anorexia so that she loses weight. Sometimes nausea but not vomiting. The patient also complained of jaw claudication during the last 2 months.

The most common symptom of GCA is headache, which is present in more than two-thirds of patients. It usually begins early in the course of the disease and may be the presenting symptom. The pain is severe and localized to the temple. However, it may be occipital or be less defined and precipitated by brushing the hair. The nature of the pain varies; some patients describe it as shooting, and others as more like a steady ache. Scalp tenderness is common, particularly around the temporal and occipital arteries, and may disturb sleep. Tender spots, or nodules, or even small skin infarcts may be present for several days. The vessels are thickened, tender, and nodular with absent or reduced pulsation.⁶

The patient complained about having headache since 2 months ago. Headache was like being pressed and localized to the temple. However, it may be occipital or be less defined and precipitated by brushing the hair. The pain feels heavy everyday so it is difficult to sleep.

There is a vessel that is thickened, tender, with reduced pulsation in her head.



Figure 1. Clinical picture of the patient

Vision symptoms, such as amaurosis fugax, blurred vision, diplopia, and blindness (monocular and binocular) occur in 12%–40% of patients (Arteritis et al., 2003). Sudden, severe, and sequential vision loss is the hallmark of giant cell arteritis. The vision loss is usually discovered upon awakening in the morning. Visual acuity is usually less than 20/200 in greater than 60% of patients who lose vision. The fellow eye usually gets involved within days to weeks of the initial eye. GCA may initially also present with diplopia or eye pain.³

In this case, the left eye feels more blurry than the right eye since 2 months ago. The patient admitted to having both red eyes since the headache appeared.

Original criteria	Suggested expansion
Age at disease onset ≥ 50 years	Age at disease onset ≥ 50 years
New onset headache of or new type of localized pain in the head	Any of the following: New onset headache of new type of localized pain in the head, Visual symptoms, sight loss, PMR, Constitutional symptoms, Jaw and/or tongue claudication
Abnormality of temporal artery (tenderness to palpation or decreased pulsation unrelated to arteriosclerosis)	Abnormality of temporal and/or extra-cranial arteries (tenderness to palpation or decreased pulsation, bruits of extra-cranial arteries unrelated to arteriosclerosis)
ESR ≥ 50 mm/h	ESR ≥ 50 mm/h and/or CRP levels ≥ 10 mg/l
Abnormal artery biopsy	Abnormal artery biopsy and/or abnormal imaging result (US, MRI and/or $^{18}\text{F-FDG}$ PET)^a

Table 1. Expansion of the 1990 ACR criteria items for the classification of GCA⁵

A patient shall be diagnosed to have GCA if three of the five criteria are present, as long as either temporal artery biopsy and/or imaging results are compatible with a diagnosis of GCA. F-FDG PET: 18-fluorine fluorodeoxyglucose PET/CT.

In this case, there are five criteria, among others

1. Age of disease onset 52 years
2. New onset headache of new type of localized pain in the head, visual symptoms, constitutional symptoms
3. Abnormality of temporal (tenderness to palpation)
4. ESR 54 mm/ hr and CRP levels 12 mg/l
5. Abnormality in MRI: wall thickening and mural contrast enhancement a. temporalis superficialis and a. meningea media right left accompanied by vasculitis picture of a. temporalis superficialis and a. meningea media right and left may represent temporal arteritis

The immunopathological model of GCA can be divided into four phases⁸

Phase 1: loss of tolerance and activation of resident dendritic cells of the adventitia. Phase 2: recruitment, activation and polarization of CD4⁺ T cells. Phase 3: recruitment of CD8⁺ T cells and monocytes. Phase 4: vascular remodeling⁸

People with a certain genetic background (such as female sex, northern European descent and other genetic variants) are more susceptible than others. Genetic factors might also influence the phenotype and course of GCA and PMR.⁵

The pathogenesis of GCA is still unclear, and experts believe that it may be caused by a combination of genetic and environmental factors. At present, it is generally believed that the pathogenesis of GCA is dendritic cells (DCs) which in the middle and endometrium layer of the vascular wall are activated, secrete various inflammatory factors and chemokines such as IL6 and IL8, active and recruit CD4⁺ T cells and macrophages into the vascular wall. Activated CD4⁺ T cells can polarize to Th1 and Th17 cells, and participate in vascular inflammation. DCs and CD4⁺ T cells also play a vital role in the pathogenesis of RA

and SS.¹

In this case, GCA and SS appeared on the basis of PMR and after the aggravation of PMR, which strongly suggested that there may be a common pathogenesis between the three diseases and may promote the occurrence and development of each other.

Acute-phase markers of inflammation are often significantly elevated, and a normocytic normochromic anemia and thrombocytosis may be present, as may elevation of liver transaminase levels with a reduced albumin level. Although the ESR has historically been the acute-phase measure of choice in the diagnosis of GCA, up to a quarter of patients may have a normal value and elevation of the C-reactive protein (CRP) is a better predictor of obtaining a diagnostic TAB (Temporal Artery Biopsy). The combination of an elevated CRP and positive TAB render the highest sensitivity and specificity for the diagnosis of GCA.⁹ TAB has been the gold standard test representing definitive pathologic diagnosis.¹⁰

Both MRI, MR angiography (MRA), and contrast-enhanced CTA provide useful images of mural and luminal changes suggestive of large-vessel vasculitis in GCA that include circumferential wall swelling, smoothly tapered luminal narrowing of aortic branches and aortic aneurysm formation. MRI and MRA are favored over CTA by most experts. Bright mural enhancement of the temporal artery on contrast-enhanced high-resolution MRI had comparable sensitivity and specificity to temporal artery ultrasound (TAUS) in the diagnosis of GCA.⁹

Current EULAR and British Society of Rheumatology (BSR) recommendations for GCA management suggest immediate treatment of GCA using GCs at a dose of 1 mg/kg (up to a maximum of 60 mg/day) or 40- 60 mg/ day prednisone equivalent, respectively, in order to prevent ischaemic

complications, particularly blindness.¹¹

Patients presenting early after the onset of visual symptoms may require GC pulse therapy with 0.5- 1 g methylprednisolone for 3- 5 days. EULAR recommends considering MTX in every GCA patient, whereas BSR has reserved this treatment for relapsing patients.¹²

According to the recently published 2015 EULAR- ACR recommendations for PMR, the initial GC dose is 12.5- 25 mg/day prednisone equivalent followed by gradual dose tapering.¹³ MTX may be used in patients at risk of relapse and/or GC-related adverse events. BSR recommends an initial GC dose of 15 mg/day for PMR, and the introduction of MTX after the second relapse.¹¹

Glucocorticoids (GC) are the cornerstone of the treatment of GCA as they are very effective but are often given for 1 year to avoid relapses. As a result, 86% of patients develop ≥ 1 GC-related complication(s) after 1 year of follow-up. Therefore, GC-sparing therapeutic strategies are required to improve the management of GCA patients. Methotrexate is often used as a GC sparing agent, but its effect seems moderate and is still debated.¹⁴

In this case, the patient was given methylprednisolone 1x 4 mg and myfortic addition 2 x 180 mg, chloroquine 1x 250 mg. After a month the complaints still did not get better. So that the patient was admitted to the hospital.

Patients presenting early after the onset of visual symptoms may require GC pulse therapy with 0.5- 1 g methylprednisolone for 3- 5 days. EULAR recommends considering MTX in every GCA patient, whereas BSR has reserved this treatment for relapsing patients. According to the recently published 2015 EULAR ACR recommendations for PMR, the initial GC dose is 12.5- 25 mg/day prednisone equivalent followed by gradual dose tapering.¹³

In this case, the patient was planned for Inj. Methylprednisolone 62.5 mg for 24 hours in 3 days and was planned for inj. Cyclophosphamide 500 mg for 24 hours. One day after cyclophosphamide, headache complaint decreased so that the patient was discharged from the hospital.

Conclusion

A 52 years old woman was admitted by her family to the outpatient clinic of Dr. Soetomo general hospital, Surabaya, with a chief complaint sudden onset headache. The patient was diagnosed with giant cell arteritis, sjogren syndrome, chronic hepatitis b. TAB has been the gold standard test representing definitive pathologic diagnosis. Glucocorticoids (GC) are the cornerstone of the treatment of GCA as they are very effective but are often given for 1 year to avoid relapses.

Ethical Clearance- Nil

Source of Funding- Nil

Conflict of Interest - Nil

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Smart Materials- A Review

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Abstract

The word “smart” refers to material that can sense changes in their environment and are responsive to these changes in a pre-requisite manner. Stimuli such as stress, temperature, moisture, pH, electric or magnetic field change the characteristics of these materials in a controlled fashion and also possess the ability to change their physical properties in a specific manner in responses to specific stimulus. In dentistry, there is no material that can be referred as ideal in nature and satisfies the properties of an ideal material. This paper attempts to highlights the use of “smart materials” to achieve a maximum advantage over conventional materials in dentistry.

Keywords: Biosmart materials, Self healing composite, Cercon, Smart burs.

Introduction

The research and development of newer and better materials is unending particularly in the field of dental science¹⁻². Materials utilized in dentistry can be categorized as bio-inert, bio-dynamic, and bio-responsive or smart materials dependent on their interactions with the environment.³

Early smart material applications began with magnetostrictive advancements. Because of the fascinating conduct of Smart materials, researchers were urged to apply them in different fields such biomedical science and dentistry⁴.

Smart materials have been around for quite a long time in dentistry, the initial term smart materials began from the 1980's. McCabe et al characterized, Smart materials as materials whose properties might be changed in a controlled design by stimuli, like pressure, stress, temperature, moisture, pH, and electric or magnetic fields⁵. Accordingly Smart behaviour happens when a material can detect and upgrade from its current circumstance and respond to intentional or unintentional changes that it is subjected to, in a helpful, dependable, reproducible and generally reversible way. A truly smart material will utilize its response to the outside environment to start or incite a functioning reaction.

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CLASSIFICATION OF SMART MATERIALS

A survey of literature by us revealed the following classifications for smart materials relating to dentistry.

ACCORDING TO THEIR NATURE⁷:

ACTIVE SMART MATERIALS	PASSIVE SMART MATERIALS
Alter their geometrical or mechanical properties after application of stimulus Ex: composites, ceramics, fluoride releasing pit and fissure ⁶ .	Materials which are dormant in their reaction and working. Self-repairing property seen. Ex: compomer, fibre optic materials, fibre optic materials ⁸ .

ACCORDING TO THEIR USE⁵:

RESTORATIVE DENTISTRY	PROSTHETIC DENTISTRY	PREVENTIVE DENTISTRY	ORAL AND MAXILLOFACIAL SURGERY	ORTHODONTICS
<ol style="list-style-type: none"> Smart GIC Smart Composite Self-healing composite Smart Prep Bur Smart Bonding System 	<ol style="list-style-type: none"> Smart ceramics Smart impression materials 	<ol style="list-style-type: none"> ACP releasing pit and fissure sealants Fluoride releasing pit and fissure sealants 	<ol style="list-style-type: none"> Smart sutures 	<ol style="list-style-type: none"> Smart memory alloys Smart orthodontic adhesives
PERIODONTICS	ENDODONTICS	IMPLANT DENTISTRY	SMART FIBRES FOR LASER DENTISTRY	BIOMEDICAL USE
<ol style="list-style-type: none"> Smart antimicrobial peptide 	<ol style="list-style-type: none"> NiTi Rotary instruments Smart seal obturation 	<ol style="list-style-type: none"> Smart coating on implants 	<ol style="list-style-type: none"> Hollow core photonic fibres 	<ol style="list-style-type: none"> Smart sutures Smart pressure bandages Hydrogel

ACCORDING TO THE NATURE⁸:

ELECTROSTRICTIVE MATERIAL	Material changes its shape when electric field is applied.
MAGNETOSTRICTIVE MATERIAL	Materials undergo mechanical changes under the influence of magnetic field.
THERMORESPONSIVE MATERIAL	At their glass transition temperature they undergo, a particular volume of change in their polymeric structure Ex. Pressure gauze
LIGHT SUSCEPTIBLE MATERIALS	Material that shows various sorts of changes to light shift.
SMART GELS	Mixture of dissolvable swollen polymer network in combination to react to different sorts of stimuli
pH SENSITIVE MATERIALS	Ability to modify color as a result of pH change and their activity is reversible.

MATERIALS:

SHAPE MEMORY ALLOYS

The shape memory effect is a very interesting phenomenon seen in metals. Greniger and Mooradian in 1938 first discovered this effect in copper-zinc and copper-tin alloys. A thermomechanical change is observed in these materials when they transform from one phase to another.

The austenite phase is a high temperature and low stress phase whereas the martensite is a low temperature and high stress form. On heating martensite phase changes to austenite phase and reverts back on cooling.(Fig 1)

Eg NiTi Alloys

In endodontics the root canal files are made from such materials.

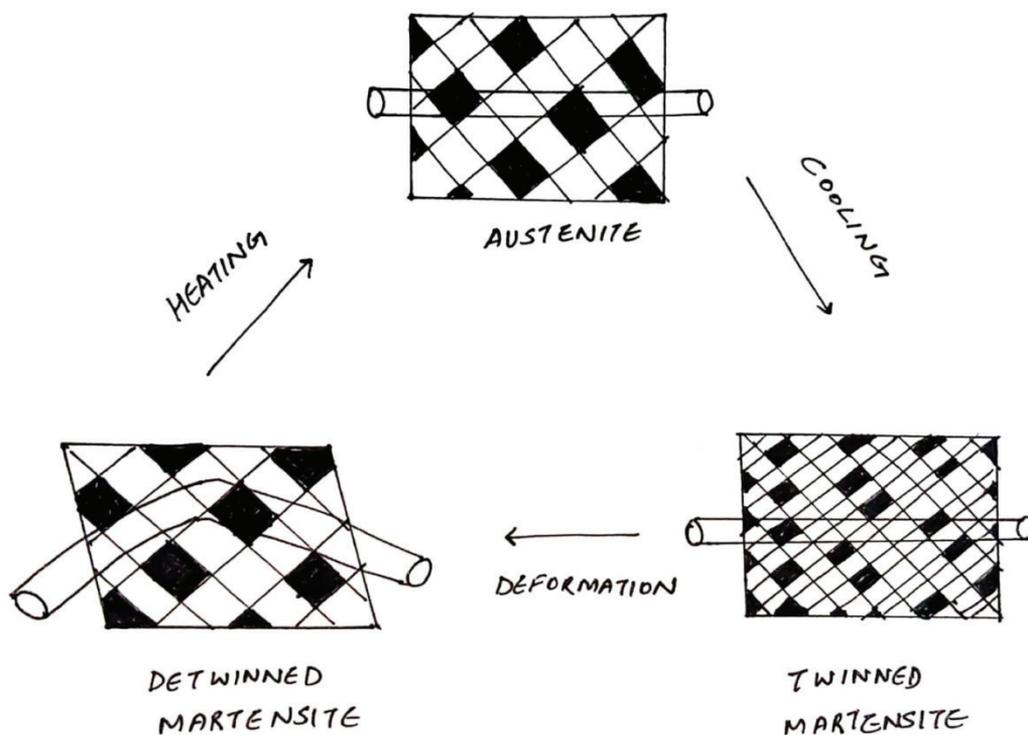


Fig 1: Phase changes in NiTi Alloy

SMART COMPOSITE

In the previous few decades, composite materials have been created to aesthetically and functionally rehabilitate damaged teeth⁹. Composite materials address one of the numerous requirements of present-day biomaterials research, since they reproduce organic tissue in both appearance and function. Half

of posterior direct restorations presently depend on composite materials.⁸ In 1998 so-so introduced ‘smart composites’ which were light activated, nano filled glass restorative materials and were of two types.⁵

1. Amorphous Calcium phosphate releasing composite

2. Self-healing composite

Amorphous Calcium Phosphate releasing composite

An inventive methodology in therapeutic dentistry was the presentation of an ion releasing composite material in 1998. ACP composite is an intelligent material, it is a light-activated filling material used for posterior fillings. The monomer grid comprises of a combination of dimethylmetacrylates and inorganic fillers incorporated with alkaline glass, Ba-Al fluorosilicate glass, Ytterbium trifluoride and scattered silicon dioxide⁷.

As mentioned in the reports, these materials discharge three distinct kinds of ions (fluoride, calcium and hydroxyl). The discharge of these particles relies upon the pH changes, at the point where pH in the oral cavity is low because of the plaque, composite discharges a fundamentally higher measure of ions

than it does with neutral pH changes⁸. The discharged particle impacts are as follows: The Fluoride particles hamper demineralisation, advance remineralisation and repress bacterial development. The Calcium particles hamper demineralisation and advance remineralisation. The Hydroxylions neutralise the production of acid from cariogenic bacteria and restrain bacterial growth⁸.

SELF HEALING COMPOSITES

Self-Healing is the property by which the materials, recuperate from any sort of harm or damage automatically without any fringe intervention. The mechanism is to deliver the repairing agents (like monomers, catalyst, hardeners containing Hollow filaments, and microcapsules) to the cracks¹². The Microcapsules release resin which reacts with the catalyst near the crack and leads to the polymerization of the resin, and in this manner the sealing of the crack takes place¹³. (Fig 2)

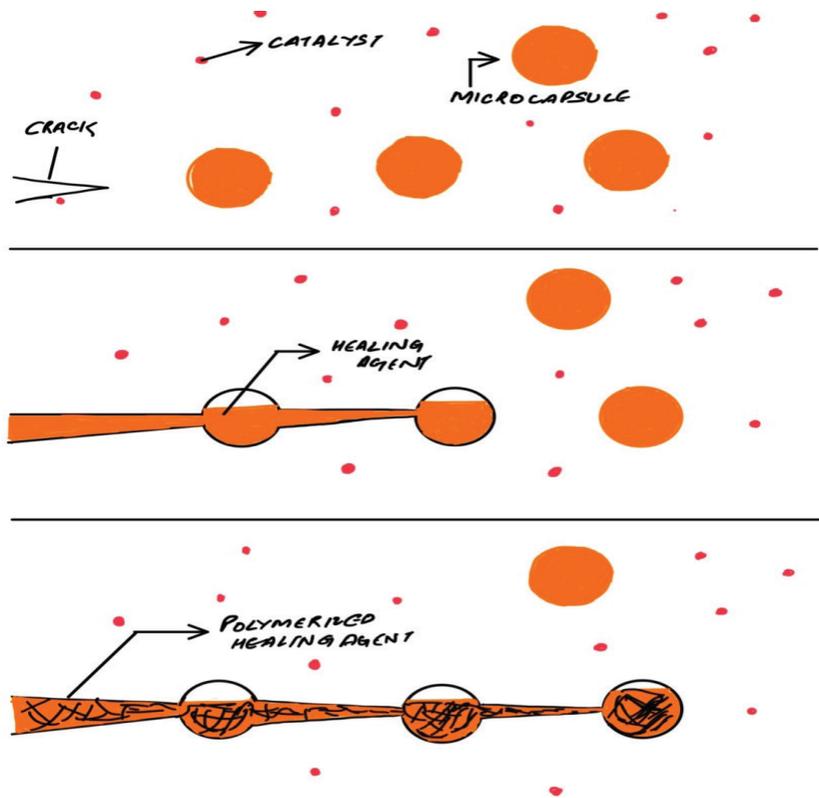


Fig 2: Mechanism depicting self healing feature of composite

SMART CERAMIC

Ceramic materials are the most biocompatible materials which is being used for quite a long time. In PFM crowns they are used with metal, which lacks in the aesthetic quality. A high-tech ceramic zirconia is now available which are much tougher, stronger and aesthetically suitable¹⁴.

Cercon

The restorations formed from Cercon smart ceramic materials appears so natural that they can't be differentiated from your natural teeth. The cercon zirconia is viewed as a smart framework because of its capacity to go through —transformation toughening when exposed to pliable stresses, it stops the crack propagation in the material and increases durability of the restoration(Fig 3). Cercon ceramic requires no metal system. This offers an incredible benefit for restoration aesthetic¹⁴.

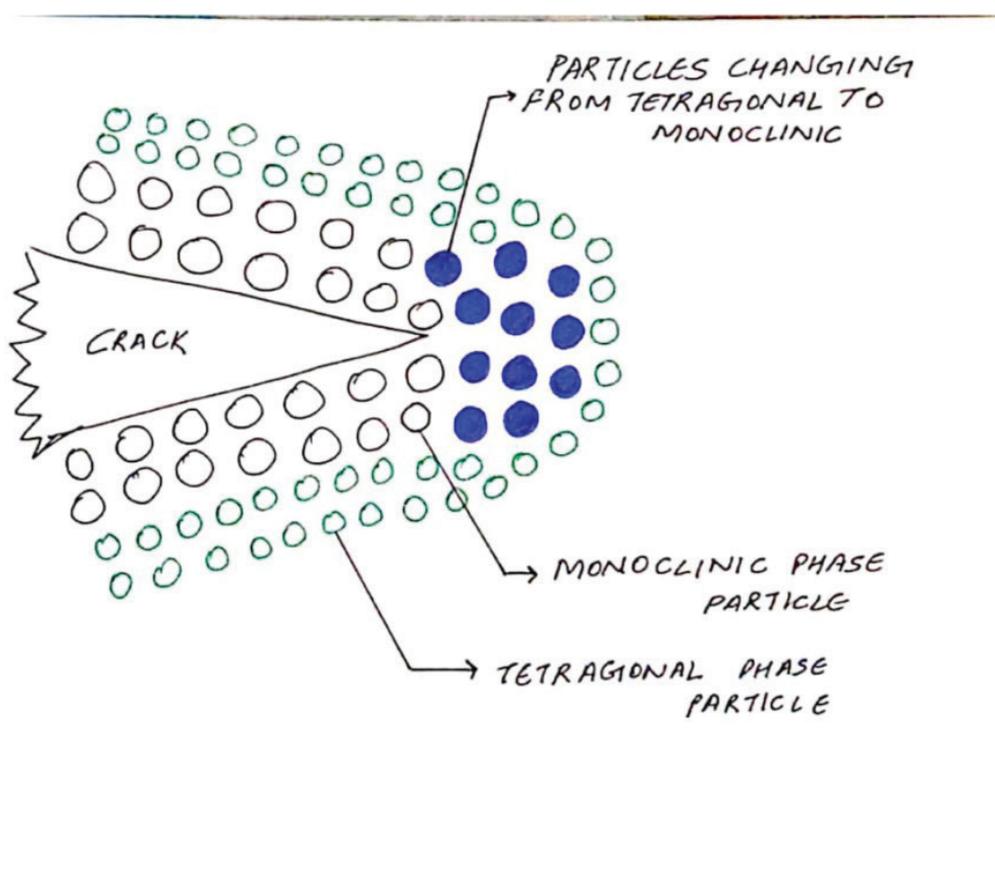


Fig 3: Transformation Toughening in Ceramic

SMART PIT AND FISSURE SEALANT

Fluoride releasing sealants

These sealants were introduced during the 1970s, fluoride catalyses the dispersion of calcium and

phosphate ions into the surface of tooth, which thus remineralizes the crystalline like structures in dental cavities. After the incorporation of fluoride, they form fluoridated hydroxyapatite and fluorapatite, which opposes the assault better than the novel teeth¹⁵.

Kadoma et al. (1983) expressed that the properties a fluoride containing sealant ought to be better than the traditional one, i.e. (a) They should have superior or possibly similar retention standards than the ordinary sealant, (b) They should give steady fluoride discharge for a delayed time frame and (c) They should work as a repository of fluoride particles to give fluoride and stimulate the fluorapatite development in enamel¹⁶.

Smart prep burs

Smart prep polymer burs are recently introduced (2006) and have exhibited excellent presentation for specific dentine caries expulsion. As of late, polymer burs are depicted as dentin safe, it implies that it eliminates just carious dentine, the bur will act naturally, restricting the cutting, when it arrives at sound, solid dentin. Its utilization has demonstrated to be effective in caries excavation. Polymer burs can eliminate mollified dentin yet can't cut hard sound dentin¹⁷.

Since this bur eliminates just carious portion of enamel and dentin, there are less possibilities that the odontoblasts are exposed which can limit the pain and discomfort while treatment and post-operatively¹⁸.

The instruments look like regular burs, yet they are not produced from metal, they are made from a polymer material i.e., polyamide/imide (PAI) polymer, having marginally lower mechanical properties than sound dentin. The cutting edges are not spiral like. The sharp edge configuration was created to eliminate dentin by locally discouraging the carious tissue.^{17,19}.

SMART SEAL OBTURATION MATERIAL

Gutta-percha is generally utilized as an endodontic obturation point, its capacity to laterally seal the root canal dentin remains questionable due to adhesion. To beat this disadvantage and to improve the nature of obturation, a root-canal obturation system called SmartsealTM (known as ProsmartTM) was created²⁰.

Smartseal is a two-section framework comprising of:

1. Propoint/C point.
2. Smartpaste/Smartpaste Bio/Hyseal-Bio.

Propoint

Also called as C points, these obturation points are built in two sections:

Central Core: It is a blend of two nylon polymers, Trogamid T and Trogamid CX. The gutta percha point are furnished with the adaptability to permit it to effectively pass around any curves in the prepared canals, while being sufficiently flexible to pass effectively to working length in smaller canals²¹. At the point when they get hydrated in the root canal, C-points grow, and adjust to canal inconsistencies thereby squeezing the hydrophilic sealer.

External Polymer Layer: It comprises of a copolymer of acrylonitrile and vinylpyrrolidone, which has been cross-connected utilizing allyl methacrylate and a thermal initiator. This layer permits the points to swell so as to adjust to the ramifications in the root canal. This coating is intended to expand horizontally, consequently self-sealing the canal.^{20,22}.

Smartpaste

Smartpaste is a resin-based sealer containing a functioning polymer that swells to make up for any shortfalls or voids in the root canal. The amount of swelling is constrained by the active polymer used. The polymer can swell at a later stage in the canal to make up for any voids that may develop²².

Conclusion

Smart materials are a new category of materials which have shown us a lot of promise²³. Dentists should be aware of these innovative materials to enable their use and utilize their optimal properties in day-to-day practice to provide quality and effective

treatment⁵.

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Effect of Oxytocin Augmentation on Neonatal Bilirubin Levels : A Case Control Study

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Abstract

Introduction - Hyperbilirubinemia is a commonly encountered problem in both term as well as preterm infants and is a source of concern to neonatologists.

Aim- to determine the relationship between the usage of oxytocin during labour and the development of neonatal hyperbilirubinemia.

Methodology- a case controlled study was conducted. 50 patients were given intravenous oxytocin for the augmentation of their labour and 50 patients delivered spontaneously without the usage of oxytocin during their labour. The neonates born to both these categories of patients were studied for their bilirubin levels of day 2 of life.

Results- hyperbilirubinemia developed in 34% of the neonates whose mothers were given oxytocin for augmentation of labour and hyperbilirubinemia developed in only 14% of the neonates whose mothers delivered spontaneously without the usage of oxytocin.

Conclusion- A positive relationship between the usage of oxytocin during labour and development of neonatal hyperbilirubinemia has been established.

Keywords – oxytocin, neonatal hyperbilirubinemia, augmentation of labour

Introduction

Hyperbilirubinemia is the most common problem encountered in both term as well as preterm infants and is a source of concern to the neonatologists^[1,2,3]. It is often a cause of readmissions as well as late discharges in newborns^[3,4]. The common cause of neonatal hyperbilirubinemia are fetomaternal blood

group incompatibility, prematurity and a previously affected sibling^[5]. There has recently been a rise in the number of newborns with neonatal jaundice and it has been postulated that the use of oxytocin during labour might be a probable cause. Oxytocin is often used intravenously to induce or augment the labour. Some studies have shown a positive relationship between the usage of oxytocin during labour and the development of neonatal jaundice^[6]. This study aims at determining the relationship between use of oxytocin during labour and the development of hyperbilirubinemia in the neonates.

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Materials and Methods

This was a case control study, consisting of A Total of 100 patients admitted to the labour ward of Dhiraj Hospital from 15th July 2020 to 15th July 2021 were studied. They were divided into two groups based on the modes of expedition of their deliveries. In order to eliminate the bias, the patients having ABO or RH incompatibility were excluded from the study^[7], the infants weighting less than 2.5kg or being born before 37 completed weeks of gestation were also excluded. Group A consisted of patients who were already in labour at the time of admission and their labour was augmented further with the use of intravenous oxytocin. Group B consisted of 50 patients who delivered spontaneously without the use of any agents for induction or augmentation. The information regarding the reason for augmentation of labour, the APGAR score of the neonates that were born, the bilirubin levels of the neonates was recorded on the second day of life. For the purpose of this study the bilirubin levels of 10 mg/dl or above were considered to be significant in the neonates^[8,9]. All the neonates born were breastfed in order to eliminate the

bias regarding breast feeding jaundice.

Results

A Strong association was found between the use of oxytocin during labour and hyperbilirubinemia in the neonates. Out of the 50 neonates in group A born to mothers who were already in labour at the time of admission with their labour augmented with Oxytocin, 17 neonates had a bilirubin levels more than 10 mg/dl on day 2 of life and the neonates born to mothers in from Group B, who did not receive any oxytocin during their course of labour, only 7 out of 50 neonates developed a bilirubin levels more than 10 mg/dl on day 2 of life. This shows a positive relationship between administration of oxytocin during labour and neonatal hyperbilirubinemia following birth. If all the 8 post dated patients whose labour was augmented with oxytocin were eliminated from the study, even then the number of neonates with neonatal hyperbilirubinemia would be 14 out of 42 augmented patients which is statistically significant. All the infants that were included in this study were breastfed so as to eliminate breastfeeding as a confounding factor for neonatal hyperbilirubinemia.

Table 1 : Percentage of hyperbilirubinemia in neonates

	Total	Bilirubin>10mg/dl	Percentage	P value
Group A (cases)	50	17	34%	<0.05
Group B (controls)	50	7	14%	>0.05

Table 2 – Association of Birthweight and Gestational Age with Neonatal Hyperbilirubinemia

		Cases	Controls	Total
Birthweight	2.5-3.0 Kg	7(14%)	3(6%)	10
	3.1-3.5 kg	10(20%)	4(8%)	14
Gestational Age	37-39+6	9(18%)	2(4%)	11
	40-42+6	8(16%)	5(10%)	13

Discussion

A positive correlation between the administration of oxytocin during labour and the increased neonatal bilirubin levels has been established. Several theories have been put forward for the same. Some authors have put forward that oxytocin caused contractions of higher intensity which causes fetal hypoxia which may be responsible for the higher bilirubin levels^[7]. However the validity of this explanation seems to be questionable. D'souza and associates have demonstrated from the umbilical cord blood samples and the venous blood samples that the hypoxia developed in contractions in both spontaneous as well as in induced labour were similar^[8].

Another explanation regarding the association of oxytocin administration with neonatal hyperbilirubinemia is that during spontaneous labour, the fetal cortisol levels are found to be higher^[9]. This fetal cortisol is required for the maturation and then for the functioning of fetal liver enzymes which are required for the excretion of bilirubin resulting in lower neonatal bilirubin levels in neonates born

through spontaneous labour. Prostaglandins have a role in increasing the cortisol levels^[10] which helps in the bilirubin conjugation and excretion. So Ideally there should not be hyperbilirubinemia in neonates delivered through induction with prostaglandins but studies^[11,12] have shown that in induction of labour with either oxytocin or prostaglandin, the neonatal hyperbilirubinemia is present.

Another explanation for the increased bilirubin levels in neonates delivered after augmentation with oxytocin is that oxytocin leads to an increase in the fetal erythrocyte fragility^[13,14] causing hemolysis, causing an increase in the bilirubin levels. This is because oxytocin has an antidiuretic property. Because of this it causes a fetal electrolyte imbalance^[15] leading to an influx of fluid into the fetal erythrocytes causing their lysis. There is also a positive relationship between the dose and duration of administration of oxytocin and the neonatal hyperbilirubinemia^[5,8,9] evident by the fact that there were increased number of infants developing hyperbilirubinemia who were born to mothers who were induced with oxytocin for a longer

period of time rather than born to those mothers who were already in labour and were augmented with oxytocin for a shorter period of time.

Conclusion

There are various factors which lead to an increase in the neonatal bilirubin levels. From this study a positive relationship has been established between the usage of oxytocin in labour and the development of neonatal hyperbilirubinemia.

Ethical Clearance: was given by the ethical committee at Dhiraj General Hospital, SBKS MI & RC.

Source of Funding : None

Conflict of Interest: None

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Study of Working Hours of Indonesian Civil Servant Doctors in Relation to the Rule of Three Licenses for Practice and Labor Law

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Abstract

There are no specific rules about doctors' working hours in Indonesia, it causes working hours of Indonesian doctors to be excessive. Following the rules of the labor law that the maximum work of a worker is 40 hours a week. However, with the applicable 3 Licenses For Practice rules, the doctor's work hours will be more than 40 hours a week. It was to examine whether the working hours of Indonesian Civil Servant doctors are rational. Based on Law No. 13 of 2003 about Manpower article 77 paragraph (2), the maximum working hours of a worker is 40 hours. In reality, with the 3 Licenses For Practice rules, if it was calculated, the working hours of civil servant of doctors in Indonesia was very excessive. In other countries, there were already standard rules. Doctors work 40 hours a week or a maximum of 80 hours a week. Therefore, it needed to be studied further, whether the 3 Licenses For Practice policy had provided a solution or had actually aggravated the condition of a civil servant doctor. The monopoly discourse of a doctor needed to be balanced with an increase in the services or incentives of a civil servant doctor. With the rule of three Licenses For Practice, the working hours of civil servant doctors in Indonesia became excessive.

Keywords: *Working Hours, Civil Servant Doctor, Three Licenses For Practice, Employment.*

Introduction

Doctors as health service providers currently have a unique position. On the one hand, civil servant doctors who are enshrined in the Civil State Apparatus Law, it can become employees or workers who are bound by the Manpower Act. Then, doctors can also become business actors for themselves, if they

carry out individual practices which are bound by the Law. Medical practice. In Indonesia, there are no specific rules about the working hours of civil servant doctors. The rules about this matter refer to Article 77 Paragraph (2) of Law no. 13 of 2003 about Manpower. The paragraph stipulates that the maximum working hours of a worker is seven hours one day (for six working days in one week) or eight hours one day (for five working days per week). However, the reality is that doctors often work longer hours than this rule. This happens because of the regulation that doctors can have a license for practice in three places as stated in the Regulation of the Minister of Health of the Republic of Indonesia Number 2052/MENKES/PER/X/2011 concerning License For Practice and

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Implementation of Medical Practice. The regulation is actually an effort to improve doctor services (which is done by limiting the places where doctors practice), but according to the author, this limitation can still trigger problems. For example, a Regional Hospital requires civil servant doctors to work 40 hours at the site in accordance with the Manpower Act and the Civil State Apparatus Law. If the civil servant doctor is still working in two other places, of course, the workload will far exceed the rules. Then, this is quite a dilemma considering that the imposition of a maximum limit of three licenses for practice is actually enough to trigger polemics among doctors. Doctor's working hours and location of practice actually affect the doctor's overall income, so if these restrictions are not implemented carefully, it can reduce the level of doctor's welfare.

The explanation above is related to the issue that Indonesia still lacks of doctors. According to data from the Indonesian Medical Council, the ratio of doctors to Indonesians per 2014 was 1 doctor for 2,358 residents; higher than the ideal ratio standardized by WHO, which was 1:1,000. This number is still far less than the number of doctors in developed countries.

Besides, the uneven distribution of doctors in Indonesia also contributes to the high workload of doctors. The ratio of doctors to patients in Indonesia which is at least above the WHO standard, it should make the workload of doctors evenly distributed. However, in reality, these doctors are concentrated in certain areas. This surely makes the burden on doctors in other areas heavier, so they have to work longer hours than they should.

Some studies had shown that the long working hours of doctors had an effect on their performance in dealing with patients. This was because the stress and fatigue of doctors could increase their error rate for losing focus. The study conducted by Lockley et al showed that residents (doctors who took specialist courses) who worked 24 hours in a row had a high

risk in various aspects: higher rates of medical treatment errors, patient misdiagnosis, to a higher risk of accidents while on the way back home after work. The study also showed that the workload reduced the performance of the residents to the level of those with a blood alcohol level of 0.05 to 0.10%.¹ Entrusting the health of someone with such a condition would of course be a cause for concern. Health services should be provided as well as possible considering that this field deals with a person's life. The ability which is not maximally deployed by the doctor will certainly have an effect on the patient's health, so it is better to consider steps to improve this.

Limitation the practice to only three places is a good start. However, according to the illustration above, these restrictions are not sufficient to reduce the workload of doctors. Taking a license for practice is actually the decision of every doctor; some doctors practice in three places, while others limit themselves to only practicing in one place. One of the considerations in this decision is the income that the doctor can earn. Monoloyalty to one practice must be accompanied by adequate income for the doctor. For maximum service, it is better if the working hours of doctors are limited in accordance with the Manpower Act; which is forty hours a week throughout the practice. The limitation of working hours in the Manpower Act is in accordance with the rules of the International Labor Organization (ILO) which accommodates the interests of world workers. If workers work maximally during that time, how can a doctor who incidentally deals with human life be required to work longer than that.

Problem Formulation

Based on the description of the background above, the authors took the formulation of the problem as follows:

1. Are the working hours of civil servant doctors

in Indonesia in accordance with the provisions of the Manpower Act?

2. Is the three licenses for practice policy correct or does it increase the working hours of doctors in Indonesia?

Method

The research method applied in this study was the conceptual method and the comparative method.

Discussion

Doctors as Civil Servant

Based on Law Number 5 of 2014 about State Civil Apparatus in Article 1 point 3, it is meant by Civil Servants (abbreviated as PNS) are Indonesian citizens who meet certain requirements, are appointed as State Civil Apparatus Employees on a permanent basis by the staffing officerto occupy government positions.²Civil Servants play a role as one of the driving forces for the wheels of the government where their position is as Servant of the Community, Civil Servant and Civil Apparatus Employees in carrying out the system of government and development in the context of National goals. Civil Servants are different from the State Civil Apparatus, Civil Servants are one of the State Civil Apparatuses where these Civil Servants are appointed as permanent employees, and they have an Employee Identification Number (abbreviated as NIP).³

Based on the Decree of the Minister for Empowerment of State Apparatus No. 139/Kep/M. Pan/11/2003 Chapter VIII about Terms of Appointment in Position, it is stated that the requirements that should be met to become a Civil Servant doctor are as follows:

1. Certified Doctor;

2. The lowest rank is Tk. I Young Arranger in room III/b class;

3. Each element of the work implementation assessment in the DP3 has at least a good value in the last 1 (one) year.

The working conditions of civil servant doctors can be viewed from the doctor's workplace environment, completeness of facilities, and infrastructure at work, work comfort and safety and workload. As mentioned by the Secretary of the Irrigation Service, Budianto explained that there are still many employees who do not comply with the provisions of working hours, and they do not come to work without information or without a permit. This is because there are still many employees who prioritize their interests, such as being outside and not being the duty of their superiors, and picking up their children from school.⁴ Therefore, work discipline is a very important foundation that should be owned by civil servant (abbreviated as PNS) because it involves the provision of public services.

Explanation of PP No. 53 of 2010 about Civil Servant Discipline does not regulate working hours, but it only mentions the obligations of Civil Servants. It is only stated "going to work and obeying working hours is that every civil servant is obliged to come, carry out duties and go home according to the provisions of working hours and not in public places not because of the service."

Presidential Decree Articles 1 and 2 Number 68 of 1995 about Working Days in Government Institutions states that the number of effective working days in five working days is 37.5 hours which is partly determined as follows:

1. Monday to Thursday: 07.30 – 16.00. Break time: 12.00 – 13.00.

2. Friday: 07.30 – 16.30. Break time: 11.30 – 13.00

Regulation of Indonesian Civil Servant Doctor Working Hours

Based on Decree of the Health Minister No. 81 of 2004, guidelines for the preparation of health human resource planning have been established at the provincial, district/city and hospital levels. In the policy, the steps for determining workload standards are stated.⁵The standard workload is determined based on the available working hours owned by each HR divided by the time needed to complete main activities. The available working hours is obtained from the number of working days during the year minus the entitlement to annual leave (12 days), education and training (6 days), national holidays, absence from work, multiplied by the working time per day.

Regulating dan determining doctors' working hours, there are values which should be considered in making decisions. These values are contained in the Indonesian Medical Code of Ethics (abbreviated as KODEKI), and they are specifically stated in Article 2 about Standards of Good Medical Service and Article 13 about Cooperation. In both articles, doctors are more emphasized to maintain professional behavior in making decisions in the best interest and patient safety, both in individual work and in collaboration with colleagues or other parties.⁶

The reason for the long working hours of doctors is undeniable because of the high workload. Based on data from the Health Ministry, the ratio of doctors to the Indonesian population in 2014 was 1:2358, which was still far from the WHO ideal limit of 1:1000.⁷In addition, the concentration of doctors in big cities in Indonesia, causing the workload of doctors in the regions to be bigger, so doctors have to work longer hours. As a result, a lot of fatigue affects concentration and the ability to complete tasks, so it becomes the root cause of mistakes made by doctors. Hereby, it is important to keep the doctor's work environment healthy and free from conditions that force doctors to work in an unhealthy work environment.

According to Colligan and Higgins, there are several factors that contribute to stress in the workplace, they are an unhygienic work environment, excessive workload, isolated work area, excessive workload, role conflict, role ambiguity, lack of autonomy, career ambiguity, barriers to self-development, difficult relationships with supervisors or coworkers, bullying, harassment, and organizational climate.⁸The importance of ensuring patient safety and creating a healthy life for doctors has been carried out in various countries with restrictions on the number of working hours.

The law regarding employment was known as labor law which was translated from *arbeidsrechts* in the past. Labor law contains three elements, they were:⁹

1. There were regulations,
2. Work for others, and
3. Salary

In Law No. 13 of 2003 about Manpower, it regulates the working hours of workers where this arrangement is immaterial which regulates working hours which affects and threatens the security, occupational health, and welfare of workers in carrying out their work. As mentioned above, the regulation regulates working hours, length of working hours, proper and safe work places for human dignity in the company as well as rest periods.¹⁰

In Indonesia, there is no regulation until now that specifically discusses the working hours of doctors, especially Civil Servant Doctors. The regulations related to working hours refer to Article 77 paragraphs (1) and (2) of the Job Creation Law No.11/2020 which requires every entrepreneur to implement the provisions of working hours. The provisions of this working hour have been regulated in 2 systems, as follows:¹¹

- The working time of 6 (six) working days in one week is 7 (seven) hours in one day and 40 (forty) hours in one week.

- Working time for 5 (five) hours in one week is 8 (eight) hours in one day and 40 (forty) hours in one week.

The working hours provisions above only regulate the working hours limit for 7 or 8 hours a day and 40 hours a week and it does not regulate when the working hours starts and ends. What is normally spent working is 6-8 hours a day and the remaining 16-18 hours is used for family and other activities outside of work.¹² Produktivitas kerjadiketahui akan menu runsetelah menjalani 4 jam bekerja.

Doctors in carrying out their profession are covered by the Medical Practice Act (Law No. 29 of 2004).¹³ Doctors are obliged to follow the standards of his profession and standard operating procedures which apply. This standard operating procedure is fully measurable and therefore every medical action/procedure can be estimated in time. The time to carry out his profession follows the general rule, 40 hours per week, and it takes into account the limitations of a human being. Therefore, it is impossible for a doctor to continuously work for 24 hours without a break because it will have an impact on patient safety. Working as a doctor is a professional job which requires physical, mental work, and it makes decisions (Judgment) in a fast time which sometimes involves death / life / disability which often causes a stress burden.¹⁴

Doctors are categorized in certain occupational sectors. Article 77 paragraph 3 of the Manpower Law explains that the provisions on working hours in accordance with the Manpower Law does not apply to certain business sectors or occupations. In Paragraph 4, it is said that the provisions on working hours in certain business sectors or jobs will be regulated

by a ministerial decision. However, until now there has been no decision from the Minister of Health to regulate the working hours of doctors. It can be said that there is a norm vacuum.

The working hours regulation adopted in the UK since 1998 and modified in 2009 is the European Working Time Directive (EWTD) which prevents employers from requiring their workers to excessively work long hours, which has implications for health and safety. The EWTD includes a doctor's working hours for 48 hours a week, 8 working hours a day, with a rest period of 11 hours a day, 1 day off a week, the right to a minimum of 20 minutes of rest where the working day is more than 6 hours, and requirements for employers to keep a record of hours worked. Where this has been implemented since 1998.¹⁵

As of 1 August 2004, junior doctors in the National Health Service (NHS) and other healthcare systems across Europe would no longer be exempt from the provisions of the European Working Hours Directive.¹⁶ Working hours were then limited by law, initially 58 hours a week and in 2009 to 48 hours. This would demand greater changes to NHS for better working conditions for junior hospital doctors.

It was initially assumed that the definition of work would be similar to that of the 1991 "new agreement" on working hours for junior doctors.¹⁷ This agreement limited the working hours of junior doctors in the UK to 72 hours on duty and 56 hours of actual work. Actual working hours were defined as time spent performing postal duties, such as receiving patients, conducting investigations, providing care, but it did not include time spent resting in the hospital or elsewhere. However, a recent European Court decision had redefined work as all time spent on duty at work.¹⁸ Thus, for junior doctors in the United Kingdom, the maximum average time spent in hospital was 56 hours per week — a 19% reduction from the current 72 hours.

Based on research conducted by Joyce et al suggest that doctors in Australia spend an average of under 7 hours per week, or 16% of their working hours, on non-clinical activities (education, administration and management). In this study, physicians who were only in public hospitals spent an average of 15.6% of their time on non-clinical activities. This figure appeared to be below the average according to several professional bodies, including the Australian Medical Association (30% benchmark for non-clinical activities) and the Victorian Department of Health (20% for nonclinical activities).¹⁹ A study published by the Singapore Medical Journal this year nearly surveyed 500 doctors. Where the average junior doctor had to work 12 to 17 hours every day on weekdays, and up to 10 hours on weekends. While on night duty you had to continuously work 30 to 36 hours, plus the usual long working hours.²⁰

THE THREE LICENSES FOR PRACTICE POLICY IN THE WORKING RULES FOR DOCTORS

In Law Number 44 of 2009 Article 37 in conjunction with Article 4 of the Health Minister Regulation No. 2052 of 2011 article 4 it is stated that:²¹

(1) Doctor and Dentist license for practice is given for a maximum of 3 (three) practice places, both in government-owned, private and individual health service facilities”

(2) 3 (three) licenses for practice places as referred to in paragraph (1) may be in the same regency/city or different in the same province or other provinces.

As for the explanation in Law (UU) No. 29/2014 about Medical Practice (articles 36 and 37), it is emphasized that doctors can only practice in a maximum of three places. Additionally, it is stated that every doctor and dentist who practices medicine in Indonesia is required to have a practice license.

Furthermore, in paragraph 2, a doctor's or dentist's practice license as referred to in paragraph 1 is only given a maximum of 3 places. Then, in paragraph 3, one Licenses For Practice is only valid for 1 practice place.

The document that guarantees the legality of a medical practice is a license for practice issued by the local Licensing Service. To get this license for practice, doctors must have a Registration Certificate (abbreviated as STR) from the Medical Council. Meanwhile, the Medical Council only issued 3 STRs. This means that a doctor can only practice in 3 places according to the number of STR.

Based on this policy, many hospitals, especially Government Hospitals implement a monoloyalty system which means that doctors at the hospital are only allowed to practice medicine at the hospital. Thus, doctors can only have 1 practice location in accordance with the work agreement between the hospital and the doctor. The usual procedure for mono-loyalty is that if a doctor wants to open or do other practices outside the hospital, the doctor must ask permission from the hospital as the first practice place. However, in practice, if the recommendation letter from the hospital concerned does not come down, then the doctor will wait until the letter can be dropped to continue the management of the next license for practice and the doctor cannot practice before the license for practice is issued. This is very detrimental to doctors, because the doctor's STR is only valid for 5 years. After 5 years the doctor must renew his STR to the Indonesian Medical College (KKI) with conditions that are quite strict so that the doctor is only faced with 2 choices, which is to face the monoloyalty system and practice according to the wishes of the hospital or even resign.

This monoloyalty policy is contrary to Law no. 29 of 2004 in article 37 paragraph 2 point 2 where it has been explained that the government in this case

the authorized health official at the district or city level gives a maximum of 3 Licenses For Practice where 1 permit is valid for 1 practice place. It can be concluded that a doctor actually has the right to get 3 practice licenses. The Mono-Loyalty System certainly limits the doctor's right to obtain a Practice License, so this system is basically not in accordance with the Medical Practice Act. This Mono-loyalty system will be good if it is followed by the fulfillment of doctors' rights both from economic aspects such as incentives, benefits, facilities and employment aspects such as a good working environment.

It should be noted that policy makers themselves are concerned about the effectiveness of the duties and profession of doctors. This can be seen from the limitation of license for practice in the Medical Practice Act. However, in practice in the field, there are still many doctors who practice with more than 3 licenses for practice. Hereby, the government as a policy maker needs to provide a solution considering that on the one hand there are areas that do not have a decent number of doctors so that doctors have to work in more than 3 places, while on the other hand there are places that use a monoloyalty system which causes doctors to not get their rights to do so. earn a decent living. A study and a win-win solution are needed so that no party is harmed, considering that the purpose of the medical practice regulation itself is to ensure that doctors can carry out their professional duties to the maximum and that the community gets the best health services from the doctors on duty.

One significant step which is important to take is to enforce the 3 licenses for practice implementation system while still paying attention to the rights of doctors which have been neglected by the government, for example receiving timely incentives, implementing standardized medical services and distributing medical services according to standards. applicable regulation. Hospitals as employers have standard rules

in distribution, and it should have a service assessment system for doctors on duty. One of the impacts that can occur if the hospital does not provide satisfactory income, then there is a possibility that the doctor will move to another hospital (turnover).²² Turnover has a detrimental impact on the organization/hospital because it can hamper the effectiveness and efficiency of work which in turn will reduce the productivity level of the organization.²³

It can be concluded that the provision of financial incentives to medical personnel, in this case doctors which is important in health services. Hospitals have to draw a common line, how a doctor will be interested in working in the government sector and minimize the use of 3 licenses for practice in accordance with the provisions of the law. Changes and studies in the law require a long process, for that the implementation of the 3 licenses for practice policy should be in line with the welfare of the actors, they are general practitioners, dentists and specialists.

If we compare the income and incentives for Indonesian civil servant doctors with other countries which apply monoloyalty, the following results are obtained: Indonesian civil servant doctors based on position range from class IIIb-IIIc to IDR 2,688,500 – IDR 4,415,600, group IIIc-IIIc ranges from IDR 2,802,300 - IDR 4,797,000 per month, for class IVa - IVc IDR 3,044,300 - IDR 5,431,900 per month and for class IVd-IVe IDR 3,447,200 - IDR 5,901,200.²⁴ The work allowance for PNS doctors at the Ministry of Health based on the Regulation of the Health Minister Number 83 of 2013 revealed that the benefits obtained reached 100% of the basic salary. For a doctor who is married, he will get a wife allowance of 10% of the basic salary and a child allowance of 2% (maximum 2 children). For food allowance, civil servant doctors will get IDR 37,000 per day (maximum 22 days).²⁵ As stated in Presidential Regulation No. 54 of 2007 states that for class IIIb doctors the allowance is IDR

325,000, for class IIIc-III d doctors IDR 750,000, doctors for class IVa-IVc IDR 1,200,000 and for doctors class IVd – IVe IDR 1,400,000. So if the total salary that will be obtained by class IIIb civil servant doctors is around Rp. 3,013,500 – Rp. 4,740,600, doctors for class IIIc-III d are around Rp. 3,552,300 – Rp. 5,547,000, doctors for class IVa-IVc are around Rp. – IDR 6,631,900, while for class IVd-IVe doctors it ranges from IDR 4,847,200 – IDR 7,301,200. This means that the income earned by PNS doctors is still much different from that of doctors in other countries.

According to a compilation of the OECD (Organization for Economic Co-Operation and Development), the Swiss Federal Office of Public Health and the Doximity Report on Physician Compensation, from May 2020 data, doctors in Melbourne-Australia earn around AUD 249,000 per year (Rp 2,727,620,790) with the lowest income of around 91,200 AUD (Rp 999,031,900) to the highest of around 420,000 AUD (Rp 4,600,806,160). Doctors in the UK typically earn around GBP 317,000 per year (Rp 6,340,55,380). The lowest income ranged from 117,000 GBP (Rp 2,340,203,870) to the highest it reached 536,000 GBP (Rp 10,720,24,050). This annual income includes housing, transportation and other benefits tunjangan.²⁶

Meanwhile, if we look at the income of our neighboring countries, Singapore and Malaysia, the income of doctors in both countries is much higher than Indonesia. Doctors in Malaysia earn an average of 15,500 MYR per month (Rp 53,533,090) with the lowest income around 5,680 MYR (Rp 19,617,290) and the highest 26,100 MYR (Rp 90,142,820). Meanwhile in Singapore, the average income of a doctor is 19,900 SGD per month which is equivalent to Rp. 213,105,500, with the lowest income of 7,290 SGD (Rp 78,067,290) and the highest of 33,500 SGD (Rp 358,745,440). In both Malaysia and Singapore, this includes housing, transportation and other

benefits.^{27,28}

The disparity in the comparison of income obtained by Indonesian civil servant doctors with these countries is very significant. This needs to be a concern and consideration for the government to regulate the amount of income of Indonesian civil servant doctors to achieve an adequate measure of welfare.

Conclusion

Based on the description above, it can be concluded that: The working hours of doctors in Indonesia is still very high, exceeding the maximum time of 80 hours a week. The policy of the three licenses for practice issued needs to be re-examined, because it results in high working hours for a civil servant doctor. On the other hand, the existence of a monoloyalty system should be accompanied by an increase in the income and incentives of a doctor as well as the fulfillment of the rights of other doctors.

Suggestions

1. It is necessary to make special rules regarding the maximum work limit of an Indonesian civil servant doctor to fill in the empty norms.
2. It is necessary to review the three licenses for practice rules in more detail.
3. The need for equal distribution of PNS doctors in Indonesia.
4. There is a need to increase the number of PNS doctors in Indonesia.
5. The monoloyalty system is very well implemented but must be accompanied by adequate doctor income.

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Profile of Pneumocystis Jirovecii Pneumonia in HIV/AIDS Patients in Dr. Soetomo General Hospital of East Java Province

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Abstract

Background: Human immunodeficiency virus (HIV)-infected patients if not treated will experience acquired immunodeficiency syndrome (AIDS). People with AIDS will suffer opportunistic infection. One of the common opportunistic infections is Pneumocystis jirovecii pneumonia (PCP). PCP is an infection caused by the fungus Pneumocystis jirovecii which infects patient's lungs. The purpose of this study is to analyze the profile of HIV/AIDS patients with PCP in Dr. Soetomo Hospital.

Methods: This is a retrospective descriptive study based on medical records obtained from the inpatient ward of Dr. Soetomo General Hospital Surabaya. A total 21 patients enrolled in this study who met the inclusion criteria. The data are retrospectively described by demographic characteristics, CD4 count, clinical symptoms, blood gas, and lungs radiographic features.

Conclusion: Major findings of PCP in HIV/AIDS patients was in the group of age 25-49 years (85,7%), male (85,7%), high school graduate (71,4%), employed and unmarried (66,7%). The most common clinical symptoms were dyspnea (100%), PaO₂ ≥ 70 mmHg (61,97%), CD4 count < 50 cells/μL (76,2%) and the most common radiographic features was bilateral interstitial infiltrates (95,2%).

Keywords: HIV/AIDS; Pneumocystis jirovecii pneumonia; Opportunistic infection; Demographic characteristics; Clinical profile.

Introduction

Pneumocystis Jirovecii pneumonia (PCP) is an infection caused by the fungus Pneumocystis jirovecii. PCP is a common opportunistic infection among immunocompromised patients such as patients receiving immune-suppressive therapy and in patients

with human immunodeficiency virus (HIV) infection¹. The incidence of PCP decreased after the discovery of prophylaxis and antiretroviral therapy (ART). However, the incidence of PCP is still being reported with varying number of cases in several countries². In Jakarta, the incidence of PCP is 20%, while in America incidence of PCP is 5%^{1,3}. Pneumocystis cannot be cultured, so definitive diagnosis requires detection and identification of the organism by polymerase chain reaction of respiratory specimens, dye staining, or fluorescein antibody staining. Definitive diagnosis

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of PCP is difficult to do, so patients with suspected clinical symptoms leading to PCP should be treated immediately⁴. PCP is generally diagnosed on the based on presumptive diagnosis. A presumptive diagnosis was made based on the patient's clinical signs and symptoms, such as dyspnea, fever, cough, and hypoxia in immunocompromised patients with cluster of differentiation 4 (CD4) <200 cells/mL and abnormal lung radiology. In Indonesia, the diagnosis of PCP is still based on a presumptive diagnosis^{2,3}. This study aims to analyze the profile of HIV/AIDS patients with PCP infection at Dr. Soetomo General Hospital of East Java Province.

Materials and Methods

The design of this study was an analytical retrospective descriptive study by conducting patient's medical records. This study was conducted on HIV/AIDS inpatient with PCP infection at Dr. Soetomo General Hospital, Surabaya admitted from November 2019 until November 2020. Patient's medical record which do not have CD4 data, clinical symptoms, radiological features and partial pressure of oxygen (PaO₂) were excluded from the study. The inclusion criteria were HIV/AIDS patients clinically diagnose with PCP infection and had complete medical records data including: age, gender, educational level, employment status, marital status, CD4 count, clinical symptoms, radiological features, and PaO₂.

Age was the patient's lifetime from birth until diagnose with HIV/AIDS and PCP, it was classified into 3 groups, 20-24 years old, 25-49 years old and ≥ 50 years old based on Indonesia ministry of health in infodatin HIV/AIDS⁵. Gender was differentiated as male and female. educational level was patient education when diagnoses with HIV/AIDS and PCP differentiated as primary school, junior high school, high school, and higher. Employment status was differentiated into employed and unemployed. Marital status was differentiated as married and unmarried. CD4 count were patient number of CD4 after diagnose with HIV classified into ≥ 200 cell/mL, 100-199 cell/mL, 50-99 cell/mL, and <50 cell/mL. Clinical symptoms were patients' complaints when diagnosed with HIV/AIDS and PCP, categorized as dyspnea, cough, fever, and weight loss. Radiological features were patient lung radiographic image by using plain x-ray differentiated into bilateral infiltrate, localized infiltrate, lung nodule, and pleural effusion. PaO₂ was patients' hypoxia severity⁶ classified into ≥ 70 mmHg (mild), <70 mmHg (moderate-severe).

Results and Discussion

A total of 50 confirmed cases of HIV/AIDS patients with PCP at Dr. Soetomo General Hospital in the period of November 2019 until November 2020 obtained in this study, 21 of which fulfilled the inclusion criteria. Clinical characteristics data presented in Table1.

Table 1. Patient's clinical characteristics

Characteristics	Frequency	Percentage (%)
Gender		
Male	18	85.7%
Female	3	14.3%

Age (Years old)		
20-24	2	9.5%
25-49	18	85.7%
>= 50	1	4.7%
Educational level		
Primary school	4	19%
Junior high school	0	0%
High school	15	71.4%
Higher	2	9.5%
Employment status		
Employed	14	66.7%
Unemployed	7	33.3%
Marital Status		
Married	7	33.3%
Unmarried	14	66.7%
CD4 count (cell/mL)		
≥ 200	0	0%
100-199	0	0%
50-99	5	23.8%
<50	16	76.2%
Clinical symptoms		
Dyspnea	21	100%
Cough	19	90.5%
Fever	14	66.7%
Weight loss	14	66.7%
Radiological features		
Bilateral infiltrate	20	95.2%
Localized infiltrate	2	9.5%
lung nodule	1	4.8%
pleural effusion	1	4.8%
PaO2 (mmHg)		
≥70	13	61.9%
<70	8	38%

Based on the data obtained from this study, majority of the patients were males (85.7%) with the most common age group was 25-49 years old found in 18 patients (85.7%). Median age in this study was 34 years old ranging from 20 years old until 50 years old. The majority of the patients were high school graduate (71.4%) found in 15 cases, primary school educational level found in 4 cases (19%) and higher educational levels only found in 2 cases (9.5%). From the data obtained, most of the patients were employed and unmarried each found in 14 cases (66.7%).

CD4 examination results showed the majority of the patient had CD4 count <50 cell/mL found in 16 cases (76.2%). The lowest CD4 count in this study was 5 cell/mL and the highest was 98 cells/mL. The median of CD4 obtained in this study was 27 cells/mL with mean number of CD4 is 32 cells/mL. The common clinical symptoms experienced by the patients were dyspnea found in 21 cases (100%), followed by cough found in 19 cases (90.5%), fever and weight loss of each found in 14 cases (66.7%). The majority of radiological findings were bilateral infiltrates in 20 cases (95.2%), localized infiltrates in 2 cases (9.5%) which distributed in the left paracardiac area, multiple lung nodules with varying sizes and pleural effusions each in 1 cases (4.8%). Based on PaO₂ results showed 13 cases (61.9%) had PaO₂ \geq 70 mmHg, ranging from 47 mmHg until 167 mmHg with median 73 mmHg. Among 21 cases, 7 cases had PaO₂ results were examined after receiving oxygenation.

The most dominant age group in this study was 25-49 years old (85.7%), the median age was 34 years old and the youngest case was 20 years old while the oldest case was 49 years old. This finding was similar in the Indonesia ministry of health in infodatin on HIV/AIDS where the most dominant age group was also 25-49 years old (70.4%)⁵. It is associated within that age a person is sexually active. So this age group becomes more susceptible to HIV infection⁷.

In this study, male patients (85.7%) was dominated compared to female patients (14.3%). Similar findings were also obtained in a study in China where the number of male patients was 93.3%⁸. The number of male patients was higher because of risky sexual behavior was more often carried out by men, such as having sex without using a condom, sex with different partners, men with men sexual intercourse, or drugs abuse. These behaviors increases the risk of HIV infection⁹.

Based on educational background, most of the patients were high school graduates (71.4%)¹⁰. Similar results were also obtained in a study in Lampung where the number of patients with a high school graduate background was 45%¹⁰. The level of education does not directly affect the prevalence of HIV infection. But through education it will be easier to understand information and education about HIV infection¹¹.

In this study, most of the patients were employed (66.7%). Ogunmola et al. (2014) stated that there was no relationship between HIV infection and employment status¹². In addition, employment status also has no effect on the prevention of HIV transmission. HIV infection is generally experienced by people in productive age. Which at that age most of the patients were employed¹³. So in this study most the patients were employed.

Based on marital status, the findings in this study showed that most of the patients were unmarried (66.7%). Similar findings were also obtained in a study in Samarinda where the number of unmarried patients was 47.1%¹⁴. Marriage can reduce risk of HIV compared to individuals who are not married, divorced, or widowed¹⁵. Marriage can prevent individuals from having sexual intercourse with different partners so it can reduce the transmission of HIV¹⁶.

In this study, the most CD4 cell count found was <math><50\text{ cells/mL}</math> (76.2%) with mean value was 32.8 cells/mL. A study in India obtained a higher mean CD4 value of 67.27 cells/mL¹⁷. The finding of a lower CD4 cell count in this study was due to the late diagnosis of HIV¹⁸. CD4 is one of defense mechanism against PCP. CD4 count is important factors in developing PCP in HIV patients. CD4 cell count <math><200\text{ cells/L}</math> increases the incidence of PCP in HIV patients. Therefore, patients with CD4 cell counts <math><200\text{ cells/L}</math> need to be screened for PCP through anamnesis complaints of lung infections, laboratory examinations, and chest X-rays¹⁹.

The most common clinical symptoms of patients were dyspnea in 21 patients (100%), followed by cough in 19 patients (90.5%), fever and weight loss in 14 patients (66.7%). A study in Switzerland found that the patient's clinical symptoms were cough (90%), dyspnea (80%), and fever (43%)²⁰. HIV patients with PCP infection generally show symptoms of dyspnea, cough, and fever or subfebrile²¹. This finding is in accordance with the findings in this study.

Based on pulmonary radiological features of HIV patients with PCP in this study, all patients showed abnormal radiological features, with the most findings were bilateral interstitial infiltrates in 21 cases (95.2%). A study in India found that the most dominant radiological feature found in HIV patients with PCP infection were bilateral interstitial infiltrates (70%)¹⁷. The most common radiological findings in HIV patients with PCP infection are interstitial infiltrates and ground glass opacities²². Other radiological findings that can be found are pulmonary nodules, lobar infiltrates, pleural effusions, and pneumothorax⁴. This finding is due to infection with other pathogens that attack the patient. Thus PCP invades the lungs of patients that have been damaged by the previous infection²³.

PaO₂ examination was performed to determine the severity of hypoxia in patients with PCP. Hypoxemia is caused by an increase in the alveolar-arterial gradient, impaired diffusion, and changes in lung compliance and total lung capacity²³. In this study, most patients showed mild hypoxia PaO₂ $\geq 70\text{ mmHg}$ (61.9%). PaO₂ <math>< 70\text{ mmHg}</math> will give a poor prognosis compared to patients with PaO₂ $\geq 70\text{ mmHg}$. Therefore, PaO₂ monitoring is needed to evaluate and assess the severity of the patient's condition²⁴.

Conclusion and Acknowledgement

In Conclusion, PCP in HIV/AIDS patients in Dr. Soetomo General hospital from November 2019-November 2020 were dominated by male, aged 25-49 years old. Most of the patients were high school graduate, unmarried and employed. All patients showed symptoms of dyspnea. Most of patient had CD4 count <math><50\text{ cells/ }\mu\text{L}</math>. Based on radiographic finding most of patients showed bilateral interstitial infiltrated. Most patients suffered from mild hypoxia with PaO₂ $\geq 70\text{ mmHg}$. HIV patients with complaints of dyspnea, hypoxia low CD4 cells and abnormal radiological features should be considered for PCP prophylaxis.

Conflict of Interest: No conflict of interest.

Ethical Clearance: This research had an ethical clearance that was approved by Dr. Soetomo General Hospital ethical committee (ethic number: 0194/LOE/301.4.2/XI/2020).

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Early Childhood Malnourishment and its Associated Factors - Uttarakhand

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Abstract

Malnutrition, a word which seems to shake our roots and disturb our future. Today the underdeveloped and many developing countries are battling this threat in many ways. The reason behind malnutrition is the determinants which make it prevail in our neighborhood and our country on the whole. Malnutrition possess a very serious threat to the future of our country especially the children between (1 – 3) years of age. This paper attempts to identify the existing prevalence of malnutrition in Uttarakhand, to explore the predisposing factors contributing to it in under five children and compare the prevalence with the selected neighboring states. An in – depth survey of all the related literatures and published articles in the selected area was made. It has been reported in recent times about the hike in India's hunger problems and the number of reported cases of stunting and wasting has also rocketed though underweight has slightly reduced. The only way to curb malnutrition is by; breaking the shackles of the determinants, thorough regular surveys and screenings, improved and adequate health care services, reaching out to the needy and spreading awareness among the masses.

Keywords: Malnutrition, Stunting, Wasting, Underweight, Children

Introduction

Children; the purest souls, are the most vulnerable section of a society. They are considered to be the future of any country and the pillars upon which the country projects its existence. Therefore, it becomes imperative for any country to focus more on this vulnerable section of the society. Only a healthy child can help in building a healthy country of the future.

A person's demands for nutrition varies according to his age.^[1] Nutrition is one important aspect in child's development apart from genetic and environmental factors. It plays a very significant role in early years of child development. It not only prepares the child to combat opportunistic infections and diseases, but lays a strong foundation for a healthy future. Child's development begins from conception itself thereby emphasizing nutrition importance during pregnancy which affects the overall child development too.^[2] In most literature studied, under nutrition is used synonymously with malnutrition.

Malnutrition refers to deficiencies, excesses, or imbalances in a person's intake of energy and/or nutrients. It refers to three broad groups i.e. under

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nutrition (weight for age), stunting (Height for age) and wasting (weight for height).^[3] Malnutrition cause is multifaceted in early childhood and is also related to socioeconomic and sociodemographic factors.^[4] Globally in the year 2020 it was found that 149.2 million children under the age of 5 years of age were stunted, 45.4 million wasted, and 38.9 million overweight.^[5]

According to recent reports, there has been a tremendous surge in levels of hunger and stunting inspite of India's economic growth. In Global Hunger Index 2020, India ranked 94th among 107 countries.^[6] According to NFHS-4, 36% under-five children are underweight, 43.3% are stunted and 24.5% are wasted. In Uttarakhand, the prevalence of underweight, stunting, and wasting as per NFHS-4 is 26.6%, 33.5%, and 19.5%, respectively and is more in rural than in urban areas.^[7] The hilly state Uttarakhand is located in northwestern part of the country. It has varied topography and is divided into two regions i.e. Garhwal and Kumaon. It has total of nine districts^[8] and the population is sparsely distributed, majority being rural.^[9] Subject to the terrain the health services are not as good as other parts of India. Uttarakhand state was found to be ranked 18th among 29 states, an indication of its indifferent health and was found to perform worse in health.^[10] The present review focuses on the prevalence and predisposing factors causing malnutrition in early childhood years specifically in the age group of (1-3) years in Uttarakhand. This review will help to consolidate the available published literature on prevalence and factors contributing to malnutrition which may help

to strengthen the programs and spread awareness to counter the menace of malnutrition in children.

Methodology

The literature regarding prevalence and factors associated to malnutrition was collected from Google search engine, Google Scholar, Pubmed and Published reports and articles. The objectives of the review were: existing prevalence of malnutrition in Uttarakhand, to explore the predisposing factors contributing to it in under five children and compare the prevalence with the selected neighboring hilly states. The neighboring states were Himachal Pradesh and Jammu and Kashmir.

Only open accessed articles were retrieved. The keywords used for search were: "malnutrition", "under nutrition", "under five children", "factors", "Uttarakhand". The literature published form 2011 till date was searched. About 518 articles were shortlisted from the electronic database. After assessment of title and abstract 514 articles were excluded. The reference list also was examined. In addition 4 studies were added from other sources. The relevant studies included for the final review based on inclusion and exclusion criteria were 7 studies. The inclusion criteria for review were: Original research studies published between 2011 to 2021, cross sectional and epidemiological studies, study conducted among children below five years of age in Uttarakhand. All the papers used the height for age, weight for height and height for age criteria for assessment of nutritional status in under five children. The process of study selection is shown in below mentioned Fig.1.

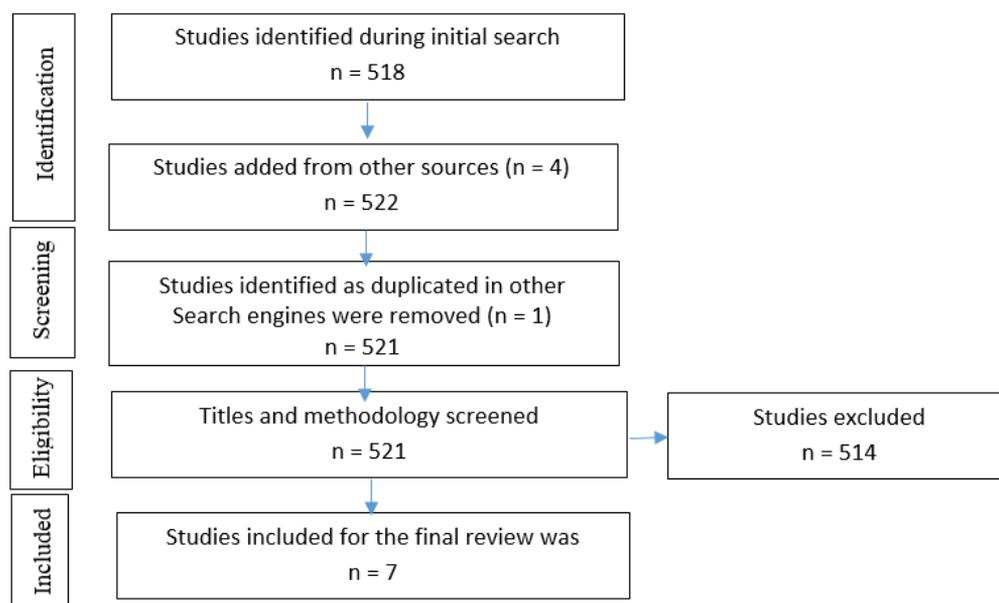


Fig.1. Flow diagram for study selection process

The review is presented in the following headings: prevalence of malnutrition in Uttarakhand, factors predisposing to malnutrition in under-fives and comparison of prevalence with the selected neighboring hilly states.

Prevalence of malnutrition in under-fives of Uttarakhand:

The studies on prevalence of malnutrition in under-five children was evaluated and results found were: in Rishikesh underweight, stunting and wasting was 37.3%, 43.3% and 24.5% respectively^[11], in Haridwar 44.82% stunted and 51.72% were underweight^[12], in Kashipur it was found that about 27.27% and 54.55% children in the age group of (0-5) years were found malnourished in mild to moderate category^[13] and in Dehradun it was found that 200 (52.5%) children having exclusively breast fed were undernourished^[14]. In another study conducted in Dehradun among toddlers regarding socio economic correlates and under nutrition it was found that 61.78% children belonging

to lower socio economic status were undernourished, 75.50% of undernourished children had uneducated fathers and 73.30% had illiterate mothers.^[15] A study of co-morbidities in children conducted in Dehradun found that 298 (59.6%) children with co morbidities were under nourished^[16] and in study conducted in rishikesh it was found 27.38% children were under weight for their age, 52% were stunted and 17.84% were wasted^[17].

As per swasth report of Uttarakhand, it was found that there has been 10.9% drop in cases of stunting in children under 5 years of age. Also there has been 11.9% decline of underweight in children below 5 years. But there has been minor increase of 0.7% in wasting cases of NFHS -4 data when compared with NFHS – 3 data.^[18] It is not only the intake of diet which will help to solve this problem of under nutrition in early years of life but also other contributing factors. Table 1. Summarizes the prevalence of under nutrition in children under 5 years of age.

Table.1. Prevalence of under nutrition in children under five years of age.

Ref. No.	Year	Setting	Sample size	Design	Identified Prevalence
1.	2020	Rishikesh	400	Community-based Cross-sectional	Urban vs rural areas: Underweight (40.5% vs 35.0%) and 46.5% vs 40.0%, respectively. Wasting more in urban (27% V/s 22.0%) than rural areas
2.	2018	Haridwar	85	Epidemiological Study	44.82% stunted and 51.72% were underweight
3.	2016	Kashipur	100 families and 33 children (0-5) years	Cross-sectional	Out of 33 children in 0-5 age group 27.27% and 54.55% were malnourished in mild to moderate category.
4.	2016	Dehradun	381	Cross-sectional	About 47.5 % children exclusively breast fed were well nourished whereas 52.5% were undernourished.
5.	2016	Dehradun	507	Cross-sectional	Children belonging to lower class were undernourished (61.78%), maximum (88.44%) proportions of children living in poor environment were found to be undernourished
6.	2014	Dehradun	500	Cross-sectional	Out of 500 children, 202 (40.4%) were well nourished and 298(59.6%) were under nourished with or without co morbidities
7.	2012	Rishikesh	695	Cross-sectional	About 27.38% children were under weight, 52% were stunted and 17.84% showed wasting

Predisposing factors of Malnutrition:

A varying number of factors lead to malnutrition in children. The studies reviewed in Uttarakhand depicted several factors that affect the nutritional status of children under five years of age. The factors have been categorized into socio demographic factors, child related factors and environmental factors.

Socio demographic factors:

The prevalence of underweight and stunting was found higher in children belonging to families of low socio economic status i.e. 47.50% and 40%.^[12,19] The educational status of parents also had a negative impact on nutritional status of children i.e. illiterate parents had more percentage of malnourished children.^[11, 12,18] Occupation of parents^[11,17,19] and size of family^[19].

Child related factors:

Underweight/low birth weight and prematurely born children were found to be more malnourished^[11,12], exclusive breast feeding, timely

complementary feeding^[14] Anemia^[12,17] and birth order were other significant factors leading to malnourishment in children.^[12] Birth interval between children less than 2 years, not immunized children, inadequate dietary intake and monotonous diet, worm infestations, repeated illness like diarrhea, fever and cough and cold also found to be one of the significant factors leading to malnourished children^[11,17].

Environmental factors:

Poor physical environmental factors like housing, overcrowding, lighting and ventilation have an important effect on the health status of the child.^[19] The present environmental factors identified in Uttarakhand were also found in the neighboring states i.e. A study conducted in Sirmour district HP found that malnutrition was associated with the type of house, number of rooms, unsafe drinking water and lack of transport facilities.^[20] Another study conducted in Jammu and Kashmir in Doda district highlighted the significant environmental factors: nature of house, type of cooking area, cooking fuel and toilet facility at home as significant factors related to malnutrition

in children.^[21]

Comparing prevalence with the neighboring hilly states:

The available data regarding prevalence of malnutrition in under-five children of Uttarakhand is very less. Very few studies on malnutrition in children have been undertaken in this area and when it is compared with another hilly state it was found that the status of malnutrition is almost similar when compared with Himachal Pradesh. A study conducted in Sirmaur district of Himachal Pradesh found that 40% of under-five children were Stunted, 19.5% were Wasted and 10.48% were Stunted and Wasted.^[20] Another study conducted in Kinnaur district of Himachal Pradesh found that prevalence of Underweight, Stunting and wasting was 21.4%, 27.4% and 11.1% respectively in under-five children.^[22] A study in the state of Jammu and Kashmir found the prevalence of malnutrition 20.87% in under-five children of which 14.56% had Grade I, 5.83% and Grade II malnutrition.^[23] Another study conducted in the Gujars population of Jammu and Kashmir found that 10.75% of children were suffering from Grade I malnutrition, 17.5% with Grade II, 19.91% with Grade III and even 2.21% with Grade IV.^[24] The prevalence reported in these studies when compared with the above mentioned studies in Uttarakhand can be interpreted that in Uttarakhand the percentage of malnutrition in children under-five is higher compared to Himachal Pradesh and Jammu and Kashmir. But according to NFHS - 4 data 2015-16, the prevalence of malnutrition in Uttarakhand for Underweight, Stunting, and Wasting reported was 26.6%, 33.5%, and 19.5%, for Himachal Pradesh it was 36.5%, 38.6% and 19.3%^[25] and for Jammu and Kashmir was 16.6%, 23.2% and 13.7%^[26] respectively. Thus, it can be summed up that the status of malnutrition is more or less in the same alarming and despairing situation.

The data regarding malnutrition in under five children specifically in hilly areas is very less but the data reported by NFHS says a lot. The studies

conducted in Uttarakhand were limited to only prevalence and factors. There are many more hilly terrains that needs to be accessed and the status of child's nutrition needs to be assessed. The factors identified and reported in the above studies conducted in Uttarakhand are the factors which can be modified through various awareness programmes. But along with the awareness programs it also necessary to have in depth identification of other factors grounded in the culture and beliefs which thou cannot be changed easily but through constant visiting and education campaigns can be modified. Also, it should not the mother who should be involved but the entire family.

Conclusion

The first 1000 days in child's life is considered to be an important phase. In this phase steps can be taken to combat malnutrition thereby preventing harm in near future.^[27] As the child enters the toddler phase the brain development accelerates thereby enabling the child to refine his fine motor skills and perform difficult tasks.^[28] Micronutrients play a very significant role in development of brain in this early phase and iron is one of the most important of them, deficiency of which can cause depression and anxiety in later phases of life.^[29-32] Though poor nutrition is one of the major cause of malnutrition there are various other predisposing factors which directly and indirectly effect the health status of child in first five years of life. The data regarding factors contributing to malnutrition in the toddler age group has not been addressed much and needs to be explored, so that it can be dealt at a very initial stage itself with proper guidance to mothers and other family members. It is very important as some factors are modifiable, which can be modified thereby preventing prevalence in older age. Socio – cultural and behavioral factors also play a significant role in increasing the incidence of malnutrition. It is difficult to change beliefs or practices, as every geographic location in India has their own characteristic which may differ from one place to another, but with a stronger integrated approach it can be dealt effectively in bringing a change. It is therefore important to develop

intervention strategies keeping in view the cultural practice and beliefs of people residing in that area which will help in reducing shackles of malnutrition in near future.

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A Quasi-Experimental Study to Assess the Effectiveness of Guided Imagery Therapy on Infertility Related Stress and Quality of Life among Infertile Women

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Abstract

Introduction and Background: According to WHO infertility is failure to achieve a pregnancy after 12 months or more than or without use of any contraception method, it is a reproductive system disease. Infertility can cause increase level of stress and create negative thought. So this study is aimed to find out effectiveness of guided imagery technique on stress and quality of life of infertile women. **Methodology:** The research approach used was quantitative approach. Quasi experimental: nonequivalent control group, Pre-test post-test design was used. 66 infertile women were selected for both the group was selected 33 subjects in each group. Pre assessment for stress level & quality of life was done for both experimental and control group. The tool of data collection included a socio-demographic Performa, Perceived stress scale (PSM- 10) and FertiQoL scale assessment tool. Guided imagery provided to experimental group. The intervention was given to infertile women is recorded 15 minutes audio tract. Participants had listened the infertile specific GI recording once in a day for 7 consecutive days. In control group, the GI therapy did not provided. **Result:** The data was analyzed by using descriptive and inferential statistics. The study result shows that effect of GI intervention on stress intensity and on QoL are effective and P value for stress was <0.001 and for QoL p value was <0.001. **Conclusion:** The study concluded that Guided imagery technique is effective in reducing stress level and improve quality of life.

Key words: Effectiveness, Guided imagery therapy, stress associated with infertility, living standard, infertile female.

Background of the Study

According to WHO infertility is inability to get a pregnancy later than twelve months or more than or without use of any contraception method, it is a reproductive system disease.

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Infertility divided into two types: Primary infertility: Means that the couple has never conceived because of inability to become pregnant in that those mothers also include who have a miscarriages, that may result in still birth or ever having a live birth. Secondary infertility: Means that couple has experience of pregnancy before and later failed to conceive due to the some reproduction problem like impaired sperm production, fallopian tube damage or other abnormal uterine condition in women.

There are many causes for the infertility like endocrine disorder, physical disorder, ovarian disorder, defectiveness in ovum and sperm etc. But, sometime psychological problem like stressful life is also cause for infertility. There are many laboratory investigation are used for detection of the infertility cause, and many surgical and medical intervention use to treat the infertility like hormonal drug therapy, IUI & IVF.

Infertility related QoL includes an impression of infertile patient's life status during their fruitless period from a wide perspective. Countless examinations uncovered that fertile ladies experienced less fortunate QoL during the time of infertility.

Infertility is major problem in the India; in 2015, 27.5 million couples affected from the infertility in India according to Ernst and Young report. 40-50% of cases are registered due to female factor and 30-40% of cases are registered due to male factor.

Infertility is a significant general medical problem with authentic social outcome. The physical and mental effect of infertility can be pulverizing to the infertile ladies and to their partner. Infertility often brings about irritation, hopelessness, nervousness, and sentiments of worthlessness. There be a numerous psychological and social problem in the infertile women.

Infertility has many psychological effects on the women which are associated with stress and it is a more stressful event in their life. Stress reduction is possible through two methods; using pharmacological methods and complementary medicine. There is a different type of pharmacological and non- pharmacological treatment for the infertility. Previous research suggests that there are many non- pharmacological treatment use of reducing the psychological problem of infertile women. In that the guided imagery is non-pharmacological treatment which has no any side

effect.⁶ Guided descriptions is a useful during stress managing process for many reasons. It can provide relax to mind and help to reduce stress.

Infertility has been a neglected area of research when compared to research on fertility. Globally between 50 to 80 million couple at some point their reproductive lives suffer from infertility problems and also related psychological disturbance So this study aimed is to diminish the infertility related anxiety and get better the excellence of life through guided imagery intervention because the stress is the physiological or psychological tension that may lead to disturbance in physical, emotional and psychological well being of women and it affect the reproductive outcome. So guided imagery intervention is reduce the stress and get better value of life of infertile female.

Objective of the study were

- To evaluate effectiveness of GI therapy on infertility related stress amongst infertile women.
- To evaluate effectiveness of GI therapy on quality of life amongst infertile women.
- To find out the association between pre-intervention levels of infertility related stress and quality of life amongst infertile women with selected socio-demographical variable.

Material and Methods

Quantitative approach was obtained by investigator for assess the efficiency of GI therapy on the infertility related stress & improve value of well-being. Research design of the study was nonequivalent control group, Pre-test post-test design

Variable of the study:

Dependent variable: stress level and QoL of infertile female.

Independent variable: Guided imagery therapy

Socio demographic variable: socio-demographic variable included Age, height, weight, type of diet, education status, age at marriage, age at menarche, duration of marriage, living pattern, type of family, social support system, occupation, monthly family income, place of residence, duration of diagnosed infertility, use of contraception, received infertility treatment, treatment duration, causes of infertility, body mass index(BMI).

Research setting:

It is the area where the research study is conducted.

- Morpheus Usha fertility center, Anand
- Spring IVF center, Ahmedabad
- Cigna IVF center, Ahmedabad
- Ansh women hospital, Ahmedabad

Population:

Target population: primary infertility.

Accessible population: primary infertile female who be fulfilled the inclusion criteria of study

Sample: it was estimated 66 totals for each group, 33 samples were for experimental group and 33 samples were control group. Sample size be calculated on the basis of $N = 2(\sigma/\Delta)^2 * Z_{\alpha} + Z_{\beta} - 1$ formula.

Sampling technique: Non-probability convenient sampling technique

Inclusive criteria: An infertile woman

- Who is in age group 18-35 years
- Who had diagnosed with primary infertility.
- Who is agreeable to contribute in the study.
- Who can capable to read and write down English or Gujarati.

Exclusive criteria: An infertile woman

- Who is having any mental/psychological disturbance.
- Who is having any medical or surgical condition with infertility.
- Who is having secondary infertility.

Tool was used for research study: To get the baseline data socio-demographic variable structured questionnaire was used. To analyze the stress level researchers had used perceived stress scale-10 and to analyze the QOL (Quality of life) FertiQol scale used.

Procedure for data collection: Ethical approval was obtained by IEC-HR, CHARUSAT. For the data collection method first the Researcher had introduce herself to the participants and inform consent was taken after explaining the purpose of study. Evaluation of effectiveness of Guided imagery therapy on stress and Quality of life among infertile women, first the investigator has assess the stress level in infertile women by using perceived stress scale tool and QOL of infertile females assess by FertiQol scale in both experimental (33) and control group (33). For experimental group the investigator has administered the Guided imagery therapy. The intervention is recorded audio tract for 15 minutes was given to infertile women. Participants had listened the infertile specific guided imagery recording once in a day for 7 consecutive days. In control group, the guided imagery therapy was not being given. In experimental group the investigator has assess the stress level by using the PSM-10 scale & assess the Quality of life by using FertiQol scale after the last intervention. In control group, the stress level & Quality of life was assessed by using the same tool without administering the Guided imagery therapy. If after the intervention stress level still persist then investigator will be referred the participants to counselor.

Result and Discussion

Table 1: Findings related to socio-demographic variable of infertile women of both experimental and control group.

Sr. no	Demographic Variable	Experimental group N=33		Control group N=33	
		Frequency (f)	Percentage (%)	Frequency (f)	Percentage (%)
1.	Age				
	20-25 years	3	9.1	9	27.3
	26-30 years	21	63.6	14	42.4
	31-35 years	9	27.3	10	30.3
	36-40 years	0	0.0	0	0.0
2.	Height				
	<140 cm	0	0.0	5	15.2
	141cm -150 cm	24	72.7	20	60.6
	151cm-160 cm	9	27.3	8	24.2
	> 161 cm	0	0.0	0	0.0
3.	Weight				
	35kg-50kg	6	18.2	4	12.1
	51 kg-60 kg	17	51.5	20	60.6
	61 kg-70kg	10	30.3	8	24.2
	71kg-80 kg	0	0.0	1	3.0
	≥81 kg	0	0.0	0	0.0
4.	Type of diet				
	Vegetarian	27	81.8	26	78.8
	Non vegetarian/Mixed	6	18.2	7	21.2
5.	Education status				
	No formal education	0	0.0	0	0.0
	Primary education	12	36.4	9	27.3
	Secondary and/or higher secondary education	17	51.5	23	69.7
	Graduation and/or above	4	12.1	1	3.0
6.	Age at marriage				
	18 years to 22 years	14	42.4	26	78.8

Cont... Table 1: Findings related to socio-demographic variable of infertile women of both experimental and control group.

	23 years to 27 years	19	57.6	7	21.2
	28 years to 32 years	0	0.0	0	0.0
	>32 years	0	0.0	0	0.0
7.	Age at menarche				
	12 years to 15 years	33	100.0	33	100.0
	16 years to 19 years	0	0.0	0	0.0
	Above 19 years	0	0.0	0	0.0
8.	Duration of marriage				
	1-5 years	25	75.8	30	90.9
	6-10 years	6	18.2	1	3.0
	>10 years	2	6.1	2	6.1
9.	Living pattern				
	Living together	5	15.1	15	45.5
	Temporary away from each other Due to occupation	28	84.9	18	54.5
10.	Type of family				
	Nuclear family	13	39.4	8	24.2
	Joint family	20	60.6	25	75.8
11.	Social support system				
	Available	24	72.7	25	75.8
	Not Available	9	27.3	8	24.2
12.	Occupation				
	Housewife	20	60.6	15	45.5
	Related to medical profession	6	18.2	8	24.2
	Related to Non medical profession	7	21.2	10	30.3
13.	Monthly family income				
	Up to Rs 5000	0	0.0	0	0.0
	Rs 5001-Rs 15000	8	24.2	6	18.2
	Rs 15001-Rs 25000	19	57.6	17	51.5
	Rs >25000	6	18.2	10	30.3
14.	Place of residence				
	Rural area	3	9.1	6	18.2

Cont... Table 1: Findings related to socio-demographic variable of infertile women of both experimental and control group.

	Urban area	30	90.9	27	81.8
15.	A duration of diagnosed infertility[years]				
	<3	28	84.8	29	87.9
	3-5	5	15.2	4	12.1
	>5	0	0.0	0	0.0
16.	Use of contraception				
	Yes	1	3.0	0	0.0
	No	32	97.0	33	100.0
17.	Received infertility treatment				
	Semen insemination(IUI)	0	0.0	0	0.0
	Stimulate ovulation & IUI	4	12.1	8	24.2
	In vitro fertilization(IVF)	1	3.0	4	12.1
	Hormonal therapy	28	84.8	21	63.6
18.	Treatment duration [years]				
	1	33	100.0	33	100.0
	2	0	0.0	0	0.0
	>3	0	0.0	0	0.0
19.	Causes of infertility				
	Female factor	33	100.0	33	100.0
	Male factor	0	0.0	0	0.0
	Reason not given	0	0.0	0	0.0
	Cause of male & female factor	0	0.0	0	0.0
20.	Body mass index(BMI)(kg/m ²)				
	Low weight (below 18.5)	0	0.0	0	0.0
	Normal(18.5-24.9)	13	39.4	11	33.3
	Overweight(25.0-29.9)	19	57.6	19	57.6
	Obese (30.0-above)	1	3.0	3	9.1

As per the Table 1 Findings regarding age were Majority of the infertile women 21(63.6%) were from the 26-30 years of age group in the Ex. group and In the control group greater part of female were from 26 to 30 years of age group which is 14(42.4%) of age group. Most participants in experimental 27(81.8) and control group 26(78.8) are vegetarian. Majority of participants in both the group have occupation related to Housewife 20(60.6%) in experimental group & 15(45.5%) in control group. In both the group causes of infertility is female factor.

The study finding supported by study conducted by Jones A, Karla K, et al. (2017) regarding age were belongs to 26-30 years in experimental group 21(63.6%) and control group 19 (63%). Regarding type of diet, experimental group 27(81.8%) and control group 13(39.4%) were non-vegetarian. About occupation majority in group 20 (67%) and control group 20 (67%) were housewife. Majority of experimental group 20 (67%) and control group 20(67%) has causes of infertility is female factor.¹¹

Table 2 Effectiveness of guided imagery therapy on level of stress in experimental and control group

N=33

Group		Mean	Std Deviation	t-test	P value
Experimental	Stress level before intervention	29.84	2.92	25.934	<0.001
	Stress level after intervention	13.33	2.74		
Control	Stress level at Day-1	29.30	2.13	1.294	0.205
	Stress level at Day-7	29.70	2.74		

As per Table 2 findings related to effect of GI therapy on stress intensity there was statistically significant difference found in experimental Group Pre test mean score is 29.84 which reduced to become 13.33 on 7th day after intervention. In control group mean score on 1st day is 29.3 which are not reduced but it is increased to become 29.7 on 7th day. In experimental group p test was 25.934 with p value was <0.001 while in control group it was 1.294 with p value was 0.205.

This findings are predictable with Porat-katz a, paltlel O, Kahane A, et al. (2016) there is a noteworthy decrease of anxiety after the guided symbolism intercession in exploratory gathering the post test feeling of anxiety (M=164.30, SD=19.03) was not exactly the pretest feeling of anxiety (M=247.51,

SD= 23.14) and the thing that matters was measurably critical at p<0.001 level, in charge gathering, there was no factual contrast between the pretest (M= 246.65, SD=22.18) and posttest (M=247.06, SD=21.89) feelings of anxiety.¹²

This is also supported by study done by Dr. Rabin B (2019) the mean score in experimental group was 1.73+0.81 and the mean score in control group 3.13+2.16, The ‘t’ value was 3.41 which is significant at P<0.05 level. Thus it becomes evident that GI was effective in lessening the stress level in experimental group.¹³

Hence 1H₀. There is no statistically significant effect of GI therapy on reduction of stress level of infertile female at 0.05 level of significant was rejected.

Table 3 Effectiveness of guided imagery therapy on quality of life among infertile women in experimental and control group **N=33**

Group		Mean	Std Deviation	t-test	P value
Experimental	QoL score before intervention	32.96	8.57	26.926	<0.001
	QoL score after intervention	85.00	6.25		
Control	QoL score at Day-1	22.63	4.96	1.67	0.105
	QoL score at Day-7	22.18	5.51		

As per Table 3 findings related to effect of GI on QoL there was statistically considerable variation found in experimental group pre test mean score is 32.96 which increased to become 85.00 on 7th day after intervention. In control group mean score is 22.63 which decreased minor 22.18. In experimental group p test was 26.926 with p value was <0.001 while in control group it was 1.67 with p value was 0.105.

A Study finding also supported study conducted by RahmaniFard T, Kalantarkousheh M, et al. (2016) Pre-test mean and SD of control group was 67.9 and 5.9617 and in study group was 68.4 and 6.688. In post test it's observed that mean and standard deviation of study group QoL level was found to be enhanced distinctly in 3 months, mean and standard deviation was 97.43 and 7.7446. Post test t value 16.5108 was originate to be considerable at $p < 0.05$ intensity. Studies outcomes suggest the guided imagery program significantly improved quality of life of infertile women.¹⁴

Hence $2H_0$. There is no statistically significant effect of GI therapy on QoL of infertile women at 0.05 point of significant was rejected.

Fisher's chi square test was used to find out the association between pre-intervention levels of infertility related stress & QOL among infertile female of both experimental & control group with selected

socio-demographical variable. The all p value is more than 0.05 level of considerable, which suggest that there was no association found with selected socio-demographic variable with 7th day of stress level & quality of life score.

Conclusion

The present study was conducted that guided imagery technique is effective in reducing stress level and improves quality of life. So similar study can be performing on larger scale & also the intervention days can increase to make it generalized. If after the intervention stress level still persist then investigator will be referred the participants to counselor.

Ethical Clearance: Ethical consideration was taken from Institutional ethics Committee- IEC

CHARUSAT, Charotar University of Science and Technology, Changa

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Meta Study of the Relationship between Knowledge and Incentives with Posyandu Cadre Performance

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Abstract

Background: Posyandu cadre performance refers to the role of cadres in posyandu activities as in the Posyandu cadre handbook, explaining the role of posyandu cadres is divided into three stages, namely before posyandu opening days, posyandu opening days and after posyandu opening days. Many studies report different results on the performance of health cadres related to knowledge and incentives. The purpose of this paper is to analyze the relationship between knowledge and incentives with the performance of posyandu cadres.

Method: The research method used is meta-analysis to assess how big the effect of the knowledge and incentive variables will be on the performance of posyandu cadres.

Result: Based on the results of the study of research articles, the combined P-value of 0,000 on the knowledge variable, and the combined p-value of 0,039 on the incentive variable.

Conclusion: There is a relationship between knowledge and incentive variables on the performance of posyandu cadres.

Keywords: Knowledge, incentives, performance of posyandu cadres.

Abstract

Background: According to the Indonesian Ministry of Health¹ (2012), The Posyandu cadre handbook states that Posyandu is a form of Community Based Health Efforts which is managed from, by, for and with the community, in order to empower the community and provide convenience to the community in obtaining basic health services.

Posyandu has 5 priority programs, namely maternal and child health, family planning, immunization, nutrition, and prevention and control of diarrhea. Until 2014, there were 55.517 poskesdes operating

and 289.635 posyandu in Indonesia. According to data compiled by the Central Kalimantan Provincial Health Office, the number of posyandu spread across Central Kalimantan province in 2017 was 2.645 posyandu with active coverage of 46,02% in 2019 and 34,08% in 2020.²

Posyandu cadres are community members appointed by the community to work for the community voluntarily, to carry out activities related to simple health services at the posyandu. Most posyandu cadres are married women and PKK members aged 20-40 years with a minimum education of elementary

school.¹ Based on the Minister of Home Affairs Regulation number 19 of 2011, posyandu cadres are community members who are willing, able and have the time to organize posyandu activities voluntarily.

The performance of posyandu cadres refers to the role of cadres in posyandu activities as in the posyandu cadre handbook which explains the role of posyandu cadres which is divided into three stages, namely before the opening day of posyandu such as preparing for the implementation of posyandu activities, during posyandu opening days such as registering including registration of toddlers, pregnant women, postpartum mothers, breastfeeding mothers, and other targets and after the opening day of the posyandu such as making home visits to toddlers who are not present on the day of opening the posyandu, malnourished children, or children who are malnourished outpatients, and so on.¹

Research that shows the results of a correlation between knowledge and cadre performance is Doda³ (2014) shows that there is a significant relationship between knowledge and the performance of posyandu cadres, Keswara (2014) with a p value of 0,000, and Sari⁴ (2017) with a p value of 0,001. Other studies that show that there is no relationship between knowledge and performance of cadres are Fretty⁵ (2020) p-value 0,550, Rinayati⁶ (2020) with p-value 0,468.

Similar research collected discusses knowledge and incentives affecting the performance of posyandu cadres. Several studies that were collected gave different results, where there was research that gave the results of knowledge and incentives that were not related to the performance of posyandu cadres while other studies showed that there was a relationship between knowledge and incentives on the performance of posyandu cadres. Many studies report different results on the performance of health cadres related to knowledge and incentives, which is the reason for reviewing these studies. One method that combines the results of research data quantitatively is to use meta-analysis. Based on this, meta-analysis can be used to explain heterogeneity in similar studies that discuss knowledge and incentives affecting the performance of posyandu cadres.

Method

This type of research is a literature review with a meta-analysis to assess how big the effect of the knowledge and incentive variables will be on the performance of posyandu cadres. The method used is a high-quality systematic review described as the most reliable source of evidence to guide clinical practice. The purpose of the systematic review carried out is to provide a careful summary of all the main research available in response to the research question.

Result and Discussion

Table 1. Effect Size of Combined Research Relationship between Knowledge and Performance of Posyandu Cadres

Effect Size and 95% CI					Significant Test	
Model	Number of Research	Combined Effect	Lower limit	Upper limit	Z	P
Random	10	2,786	1,674	4,638	3,941	0,000

From the 10 studies that resulted in a p-value of $0,000 < 0,05$, it was concluded that there was a tendency of a relationship between knowledge and performance of posyandu cadres and the combined ES of knowledge OR with posyandu cadre performance of 2,491 with a wide 95% CI lower limit of 1,842. So the results of the statistical test show a fairly high effect.

The results of this study are in accordance with research by Muzakkir⁷ (2013) which examined the factors related to the performance of posyandu

cadres. The results of the study stated that there was a relationship between knowledge and the performance of posyandu cadres. Research by Ilham et al.⁸ (2013) on the relationship between knowledge, training and motivation of cadres with the performance of posyandu cadres, stated that there was a relationship between knowledge, training, and motivation of cadres with the performance of posyandu cadres. In addition to the knowledge of posyandu cadres, other factors such as motivation, incentives, and education of posyandu cadres can also affect the performance of posyandu cadres.^{7,8}

Table 2. Effect Size of Combined Incentive Relationship Research on Posyandu Cadre Performance

Effect Size and 95% CI					Significant Test	
Model	Number of Research	Combined Effect	Lower limit	Upper limit	Z	P
Random	6	4,670	1,062	20-149	2,066	0,039

The analysis of the 6 (six) studies resulted in a p-value of $0,039 < 0,05$, it was concluded that incentives have a tendency to be related to the performance of posyandu cadres and ES combined from OR on incentives with posyandu cadres' performance of 4,670 with a wide CI upper and lower limits 1,082-20,149. So the results of the statistical test show a very high effect.

Based on the results of the study, there were 4 (four) research journals or 66,66% stated that incentives were related to the performance of posyandu cadres while 2 (two) research journals or 33,33% stated that there was no relationship between incentives and posyandu cadres performance.

This research is also in line with Ponto's research, (2020) the results of his research stated that there were 62 respondents who gave good and active incentives as many as 34 respondents (54,8%), good

and less active got 16 respondents (25,8%), good and inactive got 0 respondents (0%), providing sufficient incentives and active got 0 respondents, moderate and less active 8 respondents (12,9%), moderate and inactive got 4 respondents (6,5%), while incentives were less and active 0 respondents (0%), moderate and less active 0 respondents (0%), less and inactive get 0 respondents (0%) with p-value $0,271 > 0,05$. Other research conducted by Silviyani⁹ (2015) with the title of the study of factors related to the performance of elderly posyandu cadres in the Miroto Health Center Semarang area where the incentive factor did not have a significant relationship with the performance of cadres in posyandu statistical results obtained p-value $0,16 > 0,05$.

Conclusion

The results of the meta-analysis show that knowledge and incentives have a relationship with the

performance of posyandu cadres. Recommendations based on the results of this study are to hold tiered and varied short courses for cadres as an effort to in-depth knowledge about improving nutrition and food security, maternal and child health and environmental health as well as providing incentives to posyandu cadres on an ongoing basis, in the form of providing transportation costs for cadres, health insurance, and food assistance.

Ethical Clearance: There is no ethical test because it uses the journal analysis method

Source of Funding: This research used self funding

Declaration of Conflicting Interest

The authors declared no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

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Comparing Walking age, Receptive and Expressive Language Profiles between Speech Delay Children with and without Hearing Loss

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Abstract

Objectives : The study aimed to investigate whether walking age, receptive, and expressive language profiles differ between speech delay children with hearing loss and speech delay children without hearing loss; to identify walking age, receptive, and expressive profiles between speech delay children with hearing loss and speech delay children without hearing loss.

Methods : The study is an observational analytic with retrospective cross-sectional design using medical records data for two years. Data was collected using a total sampling technique.

Results : The study involved 92 children with speech delay, consisting 72 children in the hearing loss group and 20 children in the normal hearing group. The average age at walk as gross motoric profile shows that speech delays children with hearing loss have an average age that is later than speech delays children without hearing loss. Also, walking age significantly differs between children with speech delay in hearing loss group and normal hearing group. Both receptive and expressive language profiles show no different between the groups.

Conclusions : Findings have consequences for consideration motor developmental delay in children with speech delay, especially in hearing loss group. So that, the findings can be a reference to consideration in further management basis for speech delay interventions with and without hearing loss in children.

Keywords : walking age, language, hearing, speech delay children

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Introduction

Hearing loss exists in more than 5% of the world's population and 34 million of them are children.¹ This hearing loss can affect the development of speech ability in children, causing delay in speech development children. Delays in a child's speech can be an undiagnosed early symptom of some diseases, such as autism spectrum disease, global development

delay, or loss of hearing.² In children with speech delay, there were children with hearing loss.³ In children who have difficulty hearing, the development of speech ability and hearing ability is delayed compared to normal children.⁴ A child's hearing loss can have an impact on their speech ability.

Speech delay can be found as a manifestation of another disease. In 110 children with complaints of being unable to speak, unable to form sentences, and speech delay, it was clinically found that there were 28.18% of them with delay in speech development and others, such as mental retardation, pervasive developmental disorder, and phonological disorder.⁵

Some studies have shown motor delays in hearing loss children. Hearing loss or vestibular disorders especially in children with cochlear anomalies have a history of delays in motor development.⁶ Children with hearing loss who showed vestibular dysfunction, had decreased results in motor assessment, meaning that there was a delay in motor development compared to normal hearing children.⁷ In this case, hearing loss with motor development has a connection.

The ability to speak in children comes from the combination of language, and the process of producing vibrations that can be heard or known as vocalization, supported by aneural language center and the coordination of the lip muscles, tongue muscles, jaw muscles, and the vocal tract.⁸ Muscle coordination as a child's motor ability can have an impact on their speech ability. Previous research that examined the motor profile of children who experience speech and language delays, concluded that children with impaired speech skills show problems with their motor abilities.⁹ The speech impairment category is included in the primary speech delay. However, it is categorized as a secondary speech delay when there is a comorbid factor, such as hearing loss.¹⁰ There are more than one risk factor, such as hearing loss and environmental risk factors in children, causing

children to have worse developmental delays than children who have only one risk factor. Two risk factors that are in a child simultaneously can lead to worse developmental delays.¹¹

Methods

Participants

Data sources were conducted from the medical records of the occasion clinic in the audiology clinic and installation of physical medicine and rehabilitation from 2015 to 2016 at Dr. Soetomo Hospital, Surabaya, Indonesia. The research was conducted from September 2019 to March 2020. This research is an observational research with retrospective cross-sectional design. Sampling is done by a total sampling technique and taken based on inclusion criteria, which are speech delay children, aged between 18 and 60 months with or without hearing loss. Psychiatric disorders, history of seizures, abnormalities of head size or microcephaly, hydrocephalus, and Patent Ductus Arteriosus are the exclusion criteria in this study.

The whole subject is speech delay children. Speech delay is defined as a delay in speech development or receptive or expressive language. This data is obtained from complaints, diagnoses, or results of receptive or expressive language examinations were listed in medical records. Then, the speech delay children will have their hearing examined and listed in medical records.

The study was divided speech delay children into two groups of research, which are speech delay children with hearing loss (SDHL) and speech delay children with normal hearing (SDNH) group. Hearing loss is defined as a decrease in the child's hearing ability threshold of > 25 dB, indicated from the results of a Brainstem Evoked Response Audiometry (BERA) or Behavioral Observation Audiometry (BOA) examination. Normal hearing is defined as the

threshold of a child's hearing ability < 25 dB, indicated from the results of a BERA or BOA examination, and is considered normal in speech delay children at the installation of physical medicine and rehabilitation.

Materials

Motor profile, receptive, and expressive language are variables to be tested for differences. Gross motor development data is obtained retrospectively through medical record data. Developmental delay is defined by comparing normal references. The normal reference for a child's walking age is 8.2 to 17.6 months.¹²

Expressive or receptive language profiles are presented through nominal data scales (delay or not delay). Receptive or expressive language delay is defined as delay in all or part of the developmental stages of speech or language by comparison to normal references, especially the stages of the child being able to understand simple commands and the stages of the child being able to produce single words. The normal reference for the ability to understand simple commands in children as part of the stages of receptive language development and ability to produce single words as part of the stages of language development are 12 months of age and 10 until 16 months of age respectively.¹³

Statistical Analysis

The gross motor profile was shown through a scale of ratio data and was analyzed to determine the difference between the two groups with the Mann Whitney test. While the different receptive and expressive language skills were analyzed with Fisher's exact test.

Ethical Clearance

Certificate of ethics as a research license to use the medical record data has been given for this research by the Ethics Committee of Dr. Soetomo Hospital,

Surabaya, Indonesia.

Results

By Age Group

A total of 92 children had speech delay with an age range of 18 to 60 months, consisting of 72 subjects in the SDHL group and 20 subjects in the SDNH group. Mean age of the SDHL group and SDNH group were 35.31 ± 9.25 and 33.02 ± 7.40 months respectively.

The age group differences showed statistically no difference. The group of SDNH had the highest frequency in the age range from over 24 to 36 months by 16 out of 20 (80%). The group of SDHL also had the highest frequency in the same age range by 43 out of 72 (59.7%).

By Gender

Differences between the study groups by gender showed statistically no difference. The group of SDNH showed that the boy had the highest frequency by 14 out of 20 (70%). The group of SDHL showed the same results by 36 out of 69 (52.17%).

Diagnosis

Global developmental delays were found in one child in the SDNH group. Motor delay, hyperlaxity, waste or nutrition disorder, social personal domain delay, and improved bronchopneumonia are known to be found in one child in the SDNH group.

Prenatal, Perinatal, and Postnatal History

A known history of prenatal problems was found in 4 out of 20 children in the group of SDNH, and 24 out of 72 children in the group of SDHL (see table 1). The difference in the number of children who had a history of prenatal problems and children who had no history of prenatal problems between the study groups showed statistically no difference.

History of known perinatal problems is found in 14 out of 20 (70%) in the group of SDNH and were found in 41 out of 72 (56.9%) in the group of SDHL (see table 1). The difference in the number of children who had a history of perinatal problems and children who had no history of perinatal problems between the two study groups showed statistically no difference.

History of known postnatal problems were found in 10 out of 20 (50%) in the group of SDNH and in 41 out of 72 (56.9%) in the group of SDHL. Differences in the number of children who had a history of postnatal problems and children who had no history of postnatal problems in the two study groups showed statistically no difference (see table 1).

Table 1 Prenatal, Perinatal, and Postnatal History

History		N (%)		p value
		SDNH (n=20)	SDHL (n=72)	
Prenatal	No	15 (75%)	47 (65.3%)	0.286*
	Yes ^a	4 (20%)	24 (33.3%)	
	unknown data	1 (5%)	1 (1.4%)	
Perinatal	No	6 (30%)	31 (43.1%)	0.292*
	Yes ^b	14 (70%)	41 (56.9%)	
Postnatal	No	10 (50%)	31 (43.1%)	0.580*
	Yes ^c	10 (50%)	41 (56.9%)	

*comparative test with Chi Square test, without using unknown data: $p > 0.05$

^a SDNH group: Hypertension, preeclampsia, and diabetes mellitus; SDHL : Hypertension, Take Herbal Medicine, Measles, Take Medicine, History of Bleeding, History of Falls during pregnancy, Hyperemesis Gravidarum, Asthma, Hypotension, Pregnancy Induction Disorder, Typhoid, Hypercholesterolemia, Maternal Age >35 y

^b SDNH: preterm, caesarean, history of not crying immediately after birth, asphyxia, icterus, oxygen delivery, low birth weight baby; SDHL: preterm, postterm, spontaneous birth delivery with forceps or vacuum, caesarean, history of not crying immediately after birth, asphyxia, icterus, cyanosis, oxygen

delivery, low and very low birth weight baby.

^c SDNH: infection history, asthma, trauma, Neonatal Intensive Care Unit entry history, craniofacial anomalies, take medicine, allergic history, and heart disease; SDHL: infection history, asthma, craniofacial anomalies, take a medicine, trauma, allergic history, Neonatal Intensive Care Unit entry history, eyes disorder, surgery history, enlarged lymph nodes, and icterus.

Walking age in SDNH and SDHL groups

The difference in the walking age between the study groups showed statistically significant

differences (see table 2). Mean age of the walking age in SDHL group shows later than SDNH group.

Table 2 Comparative Test of Motor Profile

Variable	Mean (SD)		p-value
	SDNH (N=20)	SDHL (N=72)	
Walking Age	13.75 (±5.46)	17.24 (±5.14)	0.019*

* Comparative test using the Mann Whitney test: p<0.05

Receptive and Expressive in SDNH and SDHL groups

Differences in receptive language in the two study groups showed statistically no difference. Same results can be found in expressive language. See table 3.

Table 3 Comparative Test of Expressive and Receptive Language

Variables		N (%)		p-value
		SDNH	SDHL	
Receptive ^a	Normal	3 (23.1%)	16 (22.5%)	0.607*
	Delay	10 (76.9%)	55 (77.5%)	
Expressive ^b	Normal	1 (5%)	1 (1.4%)	0.389*
	Delay	19 (95%)	71 (98.6%)	

*Comparative Test using Fisher’s Exact Test: p>0.05

^aUnknown data were excluded so that the SDNH group consisted of 13 children and the SDHL group consisted of 71 children.

^bThe SDNH group was 20 children and the SDHL group was 7

SDHL.

From the results of this study, it is known that the frequency of boys is more than the frequency of girls in both groups: SDNH and SDHL. This result is similar to a study in the age range of 1-7 years with a mean age of 3.1 y,⁵ children with hearing, speech, or language problems,¹⁴ and children with communication, language, or speech disorders[Adani, 2019]. Other studies have also found that male gender is one of the risk factors for delay in 24-month-olds.¹⁶ There are sex hormone factors such as estrogen and testosterone, and genetic factors namely FOXP2 that

Discussion

A study of children with a diagnosis in one of the areas of hearing, speech, or language, is found the highest frequency in the age group 2-5 y.¹⁴In this study, the age range from more than 24 to 36 months had the highest frequency in both groups: SDNH and

causes differences in language development, speech, and communication between boys and girls. In estrogen hormones, there is a characteristic tendency to social and verbal abilities, and development in the language center. In genetic factors, FOXP2 has an influence on language development and is known to decrease in boys compared to girls.¹⁵

Walking Age Differences between the groups

The study of differences in motor profiles in SDNH and SDHL was done on the basis of previous research that among speech delay children were delayed in motor values,⁹ and hearing loss has an influence on the motor aspect, namely postural and balance.⁷ A study in the literature showed significant differences in the motor aspects of the 48 until 71 months of age group in two groups, children with hearing loss and children with normal hearing.¹⁷ Meanwhile, in other literature, a study showed insignificant results in the degree of hearing loss as a factor in the motor development of 3-year-olds.¹⁸ And in this study, motor differences in the SDNH and SDHL group showed differences. These results were supported by a systematic research review showing that motor profiles between children with normal hearing and those with hearing loss resulted in significant differences, especially in blindfold balance tests and one foot standing, and motor development was not achieved optimally especially in children with hearing loss.¹⁹

Children's motor development has a role in the development of their language. This is because children's exploration abilities differ in several stages of motor development and this exploration ability influences language development. Children can explore the surrounding environment, such as knowing the name of objects or knowing spatial language (above, under, or besides) on their own without the help of others as they enter the motor development stage of walking without assistance,²⁰ even before that, when children are able to sit on their

own, language skills can be explored further from the surrounding environment compared to when they could not sit by themselves.²¹

Receptive and Expressive Language Profile Differences between the groups

The results showed no difference in receptive and expressive language in SDNH and SDHL groups. This is different to other studies that compared receptive and expressive abilities between children with specific language impairment and children with hearing loss. The results showed that there is no better receptive language ability in children with hearing loss compared to children with language impairment, however, in expressive language ability there was no association with the degree of hearing loss.²² The same results were also shown in linguistic profile research in children with hearing loss and children with specific language impairment, which showed that there were differences in test scores used as linguistic profiles.²³ Children with hearing loss may show decreased language or speech skills when compared to healthy children.²⁴

Limitation in this study is the current research conducted has categorized the expressive language and receptive language only by late and not late without knowing more specifically the location of subsections of the stages of development of receptive or expressive language that have not been achieved by each child so that it may produce a clearer result between SDHL and SDNH groups.

In summary, there are no differences in language profiles between the groups. However, motor development between the groups was found to be different. It is important for health workers and parents to pay more attention to the impact of other developments, such as motor development in children with speech delays, especially those with hearing loss.

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Disclosure of Interest

The authors report no conflicts of interest, and no relevant financial and non-financial competing interest for this study. The authors are responsible for the content and writing of this article.

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Association of Plasma Interferon- α (IFN- α) with C-Reactive Protein (CRP) Level and Disease Activity in Systemic Lupus Erythematosus (SLE) Patients

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Abstract

Background: CRP is normal or only slightly increased in active SLE. It is presumed that IFN- α may inhibit the transcription process during CRP synthesis. There is also increasing in IFN- α -gene expression in active SLE. This study examined the correlation of plasma IFN- α with CRP and SLE activity.

Methods:Forty SLE patients were included. SLAM and SLEDAI were used to measure SLE disease activity. Laboratory tests were examined at dr. Soetomo Hospital Surabaya. CRP was measured using immunoturbidimetry. C3 and C4 were measured by radial immunodiffusion technique. IFN- α was measured using ELISA.

Results and conclusion:Twenty-six patients from the outpatient clinic and 14 from wards were included from August 2019 to February 2020. The median age was 31.5 years old. The median SLAM score was 8.5. Mean CRP was 5.19 ± 2.69 mg/L. Median plasma IFN- α was 46.02 (16.43-177.96). Spearman correlation test revealed a moderate negative correlation between plasma IFN- α and CRP level ($p=0.003$; $r=-0.455$). A moderate positive correlation was showed between plasma IFN- α level and SLAM score ($p=0.001$; $r=0.568$). No correlation found between CRP and SLAM. There was a strong correlation between complement levels with SLEDAI. Linear regression revealed a significant association of IFN- α and C3 (not C4) level with SLEDAI.

Keywords: *interferon- α , CRP, systemic lupus erythematosus, disease activity*

Introduction

Interferon- α (IFN- α) is a pleiotropic cytokine that can influence various cells involved in systemic lupus erythematosus (SLE) pathogenesis. A high level of IFN- α can directly cause immune dysregulation in SLE from various pathways. IFN- α may activate T-cell and B-cell, hinder Treg, and increase toll-like receptor (TLR) signaling¹⁻³. C-reactive protein (CRP), as one of the acute-phase reactant, will increase

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fast during inflammation. Unlike other autoimmune diseases, active SLE patients generally have normal serum CRP level despite high disease activity unless there is bacterial infection⁴⁻⁶.

There are three hypotheses that explain unresponsiveness of CRP in SLE, i.e.: (1) genetic variation from CRP gene, (2) antibody anti-CRP existence, (3) interferon- α (IFN α) that can decrease CRP secretion by hepatocytes. From studies within the various population, CRP gene polymorphisms have shown various results regarding their relationship with serum CRP level^{5,7}. Antibody anti-CRP is often found in SLE, but its relationship with CRP level is not clear. It is said that anti-CRP correlates with SLE disease activity but does not correlate with CRP level. When produced from the liver, CRP form is pentamer (native-CRP). During special condition like inflammation, this native pentameric CRP will dissociate into monomeric CRP (mCRP) that is more functional biologically. Antibody anti-CRP measured from ELISA is the antibody to mCRP, while the plasma CRP level measured is native pentameric CRP^{5,8,9,10}.

Another theory that may affect CRP level in SLE is IFN- α . Studies show that despite a high level of IL-6 and wide systemic inflammation during SLE flare and viral infection, the plasma CRP level is still normal. There is an increasing level of IFN- α either in SLE or viral infection, so it is assumed that IFN- α may decrease CRP production by hepatocytes^{3,5}. This study objective was to analyze the association between IFN- α level with plasma CRP level and SLE patients' disease activity.

Patients and Methods

Subjects

Since there was no previous study using a correlation test to analyze the direct correlation between plasma IFN- α level and CRP level, we use

$r=0.5$ to calculate the sample size. Hence, the minimal subject was 38, and we agreed to use 40 SLE patients for this study cumulatively. All patients came to either the rheumatology outpatient clinic for routine control or admitted to the hospital ward due to SLE flare. Diagnosis of SLE was made using ACR classification criteria for SLE 2019¹¹. Demographic data and clinical manifestation were obtained from history taking, physical examination, and medical record. SLAM (Systemic Lupus Activity Measurement) and SLEDAI (SLE Disease Activity Index) were used to measure the SLE disease activity score.

Laboratory examination

Blood samples were taken from the subject to check CBC, ESR, CRP, complement C3, and C4 level. All of the examinations were done at Clinical Pathology Laboratory at dr. Soetomo Academic General Hospital Surabaya. CRP level was measured using the immunoturbidimetry method with an upper normal limit level was 5 mg/L. Complement C3 and C4 level were measured using radial immunodiffusion technique, and the normal limit for C3 was 90-180 mg/dL and for C4 was 9-36 mg/dL. Plasma samples were stored at -800C to measure IFN- α level until ELISA was performed. ELISA kit was Human IFN alpha ELISA Kit Invitrogen BMS216/BMS216TEN. Upon completing a sample assay using the kit protocol, absorbance was determined at 450 nm on Microplate reader: iMark (BioRad).

Statistical Analysis

We used SPSS.21 for data analysis. First, we tested the data distribution. If homogenous, we used Pearson correlation, and if not, the Spearman correlation test was used to analyze the association between IFN- α and CRP level and disease activity score. Then multivariate analysis using linear regression was done to analyze the association of some independent variables (IFN- α , C3, C4) with SLE disease activity.

Results

This study had received approval from the Ethics Committee of dr. Soetomo Academic General Hospital with Ethical Clearance Number 1014/KEPK/III/2019 dated March 8, 2019.

Forty SLE patients from the rheumatology outpatient clinic (26 patients) and those admitted to the

internal medicine ward (14 patients) were included in this study from August 2019–February 2020. Diagnosis of SLE was made based on SLE classification criteria from ACR 2019 (Fanouriakis, 2020). All patients were women with a median age was 31.5 years old (range 18-59). The clinical characteristic of the subjects is shown in Table 1.

Table 1. Clinical characteristics of subjects

Clinical manifestation	Median	Number (%)
Duration of illness (months)	23	
Body mass index	23.19	
Underweight		4 (10)
Normal		22 (55)
Overweight		14 (35)
Skin and mucosa manifestation		
Malar rash		9 (22,5)
Discoid rash		6 (15)
Oral ulcer		4 (10)
Hair loss		17 (42.5)
Photosensitivity		1 (2.5)
Musculoskeletal manifestation		
Arthritis		22(55)
Muscle pain		2 (5)
Fatigue		2 (5)
Panniculitis		2 (5)
Lung manifestation		2 (5)
Heart manifestation		
Heart failure		2 (5)
Hypertension		2 (5)
Serositis		5 (12.5)
Nephritis		13 (32.5)
Neuropsychiatric		2 (5)
SLAM score	8.5(0-26)	
Mild (<7)		16 (40)
Moderate (7-20)		21 (52.5)
Severe (20)		3 (7.5)
SLEDAI	2 (0-28)	
Mild (1-5)		28 (70)
Moderate (6-10)		3 (7.5)
Severe (>10)		9 (22.5)

Laboratory result of the patients is revealed in Table 2.

Table 2. Laboratory characteristic of subjects

Laboratory examination	Mean \pm SD	Median	Number (%)
Positive anti ds-DNA			13 (32.5)
Hematology			
Hb (g/dl)			
Anemia			
Leukocytes ($\times 10^3$ /ml)	10.78 \pm 2.92		22 (55%)
Leukopenia			
Lymphocyte	8.92 \pm 4.68	1715	5 (12.5)
Lymphopenia	252,150 \pm 144,462		17 (42.5)
Thrombocyte			11 (27.5)
Thrombocytopenia			
Serum complement			
C3			
low (<90 mg/dL)	88.41 \pm 44.28		16 (40)
C4	20.76 \pm 12.22		7 (17.5)
low (<9 mg/dL)			
ESR (mm/h)		24 (6-155)	
High (>20mm/h)			26 (65)
C-reactive protein (mg/L)	5.19 \pm 2.69 (0.5-9.8)		
IFN- α (pg/ml)		46.02(16.43-177.96)	

Shapiro-Wilk test showed $p < 0.05$ for IFN- α level, so we used the Spearman correlation test. It revealed a significant association between plasma IFN- α and CRP level ($p = 0.003$ and $r = -0.455$ (moderate negative correlation)). Spearman test also showed a moderate positive correlation between plasma IFN- α level and SLE disease activity (SLAM score), with $p = 0.001$ and $r = 0.568$ and with SLEDAI $p = 0.004$ and $r = 0.440$. There was no significant association between CRP

level and SLE disease activity ($p = 0.903$).

Many studies have shown the importance of complement C3 and C4 in SLE, and they have already used as biomarkers in SLE activity. In this study, there was also a strong correlation between serum C3 and C4 level with SLEDAI score (both had $p = 0.001$ and $r = -0.735$ for C3 and -0.773 for C4). Multivariate analysis was done to analyze the association of serum

IFN-a, C3, and C4 level with SLEDAI score. Linear regression revealed a significant association of IFN-a and C3 level with SLEDAI score, but not for C4.

Normal P-P Plot of Regression Standardized Residual for SLEDAI score is shown in Figure 1. The complete multivariate analysis is seen in Table 3.

Table 3. Multivariate analysis of this study

Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.	Collinearity Statistics	
		B	Std. Error	Beta			Tolerance	VIF
1	(Constant)	14.732	2.638		5.585	.000		
	IFN-a	.039	.019	.220	2.052	.048	.899	1.112
	C3	-.118	.027	-.645	-4.369	.000	.472	2.118
	C4	-.046	.097	-.070	-.478	.635	.479	2.086
2	(Constant)	14.500	2.565		5.652	.000		
	IFN-a	.039	.019	.224	2.128	.040	.907	1.102
	C3	-.127	.019	-.694	-6.584	.000	.907	1.102
a. Dependent Variable: sledai								

Mathematic model for this linear regression was $SLEDAI = 14.5 + 0.224 \text{ IFN-a} - 0.694 \text{ C3}$.

Discussion

Hepatocytes synthesize CRP molecule as a response to IL-6 during inflammation. In viral infection and SLE flare, plasma CRP is normal though there is increasing in IL-6. It is caused by abundant IFN-a during SLE flare and viral infection. All subtypes of IFN- α can inhibit CRP promoter gene activity. This inhibition depends on the dose and mediated by type-I IFN receptors. The IFN-a-dependent inhibition

of CRP promoter activity was confirmed by studies of CRP secretion in primary human hepatocytes. IL-1 β -induced CRP secretion was inhibited by 49.2%, and IL-6-induced secretion was inhibited by 51.5%, whereas the inhibition induced by IL-1 β plus IL-6 was moderate (21.1%). After preincubation of IFN- α for 6 hours, there is suppression of promoter activity despite stimulation of IL-6 and IL-1 β . CRP itself also may inhibit the production of IFN- α by pDC induced

by the immune complex. There is no study directly compares serum IFN- α and CRP level. A study by Enocsson et al. above showed inhibition of the transcription process in CRP promoter during CRP synthesis by hepatocytes. This is one of the reasons why CRP level is normal in SLE flare ^{7,10}.

In a longitudinal study, it is reported that there is increasing of IFN- α in active SLE patient serum. Plasma IFN- α level also correlates with SLEDAI score. There is also increasing IFN- α gene expression in active SLE patients, and there is a significant difference in SLEDAI scores between patients with high and low plasma IFN- α level ($p=0,0038$). Direct measurement of plasma IFN- α has shown more accurate and specific than IFN- α signature ^{12,13,14}. All type I IFN will increase during SLE flare related to higher disease activity. IFN signature gene expression is also high in severe organ disturbance in lupus-like nephritis or neuropsychiatry ^{15,16}. Our study also showed a moderate positive correlation between IFN- α and disease activity in SLE patients. Also in linear regression, it showed a positive correlation and complement C3.

Conclusion

A moderate positive correlation was showed between plasma IFN- α level and SLAM score ($p=0.001$; $r=0.568$). No correlation found between CRP and SLAM. There was a strong correlation between complement levels with SLEDAI. Linear regression revealed a significant association of IFN- α and C3 (not C4) level with SLEDAI.

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Assessment of Factors Influencing Uptake of National Programme on Immunization among People in Chanchaga L.G.A., Niger State, Nigeria

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Abstract

This study assessed the Factors influencing uptake of national immunization programme among people in Chanchaga L.G.A., Niger state. Immunization has brought sound health to many children in the world, reduced the agony experienced by parents during child rearing and reduced the mortality rate among children. The purpose of this study was to examine cultural belief, fear of parents, religion, level of service and uptake of national immunization programme among people in Chanchaga L.G.A., Niger state.

Descriptive research design of survey type was adopted for the study. The population comprised of all people in Chanchaga L.G.A., Niger state. A multistage sampling technique which consist of simple random sampling technique, purposive and convenience sampling technique was used to select 384 respondents for this study. Questionnaire was validated by three experts in the Department of Health Promotion and Environmental Health Education for data collection from the respondents. A reliability coefficient (r) of 0.76 was obtained through split half method using Spearman Brown for analyzing data generated. The inferential statistics of Chi-square was used to analyze the data collected for the postulated null hypothesis at 0.05 alpha level.

The findings revealed that:

1. Cultural belief of people is a significant factor influencing the uptake of National Immunization Programme among people of Chanchaga Local Government Area, Niger State because the calculated value 271.20 is greater than the table value 21.3
2. Fear of parents about immunization is a significant factor influencing the uptake of National Immunization Programme among people of Chanchaga Local Government Area, Niger State because the calculated value 175.76 is greater than the table value 21.3
3. level of coverage of service of people will significantly be a factor influencing the uptake of National Immunization Programme among people of Chanchaga Local Government Area, Niger State because the calculated chi-square value 247.09 is greater than critical table value of 21.03 (Cal χ^2 val > Tab χ^2 val)

The study concluded that cultural belief, fear of parents, religious belief and level of service are factors influencing the uptake of National Immunization Programme among people of Chanchaga Local Government Area, Niger State. It was therefore recommended among others that there should be

sensitization programme by health workers to community leaders and indigenes in villages in order to publicize the benefits of immunization, parents should be well informed by health workers or through media before the start of immunization programmes to reduce the fear about immunization among others.

Keywords: *Assessment, Factors, Uptake, National, Programme, Immunization*

Introduction

Immunization is an effective public health intervention to reduce morbidity and mortality among infants. It is an important means of controlling diseases, and has been considered the most cost-effective health intervention programme¹. Immunization has brought sound health to many children in the world, reduced the agony experienced by parents during child rearing and reduced the mortality rate among children². The use of immunization services however requires acceptability from the target community. This means that for immunization services to be generally accepted, there must be a clear understanding of the benefits of vaccination among community members, a readiness for providing vaccination by the health services, and intervention to overcome barriers to immunization services³.

The Increasing uptake of immunization and coverage for childhood diseases has become an important developmental issue and an area that requires more research⁴. Based on World Health Organisation/ United Nations Children's Emergency Fund (2008) report, there have been considerable increase in the global immunization coverage. The report shows that an infant under age 0-1year immunized with DPT increased from 20% in 1980 to 79% in 2006⁵. It was further revealed that percentage of children immunized with three doses of polio vaccine in 2006 rose from 22% in 1980 to 80%. Global coverage for measles increased from 16% in 1980 to 80% in 2006. However, these increasing coverage are still falling short of the 2010 target of 90% set by WHO/UNICEF Global Immunization Vision and Strategy. It has been

argued that further increases in coverage of DPT, Polio and Measles would save millions of infant lives. The current immunization coverage in Africa is 70%, the acceptable minimum coverage of 80% is yet to be reached.

The Inadequacies in immunization coverage are due to a variety of variables, including health workers' degree of sensitization and political leaders' unwillingness to mobilize and support immunization services⁶. Other factors that influence the coverage of immunization programmes are low parent acceptability in terms of expected benefits, social mobilization of various elements of society for a common developmental goal, insufficient community participation due to lack of awareness, distance from the health facility, place of delivery, migration of families, mothers knowledge and attitudes towards immunization, weather conditions and low literacy levels of the parents⁷. Social mobilization of various elements of society for a common developmental goal can overcome long odds and reach goals hitherto thought unattainable in a limited time-frame. The emphasis on attaining universal coverage of immunization programmes had helped to induce improved programme management and there was a noticeable increase in equitable access⁸. However, sustained immunization coverage especially at the peripheral levels - can be challenging as it significantly increases the costs, and requires improvements in staffing, financing and guidelines, as well as in the ability to procure a constant vaccine supply⁹.

One possible explanation for the failure to improve access to the immunization system may be related to the socio-cultural acceptability of some of the new vaccines¹⁰. For example HPV vaccines – may be associated with Sexually Transmitted Infections [STIs] and therefore can encourage stigmatization. It has been found that the introduction of HPV led to media messages that adversely affected HBV uptake. Similarly, messages need to be more pertinent to the situations of migrant and ethnic minorities¹¹. Opposition from socially conservative groups and ethical considerations have been found to negatively affect the social acceptability of new vaccines, indeed, it has been found that an increase in uptake sometimes required changes in the types of messages provided¹².

If the training programmes are not accompanied by necessary adjustments to the human resource frameworks and career paths, or to adequate remuneration arrangements¹³. In some instances especially where the levels of financial involvement and commitment by the national governments have been very limited, it may not be possible to sustain the necessary level of effort - with negative outcomes for the longer term sustainability of the immunization programme. A study on health infrastructure and immunization coverage of 43,416 children aged 2-35 months residing in rural India was conducted¹⁵. The researchers found out that the availability of health infrastructure significantly improved immunization coverage for non-Polio vaccines. The study further revealed that larger and better equipped facilities such as hospitals and health centres had bigger effects on immunization coverage including the nature of health infrastructure i.e. hospitals and health centres play an important role in increasing immunization coverage.

Parental or caregiver's factor such as knowledge is another factor which influence the immunization status of the child. These include knowledge and attitude toward vaccination and vaccine preventable

disease. Study done in Nigeria on determinants of immunization status children in rural areas showed that mothers with high knowledge level score have fully immunize their children. Also more than half of mothers can correctly call the symptoms of vaccine preventable disease. And 99% of the mothers felt immunization is good for the child¹⁵. Health facility is another factor which contributed to full immunization of the child. Different studies showed the importance of availability and accessibility of health facility in immunization coverage. Families nearer to the health facility are more likely to complete the immunization than those far from it. In a cross sectional study done in India, Assam district showed that immunization status of the children was significantly higher where the distance of the health centre was less than 2km compared with those residing in remote inaccessible areas with a distance of more than 5km to the health centre¹⁶.

Maternal characteristics is one all known determinants of uptake National Programme immunization. A comparative study done among slum and non-slum dwellers in Bangladesh shows that children age below 2 years in three zone of Dhaka demonstrated that complete immunization coverage is associated with educational status of the mother, income and living conditions. The study revealed that mothers with lowest education, households with limited monthly income and people living in slum area were less likely to complete a child immunization¹⁷. The report of the study also indicated that children whose mothers were born in a rural area or an urban slum, and those whose mothers were aged less than 30 years are 0.35 and 0.43 times less likely to be fully immunized respectively¹⁷. The study outcome revealed that poor uptake of immunization in urban areas was associated with lack of mother's awareness about repeat visits to achieve complete immunization rather than overall vaccine awareness. Furthermore, anti-vaccine rumours such as pathogenicity of a

vaccine and propaganda of vaccines weakening their children which were encountered in the community, affected immunization coverage. Negative perception about vaccination and antivaccinerumours in some communities were found to affect the level of immunization coverage. Mis-information about the side effects of vaccine during illness and false contraindications also contributed to the level of immunization coverage.

The report of 2007 found out that immunization coverage was 95% for BCG, 82% for DPT, 81% for Polio and 77% for Measles. The study revealed that immunization coverage was due to knowledge of immunization, attitudinal beliefs and social influence of the mothers and fathers. The mothers and fathers believed that routine immunization were well conceived and meant to eradicate childhood diseases. In terms of social influence, the study revealed that while it was the woman who decides the issue of routine immunization, the man was regarded as the one who makes the very important decision not to immunize in exceptional situation when immunization strengthen endication of diseases¹⁸.

A study that was meant to assess the immunization coverage of BCG, DPT, OPV and Measles, and factors affecting the coverage in 693 children aged 24 to 47 Months in two urban villages of East Delhi was carried out¹⁹. The authors revealed that immunization coverage was: 82.7% for BCG, 81.5% for DPT1/OPV1, 76.8% for DPT2/OPV2, 70.7% for DPT3/OPV3 and 65.3% for Measles vaccine. The coverage levels were associated with education of mothers and fathers, father's occupation, residential status and place of delivery. A survey to describe the immunization coverage in a rural part of North India was undertaken²⁰. The study sampled 747 children and the results revealed that 94.8 % eligible children were immunized and had received the required doses of the primary schedule vaccines. The coverage was

(BCG (94.8%), OPV/DPT (91.6%), and Measles (72.6%). Only 39 (5.2%) of the eligible children had not completed immunization schedule for BCG, DPT, Polio and Measles due to temporary or permanent migration of the children or family to the village or went back to the parents' home or divorce or the child was adopted by relative.

Scientists conducted a study to explore factors influencing urban and rural immunization coverage in 220 households with children 12-13 months of age in Ethiopia. The authors revealed that higher community awareness was associated with effective community mobilization for immunization. The study also found out that immunization service for DPT, Polio and Measles in these areas were 97.3% for DPT1/OPV1, 92.7% for DPT3/OPV3 and 75.5% respectively and the reason for this high coverage was that mothers were literate²¹.

Statement of the Problem

It is a known fact that prevention is better and cheaper than cure. The pattern of illness and diseases outbreak have made initiation of preventive and control measures inevitable in Chanchaga Local Government Area, Niger State. The researchers observed that despite government efforts towards achieving hundred percent coverage rate, the acceptance rate is too low in the Local Government Area of the state. There are many reasons responsible for the poor uptake of National Programme on immunization in Chanchaga Local Government Area, Niger State. The researchers observed that one of the factors responsible for poor uptake of the programme is strict adherence to cultural norms and values of the community. The people of Chanchaga Local Government Area have the erroneous belief that God is the utmost healer and has given them natural healing herbs and concoctions. This binding force has always reflect on their decision on issues relating to intervention programmes such as immunization programme, distribution of free

preventive drugs, free medical services and so on.

The researchers also observed from the field experiences that majority of immunization defaulters mostly nursing mothers among people of Chanchaga Local Government Area, Niger State have the fear that the vaccine given to them and the children often affect their physiological and psychological wellbeing. The parents fear that the vaccine are sterile induce drugs aim at weakening the reproductive health efficiency of the people. This fear by the parents, the researchers noted as one of the reasons for low services coverage.

The quality of immunization services rendered in some of the designated health centres are inadequate and low when compared with the target population. The poor service provision by the health workers account for high rate of immunization defaulter and non-acceptance of National Immunization Programme. In line with the observations above, this study was carried out to assess factors influencing uptake of National Programme on Immunization among people of Chanchaga Local Government Area, Niger State.

Research Questions

The following research questions were answered in this study:

1. Will cultural belief of people influence the uptake of national programme on immunization among people of Chanchaga Local Government Area, Niger State, Nigeria
2. Will Fear of parents about immunization influence the uptake of national programme on immunization among people of Chanchaga Local Government Area, Niger State, Nigeria
3. Will Quality of services rendered influence the uptake of national programme on immunization among people of Chanchaga Local Government Area, Niger State, Nigeria

Research Hypotheses

The following research hypotheses were tested in the study:

Ø Cultural belief of people will not significantly influence uptake of national programme on immunization among people of Chanchaga Local Government Area, Niger State, Nigeria.

Ø Fear of parents about immunization will not significantly influence uptake of national programme on immunization among people of Chanchaga Local Government Area, Niger State, Nigeria.

Ø Quality of services rendered will not significantly influence uptake of national programme on immunization among people of Chanchaga LGA, Niger State, Nigeria.

Methodology

A descriptive research design of survey type was adopted for this study. The population for the study comprises of all residents of the L.G.A totally Three Hundred and thirty two thousands, six hundred and six (332,606) according to the National Programme on Immunization (NPI Office) in Chanchaga local government area. A multistage sampling technique that comprises simple random sampling technique, purposive and convenience sampling technique was used to select sample of 384 respondents used for the study. The instrument used for data collection was researchers structured questionnaire adequately validated by three experts in the department of Health Promotion and Environmental Health Education, and department of Epidemiology and community Health in University of Ilorin, Nigeria. The suggestions of the jurors were incorporated to the final draft of the instrument used for the study. The reliability of the instrument was carried out using split-half method of reliability in which a reliability correlation coefficient of 0.76 were obtained, making the instruments

reliable enough for the study. The data generated for test of hypotheses formulated were analyzed using inferential statistics of Chi-square (χ^2) at 0.05alpha level. The result of the analysis were presented below.

Results/Discussion of Findings

Hypothesis 1: Cultural belief of people will not significantly be a factor influencing the uptake of National Immunization Programme among people of Chanchaga Local Government Area, Niger State. The hypothesis which was tested at 12 degree of freedom of 0.05 alpha level of significance was rejected because the calculated value (271.20) is greater than the table value (21.3). The result revealed that cultural belief of people will not significantly be a factor influencing the uptake of National Immunization Programme among people of Chanchaga Local Government Area, Niger State. Cultural beliefs in herbs, witches as causes of diseases, anger of ancestors, preaching of priests and traditional birth attendants against immunization were found to be common influencing the uptake of National Immunization Programme among people of Chanchaga Local Government Area, Niger State. This in line with findings of ²², who found out that misconceptions regarding the aetiology of disease apply to most Nigerian cultures and societies. Belief in the efficacy of native medicine and patronage of traditional health care providers is common among Nigerians.²³ It was observed that what the Yorubas of Western Nigeria perceive about the aetiology of most childhood diseases constitute a great hindrance to national immunization programme and other intervention by the government of Nigeria ²⁴.

Hypothesis 2: Fear of parents about immunization will not significantly be a factor influencing the uptake of National Immunization Programme among people of Chanchaga Local Government Area, Niger State. The hypothesis which was tested at 12 degree of freedom of 0.05 alpha level of significance was rejected because the calculated value (175.76)

is greater than the table value (21.3). The result revealed that fear of parents about immunization will not significantly be a factor influencing the uptake of National Immunization Programme among people of Chanchaga Local Government Area, Niger State. Parents who disallowed their children from being immunized, negative perception of parents and guardians towards immunization, inadequate knowledge of parents were mostly found factors influencing the uptake of National Immunization Programme among people of Chanchaga Local Government Area, Niger State. This in line with findings of ²⁵, who found out that parents play a vital role in the uptake of immunization by children under their care. It is often necessary to take children to the health facility for vaccination since they cannot do so by themselves. Their perception of immunization is therefore an important determinant of whether or not they will avail their children of immunization services. The findings in a study are similar to that of a study in New Zealand, where a survey of healthcare providers showed that 53% of them believed that parental fear was the greatest barrier to uptake of immunization programme.

Hypothesis 3: Level of coverage of service will not significantly be a factor influencing the uptake of National Programme on Immunization among people of Chanchaga Local Government Area, Niger State. The hypothesis which was tested at 12 degree of freedom of 0.05 alpha level of significance was rejected because the calculated value (154.47) is greater than the table value (21.3). The result revealed that level of coverage of service of people will significantly be a factor influencing the uptake of National Immunization Programme among people of Chanchaga Local Government Area, Niger State. Inaccessible road to health centres, inadequate awareness, low standard of health centres were found to be factors influencing the uptake of National Immunization Programme among people of Chanchaga Local Government Area,

Niger State. This in line with findings that ascertained that strengthening surveillance systems as part of improvement of immunization programs is therefore of vital importance. The author further explained that achieving high levels of coverage is, by itself, not a sufficient indication of the effectiveness of a health care system, as deficiencies in other areas could be widespread. However, lack of progress in moving towards high levels of coverage is a strong indication of failure to provide essential services to protect the health of the most vulnerable segment of a population. For diphtheria, pertussis, tetanus (DPT), a minimal coverage goal of 80 percent (three doses) as at 2005 has been proposed by the Global Alliance for Vaccines and Immunization (GAVI), to be achieved in all districts in all countries across the world²⁶

Recommendations

Based on the findings, the following recommendations were made:

1. There should be sensitization programme by health workers to community leaders and indigenes in villages in order to publicize the benefits of immunization

2. Parents should be well informed by health workers or through media before the start of immunization programmes to reduce the fear about immunization.

3. There should be government intervention in the provision of adequate social amenities in order to promote the coverage of immunization programme.

Ethical Clearance- Taken from Faculty of Education Ethical Review Committee, University of Ilorin.

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Conflict of Interest- Nil

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Analysis of *Staphylococcus aureus* and *Escherichia Coli* and its Susceptibility to Antibiotic in Catheter-Associated Urinary Tract Infection Patients at Hospital in Province of West Nusa Tenggara

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Abstract

Objective – This study aimed to analyze the proportion of *Staphylococcus aureus* and *Escherichia coli* from urine samples of catheter-associated urinary tract infections (CA-UTI) patients at the General Hospital at Province of West Nusa Tenggara. Also measured the pattern of sensitivity to several antibiotics.

Methods – This study is a descriptive observational with a cross-sectional approach. A total of 60 samples were used in this study. Bacterial identification was carried out according to standard bacteriological culture techniques. Furthermore, the antibiotic sensitivity test following the Kirby-Bauer disc diffusion method using several antibiotics, including amoxycillin (AMP), ciprofloxacin (CIP), ceftriaxone (CRO), and sulphamethoxazole trimethoprim (SXT).

Results – The results showed that bacteria causing CA-UTI were *Staphylococcus aureus* (43.33%), *Escherichia coli* (21.67%), *Staphylococcus epidermidis* (10%), *Proteus mirabilis* (6.67%), *Enterobacter aerogenes* (6.67%), *Serratia marcescens* (5%), *Klebsiella sp* (3.33%), and *Pseudomonas sp* (3.33%). The antibiotic susceptibility test found that 4.58%, 11.25%, 9.16%, and 3% of bacteria were resistant to amoxycillin, ceftriaxone, ciprofloxacin, and sulphamethoxazole trimethoprim, respectively.

Conclusion – *Staphylococcus aureus* and *Escherichia coli* were the most gram-positive and gram-negative bacteria causing CA-UTI, respectively. The most resistant antibiotic was ciprofloxacin, and the most sensitive antibiotic is sulphamethoxazole trimethoprim.

Keywords: antibiotic-resistance, catheter-associated urinary tract infection, *Escherichia coli*, *Staphylococcus aureus*

Introduction

Urinary tract infection (UTI) is a common

bacterial infection. About 35% of nosocomial infections are urinary tract infections, and 80% of them are related to catheterization¹. UTI can progress to bacteremia, sepsis, and death². As many as 20% of hospital-acquired bacteremia with a mortality rate of about 10% are due to CA-UTI. CA-UTI is defined as a patient with urinary tract infection caused by a

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catheter³ and is one of the most common nosocomial infections worldwide that can increase morbidity and mortality^{4,5}. In the United States, about 11 million people have urinary tract infections⁶ and affect around 150 million people annually worldwide⁷. Based on data from the Indonesian Ministry of Health, around 90-100 cases of urinary tract infections in 100,000 population for each year in Indonesia⁸.

According to the CDC, 12-16% of hospitalized patients use a catheter and have about 3-7% risk for CA-UTI, increasing every day⁹. Important risk factors for CA-UTI are persistent catheterization and duration of catheterization¹⁰. Other factors include female gender, age, diabetes mellitus, impaired kidney function, and lack of antimicrobial therapy^{4,9}.

In normal conditions, the urinary tract is usually sterile, except for normal flora such as digestive flora, skin flora and genital flora¹¹. UTI is characterized by the presence of infection by pathogenic microorganisms in the urinary tract, including bacteria, fungi or parasites¹². However, it is usually caused by bacteria, both by gram-positive and gram-negative bacteria¹³. The most common bacteria found as the cause of CA-UTI were *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus vulgaris*, *Providentiaertgeri*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, *Mycobacterium tuberculosis*^{11,12,13}.

UTIs are generally treated with antibiotics. Patients with catheterization have a three times greater risk for more extended hospitalization and prolonged use of antibiotics¹⁴. Inappropriate use of antibiotics creates problems, one of which is the emergence of microorganisms resistant to antibiotics^{15,16}.

Based on this description, that is important to know the pattern of bacteria and also the pattern

of antibiotic resistance, especially for a rational treatment of CA-UTI. For this reason, this study aimed to determine the proportion of *Staphylococcus aureus* and *Escherichia coli* that cause CA-UTI at the Hospital at Province of West Nusa Tenggara, also to analyze the pattern of resistance of these bacteria to antibiotics.

Materials dan Methods

2.1 Ethical clearance and study design

This research has been approved by the Ethics Committee of the Regional General Hospital of West Nusa Tenggara Province with reference number 070.1/05/KEP/2021. This research was conducted from January until March 2021 at Biomedical Research Unit, West Nusa Tenggara General Hospital, Indonesia. We made a descriptive observational with a cross-sectional approach.

2.2 Sample collection

Sample collection used a purposive sampling method. A total of 60 samples were collected. The inclusion criteria were patients with catheterization \geq 72 hours with a diagnosis of urinary tract infection, age $>$ 14 years, willing to provide urine (samples) and fill out an informed consent first.

2.3 Microbiological analysis

Sample (urine) was cultured on Mannitol Salt Agar (MSA) and Eosin Methylene Blue (EMB), incubated at 37° C for 18-24h. Bacterial colonies from media of MSA and EMB then inoculated into Nutrient Agar Plate (NAP). Then gram staining was performed. If gram-positive bacteria are found, it is continued with catalase and coagulase tests. Meanwhile, if gram-negative bacteria are found, it is followed by the biochemical test.

2.4 Antibiotics sensitivity testing

Kirby-Bauer disc diffusion method using several

antibiotics, including amoxicillin (AMC) 30 µg/disc, ciprofloxacin (CIP) 5 µg/disc, ceftriaxone (CRO) 30 µg/disc, and sulphamethoxazole trimethoprim (SXT) 25 µg/disc. The bacterial suspension was made from colonies bacteria on NAP was diluted with sterile physiological NaCl to make 0,5 McFarland standard. The bacterial suspension was cultured on Muller Hinton Agar media using a sterile cotton bud, and the antibiotic discs were placed on the agar surface then incubated at 37°C for 24h. Antibiotic inhibition zones formed were measured and adjusted to the Clinical and Laboratory Standards Institute (CLSI).

Results

A total of 60 subjects consisted of three categories: category of gender, age, and duration of catheterization. Table 1 shows the subjects consisting of 30 (50%) men and 30 (50%) women. Based on age category, UTI's patients in the range of 15-30 years were found 7 patients (11.67%), 31-45 years were 15 patients (25%), 46-60 years were 28 patients (46.67%), and range of 61-75 years were 10 patients (16.66%). Meanwhile, the duration of catheterization category consisted of 42 patients (70%) and 18 patients (30%) with a catheter duration of ≤7 days and ≥7 days, respectively (Table 1).

Table 1. General characteristics of subjects

Category		Total (n)	Percentage (%)
Gender	Men	30	50
	Women	30	50
Age	15-40 years	7	11,67
	31-45 years	15	25
	46-60 years	28	46,67
	61-75 years	10	16,66
Duration of catheterization	≤7 days	42	70
	≥7 days	18	30

Staphylococcus aureus (43.33%) was the main bacterial pathogen causing catheter-associated urinary tract infections, followed by *Escherichia coli* (21.67%), *Staphylococcus epidermidis* (10%), *Proteus mirabilis* (6.67%), *Enterobacter aerogenes* (6.67%), *Serratia marcescens* (5%), *Klebsiella sp* (3.33%), and *Pseudomonas sp* (3.33%) which can be seen in table 2.

Table 2. Bacterial identification that cause CA-UTI

Microorganism	Total (n)	Percentage (%)
Gram Positive		
Staphylococcus aureus	26	43,33
Staphylococcus epidermidis	6	10
Gram Negatif		
Escherichia coli	13	21,67
Proteus mirabilis	4	6,67
Enterobacter aerogenes	4	6,67
Serratia marcescens	3	5
Klebsiella sp	2	3,33
Pseudomonas sp	2	3,33
Total	60	100

Based on the results of the antibiotic sensitivity test in table 3, it was found that 63 isolates (26.25%) were resistant to antibiotics, of which 11 (4.58%), 27 (11, 25%), 22 (9.16%), and 3 (1.25%) were resistant to amoxicillin, ceftriaxone, ciprofloxacin, and sulphamethoxazole trimethoprim, respectively. Also,

90 bacterial isolates that were sensitive to antibiotics, as much as 27 (11.25%), 10 (4.17%), 10 (4.17%), and 43 (17,92%), were sensitive to amoxicillin, ceftriaxone, ciprofloxacin, and sulphamethoxazole trimethoprim, respectively.

Table 3. Antibiotic Sensitivity Test Results

Microorganism	n	Antibiotic Sensitivity Based on CLSI											
		Amoxycillin (AMC)			Ceftriaxon (CRO)			Ciprofloxacin (CIP)			Sulphamethoxazoletri methoprim(SXT)		
		S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
<i>S.aureus</i>	26	8 (7,72)	12 (11,54)	6 (5,77)	4 (3,85)	6 (5,77)	16 (15,39)	4 (3,84)	9 (8,65)	13 (12,5)	19 (18,27)	5 (4,80)	2 (1,92)
<i>S.epidermidis</i>	6	3 (12,5)	1 (4,16)	2 (8,34)	1 (4,16)	4 (16,67)	1 (4,16)	2 (8,34)	4 (16,67)	-	5 (20,84)	1 (4,16)	-
<i>E.coli</i>	13	6 (11,54)	5 (9,61)	2 (3,85)	2 (3,85)	8 (15,38)	3 (5,77)	2 (3,85)	7 (13,46)	4 (7,70)	9 (17,30)	3 (5,77)	1 (1,92)
<i>Klebsiella sp</i>	2	-	1 (12,5)	1 (12,5)	-	-	2 (25)	-	1 (12,5)	1 (12,5)	1 (12,5)	1 (12,5)	-
<i>Pseudomonas sp</i>	2	-	2 (25)	-	-	-	2 (25)	-	-	2 (25)	1 (12,5)	1 (12,5)	-
<i>Proteus mirabilis</i>	4	3 (18,75)	1 (6,25)	-	1 (6,25)	1 (6,25)	2 (12,5)	1 (6,25)	1 (6,25)	2 (12,5)	1 (6,25)	3 (18,75)	-
<i>Serratia marcescens</i>	3	3 (25)	-	-	1 (8,33)	1 (8,33)	1 (8,33)	1 (8,33)	2 (16,68)	-	3 (25)	-	-
<i>Enterobacter aerogenes</i>	4	4 (25)	-	-	1 (6,25)	3 (18,75)	-	-	4 (25)	-	4 (25)	-	-
Jumlah	60	27 (11,25)	22 (9,17)	11 (4,58)	10 (4,17)	23 (9,58)	27 (11,25)	10 (4,17)	28 (11,67)	22 (9,16)	43 (17,92)	14 (5,83)	3 (1,25)

S: Sensitive; I: Intermediate; R: Resistant

Discussion

Based on this study that has been conducted from January to March 2020, there were 60 subjects, consisting of 30 men and 30 women, UTI mostly occurred in the age range of 46-60 years, and the duration of catheterization was ≤ 7 days (Table 1). Women is a risk factor for CAUTI. Data from clinical epidemiological studies report that around 25-35% of adult women have experienced UTI at least once in their lives and have a risk for recurrent UTI¹⁷. UTI is more common in women than men, this is due to clinical factors such as hormonal, different anatomical variations, and different behaviour patterns between men and women¹⁸. Based on anatomical and physiological, women have a shorter urethra than men, drier urethral meatus, lack of personal care, especially intimate organs, decreased estrogen hormone, which causes an increase in vaginal pH, making it easier for microorganisms to colonize in the vagina, and the absence of antibacterial substances in vaginal fluids, that causes women more at risk for UTI^{19,20}. UTI can occur in teenagers about 1-3%, then increases with the

start of sexual activity, about 25-30% for age 20-40 years and up to 43% in women aged above 60 years²¹. Postmenopausal women and men over 50 years are at risk for CAUTI²².

Approximately 70-80% of complicated UTI are caused by catheters¹⁶. Catheterization is the most common cause of nosocomial infections because of its prolonged use and not under the indications²³ because of the risk of bacteriuria which increases by about 5% every day²⁴. Microorganism entry through the catheter with two routes; extraluminal route (through the lumen of the catheter) and intraluminal route (through the outer surface of the catheter). Microorganisms can attach to the catheter surface and colonize the bladder within three days from bacteria entry²⁵.

In this study, *Staphylococcus aureus* and *Escherichia coli* were the most common bacteria causing CA-UTI. The presence of *Staphylococcus aureus* in catheter urine can be caused by direct contact or through catheter tubes or other medical equipment used by nurses²⁶. Bacteriuria due to *S. aureus* can

occur in long-term care patients and is significantly associated with catheterization and antibiotic use²⁷. *Uropathogenic E. coli* (UPEC) is a strain of *E. coli* that often causes of urinary tract infections. This strain has the necessary factors for bacteria to colonize the urinary tract, overcoming the host defence system and causing more severe infections. These factors include fimbrial adhesin (fimbriae S, P and type 1), afimbrial adhesin, toxins (haemolysin and cytotoxic necrotizing factor), siderophores (aerobactin system) and capsular polysaccharide (group II capsules)²⁸.

Several types of antibiotics were used in this study, including amoxicillin, ceftriaxone, ciprofloxacin, and sulphamethoxazole trimethoprim. Ceftriaxone works by inhibiting the synthesis of mucopeptides in the bacterial cell wall²⁹. Ciprofloxacin by inhibiting the production of DNA gyrase and DNA topoisomerase IV enzymes that assist in the process of DNA replication, transcription, repair and bacterial recombination³⁰. Amoxicillin works by binding to penicillin-binding protein (PBP), which inhibits the transpeptidase process, thereby causing the activation of autolytic enzymes in the bacterial cell wall then causes lysis and death of bacteria³¹. Meanwhile, sulphamethoxazole trimethoprim which is a combination of sulphamethoxazole and trimethoprim, works by inhibiting the entry of PABA (paminobenzoid acid) molecules into the folic acid and inhibiting the formation of tetrahydrofolic acid³². From table 3, the bacteria were mostly resistant to ceftriaxone and sensitive to sulphamethoxazole trimethoprim.

Antibiotic resistance is still a problem in many countries. The existence of genetic mutations and the genetic transfer of microbes cause microorganisms to become more resistant. These resistance genes can be inherited or obtained from cellular genetic elements that can occur between bacteria³³. Several mechanisms are involved in antibiotic resistance,

(1) microorganisms produce enzymes that destroy the antibiotic substance, (2) microorganisms alter the permeability of the antibiotic substance, (3) microorganisms alter the structural target for the antibiotic substance, (4) microorganisms alter the metabolic pathways traversed by the reaction from inhibition of antibiotics, (5) microorganisms that change enzymes that can still carry out their metabolic functions are less affected by antibiotics³⁴.

Conclusion

CA-UTI are mostly caused by gram-positive and gram-negative bacteria; *Staphylococcus aureus* and *Escherichia coli*. Sulphamethoxazole trimethoprim is the antibiotic with the greatest sensitivity to bacteria that cause CA-UTI.

Authors statements

There is no conflict of interest to declare. All of the authors agreed that the manuscript is submitted to the Indian Journal of Forensic Medicine and Toxicology.

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The Outcome of Limberg Flap Procedure in the Management of Primary Chronic Sacrococcygeal Pilonidal Sinus Disease

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Abstract

Introduction: Sacrococcygeal pilonidal sinus (PNS) is a common chronic benign disease of young age group, associated with morbidity, often with a prolong loss of normal activity. Still there is ongoing debate regarding the best treatment option as all the methods have complications, although; the current evidence supports the use of off- midline techniques because of lower recurrence rates and avoidance of all disadvantages of open treatment. This study was done to determine the postoperative outcome of rhomboid excision and Limberg flap reconstruction as a main procedure in the treatment of PNS disease in our institute.

Patients and Method: A prospective study conducted at the Department of General Surgery- Baquba Teaching Hospital- Diyala- Iraq, from January 2017 to June 2019, in which 98 patients (78 male and 20 female) with chronic primary sacrococcygeal PNS were enrolled. The age of the patients ranged between 16 – 38 years. Patients with acute abscess were first treated by incision and drainage before definite surgery. Patients were operated by rhomboid excision and Limberg flap reconstruction.

Results: In this study, 98 patients were enrolled, 78 male (79.59 %) and 20 female (20.4%) with male to female ratio of 3.9:1. The age of the patients ranged between 16 to 38 years with the mean age of 25 years. During the follow up period; 6 patients (6.12%) developed seroma, 4 patients (4.08%) had infection and 4 patients (4.08%) had recurrent sinus after few months and one patient (1.02%) had wound disruption.

Conclusion: Rhomboid excision and Limberg flap reconstruction of PNS disease is safe and reliable technique with low complication and recurrence rates if performed according to appropriate surgical principles.

Key words: Pilonidal sinus, Limberg flap, postoperative complications.

Introduction

Pilonidal sinus (PNS) is a common, benign disease^[1-3] most frequently observed in the

sacrococcygeal region^[2], but also has been seen in the axilla, suprapubic area, periumbilical zone and between the fingers in the barbers^[4]. The onset of PNS is rare both before puberty and after the age of 40, the disease is more common in male probably due to their more hirsute nature [5-8], and more common in Caucasians than Asians and Africans due to differing hair characteristics and growth pattern^[5].

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For unknown reasons, the incidence of PNS has increased continuously during the last few decades, particularly in European and North American young men⁽⁹⁾ and now the incidence is estimated to be 26 per 100000 people^[10].

The etiology of PNS is uncertain; the current concept is that it is acquired pathology with a combination of local forces and friction acting on the topography of the natal cleft leading to implantation of loose hair into the depth of natal crease with subsequent inflammatory reaction^[1,10,11]. Obesity, local trauma, poor personal hygiene, narrowness of natal cleft and sweating with sitting and friction as in drivers and sedentary occupation are all risk factors for the development of the disease^[8,12].

Clinically, PNS can present as a cyst, acute abscess or chronic discharging sinus^[13] all are associated with morbidity, often with a prolonged loss of normal activity^[14]. Acute abscess should be drained, while chronic PNS should only be subjected to definite surgery^[4,8,13].

The ideal treatment for chronic PNS should ensure low pain, limited wound care, short hospitalisation period, low complication and recurrence rates and rapid return to normal activities^[14-16] and the available options like excision alone, excision with primary closure, marsupialisation, excision with closure using various types of flaps (fasciocutaneous V-Y flap, Z- or W- plasty, rhomboid flap and gluteus maximum muscle myocutaneous flap)^[8-13], all these methods have complications, postoperative infection and recurrence^[17]. So there is no consensus as to the best method of management of this problem^[18] and the choice of the surgical method primarily depends on the experience of the surgeon and on the content of the patient^[7].

The Limberg flap (named after its early 20th century inventor Prof. Aleksander Limberg of

Leningrad) is a rhomboid transposition flap and was first published as a treatment for PNS by Azabetal^[19] then became the most common off- midline procedure used to treat sacrococcygeal PNS as this flap is safe and reliable technique with low complication and recurrence rates if performed according to appropriate surgical principles^[1,4].

This study was done to determine the postoperative outcome of rhomboid excision and Limberg flap reconstruction as a main procedure in the treatment of PNS disease in our institute.

Patients and Methods

A prospective study conducted at the Department of General Surgery- Baquba Teaching Hospital- Diyala- Iraq, from January 2017 to June 2019, in which 98 patients (78 male and 20 female) with chronic primary sacrococcygeal PNS were enrolled. The age of the patients ranged between 16 – 38 years. Patients with acute abscess were first treated by incision and drainage before definite surgery.

The surgical treatment involved using the Limberg flap i.e after a rhomboid excision of diseased tissue down to presacral fascia, a rhomboid subcutaneous flap was mobilized and transposed to cover the defect with subsequent flattening of the natal cleft and lateralization of the wound. Good hemostasis was achieved by the use of electrocautery and a suction drain was placed in the wound cavity through a separate stab incision, the drain was removed after 48-72 hours while the sutures were removed on 10th- 12th postoperative day. Follow up continued for up to one year in outpatient basis.

Results

In this study, nintyeight patients with chronic PNS, were operated by rhomboid excision and Limberg flap reconstruction, 78 male (79.59 %) and 20 female (20.4%) with male to female ratio of 3.9:1.

The age of the patients ranged between 16 to 38 years with the mean age of presentation was 25 years.

The operative time ranged from 26 to 40 minutes

and the patients were discharged after 24 to 48 hours while the drain was removed after 48 to 72 hours and the sutures were removed after 10 to 12 days. As shown in table 1.

Table 1: Operative and postoperative data

<i>Data</i>	<i>Range</i>
Operative time	26 – 40 minutes
Hospital stay	24 -48 hours
Drain removal	48 – 72 hours
Suture removal	10 – 12 days

The follow up period was 12 months; during this period 6 patients (6.12%) developed seroma in first few weeks that responded to conservative measures, 4 patients (4.08%) had infection within the first few weeks and managed by local wound

care and antibiotic cover. Four patients (4.08%) had recurrent sinus after few months and were managed by excision followed by healing by primary intention. One patient (1.02%) had minor wound disruption and treated conservatively. As shown in table 2.

Table 2: Postoperative complications

Complication	Number of patients	Percentage
Seroma	6	6.12 %
Infection	4	4.08%
Recurrence	4	4.08 %
Wound disruption	1	1.02%

Discussion

Sacrococcygeal pilonidal sinus is a common chronic benign disease of young age group [1-8,15], a finding that is also observed in our study as our patients were young with age ranged between 16 – 38 years. The disease is more common in male [5,8], this is also observed in our study as male to female ratio

was 3.9:1.

While various methods have been described for the treatment of sacrococcygeal PNS, there is ongoing debate regarding the best treatment method [8,13] as all the methods have complications, postoperative infection and recurrence [10].

Thus, the gold standard treatment modality has yet to be established [10] although; the current evidence supports the use of off- midline techniques because of lower recurrence rates and avoidance of all disadvantages of open treatment^[4,19].

Rhomboid excision of the diseased tissue with Limberg flap reconstruction meets the requirement for being the ideal procedure for sacrococcygeal PNS if performed according to appropriate surgical principles^[1].

In this study, nintyeight patients with chronic primary PNS were operated by rhomboid excision with Limberg flap reconstruction and during the follow up period which extended for 12 months, 6 patients (6.12%) developed seroma in the first few weeks after the removal of the drain. These patients were treated conservatively. Wound seroma was a problem in the studies of Jethwanietal^[1] who reported seroma in 4.47% of patients, Yogishwarappaetal^[4] which was found in 3.8% of patients and İlhan Bali etal^[3] in 8% of patients.

Wound infection was a complication in 4 patients (4.08%) in this study who were treated by local wound care with antibiotic cover. Wound infection was also observed in the studies of Yogishwarappaetal^[4], Jethwanietal^[1], Sriharietal^[10], Gopal Rametal^[2] and İlhan Bali etal^[3] in which wound infection was seen in 1.92%, 2.9%, 6.5%, 10%, and 10.8% of patients respectively.

Recurrence of PNS is the main problem associated with all surgical methods including Limberg flap reconstruction [10]. Recurrence can be divided into two groups: early and late. Early recurrence is usually due to failure to identify one or more sinuses at incision and drainage, which was not followed by a second – look procedure. Late recurrence is usually due to secondary infection caused by residual hair or debris that was not removed at operation, inadequate

wound care or insufficient attention to depilation^[5]. In our study 4 patients (4.08%) developed recurrence few months postoperatively. The recurrence rate was 7.1% in the study of AhmetSerdarKaracaetal^[15] and in 10% of patients in Gopal Rametal^[2], recurrence rate was low, 1.49% in Jethwanietal^[1], 2.1% in Srihari RSetal^[10] and 0.6% at 12 month and 1.8 % at 24 months postoperatively in the meta-analysis of V. K. Staufferetal^[9]. While the studies of İlhan Bali etal^[3] and Yogishwarappaetal^[4] reported no recurrence.

One patient had minor wound disruption in our study (1.02%), while it was reported to occur in 3.3% in Gopal Rametal^[2] and in 2.7% of patients in İlhan Bali etal^[3].

Flap necrosis was observed in some other studies as in Jethwanietal^[1], Yogishwarappaetal^[4] and Sriharietal^[10], but was not a complication in this study. Postoperative hematoma was a complication in up to 21.6% of patients in İlhan Bali etal^[3] but did not occur in our patients probably due to meticulous hemostasis achieved intraoperatively by electrocautery.

Conclusion

Rhomboid excision and Limberg flap reconstruction of PNS disease is safe and reliable technique with low complications and recurrence rates if performed according to appropriate surgical principles.

Ethical Clearance: Taken from Baquba Teaching Hospital- Diyala- Iraq

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Conflict of Interest: The author declares no conflict of interests.

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Effectiveness of Educational Intervention Regarding Child Sexual Abuse on Knowledge and Attitude of Parents

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Abstract

Background: Child sexual abuse is a serious violation of children's rights and abuse of power. CSA is a matter of global concern and occurs across all socio-economic, educational, racial, and ethnic groups. There is a lack of knowledge and awareness regarding child sexual abuse and its preventive aspects. Parent education regarding CSA is important for keeping children safe. The study aimed to evaluate the effectiveness of information package as an educational intervention, on the knowledge and attitude of parents regarding CSA.

Methodology: An experimental, pre-test post-test control group design was adopted for the study. The setting of the study was selected schools of Bangalore. The sample included 300 parents (150 in experimental and 150 in control group). The outcome variables were knowledge and attitude. The data was collected using socio-demographic performa, knowledge questionnaire and attitude scale.

Results: The majority of the parents in both the experimental and control group had an average level of knowledge and a moderately favorable attitude about prevention of CSA. There was a significant difference in the post-test knowledge (22.10 ± 3.15 and 12.19 ± 3.20 ; $F = 101.33$; $p < 0.001$) and attitude scores (102.23 ± 10.67 ; 84.23 ± 15.18 ; $F = 54.88$; $p < 0.001$) of parents regarding CSA in the experimental group when compared to the control group. **Conclusion:** The Educational intervention was effective in improving the knowledge and bringing a favorable change in the attitude of parents regarding CSA in the experimental group. The Information package is recommended for use as a primary prevention effort for educating parents on CSA.

Key Words: CSA (Child Sexual Abuse), Prevention, Education, Information Package, Parents, Knowledge, Attitude.

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Introduction

Child abuse and neglect is a global public health concern. It is a prevailing problem in all generations, socioeconomic strata, and societies. Child sexual abuse is the involvement of a child in sexual activity that he or she does not fully comprehend, is unable to give informed consent to, or for which the child is not developmentally prepared and cannot give

consent, or that violates the laws or social taboos of society. It is the cruelest and tragic occurrence and a serious infringement of a child's rights to health and protection.¹

CSA is prevalent in all countries of the world and has a significant impact on the health and wellbeing of children. Globally 1 in 5 girls and 1 in 13 boys have been sexually abused or exploited before reaching the age of 18 years.²

A common misperception about CSA is that it is a rare event perpetrated against girls by male strangers in poor, inner-city areas. On the contrary, CSA is a common occurrence that results in harm to millions of children, boys and girls alike, in all communities, and across a range of cultures and socioeconomic backgrounds. These acts are perpetrated by many types of offenders, including men and women, strangers, trusted friends or family, and people of all sexual orientations, socioeconomic and cultural backgrounds.^{3,4}

Several studies and newspaper reports have highlighted the high prevalence of CSA across the globe. Global Scenario of CSA: A meta-analysis of 65 studies in 22 countries, estimated an overall international figure. The main findings described that an estimated 7.9% of males and 19.7% of females universally faced sexual abuse before the age of 18 years. The highest prevalence rate of CSA was in Africa (34.4%). Europe, America, and Asia had a prevalence rate of 9.2%, 10.1%, and 23.9%, respectively.⁵

Indian Scenario of CSA: The first National Study on Child Abuse, covering 13 states in India and a sample size of 12,446 children, showed that more than 53% of children faced one or more forms of sexual abuse, almost 22% faced severe sexual abuse, 6% were sexually assaulted, 50% of sexual offenders were known to the victim or were in

positions of trust.³ Studies by Tulir CPHSA revealed a prevalence of 42%,⁶ Kerala 36% boys and 35% girls had experienced sexual abuse.⁷

Childhood sexual abuse is considered to be a central issue of mental and physical problems which may carry on up to adult life of men and women which include, depression, low self-esteem, anxiety, behavioral problems, social problems, drug use, impaired relationships, a range of sexual relationships, suicidal tendency, eating disorders, post-traumatic stress disorders,⁸ GI problems-IBD, chronic pelvis pain, musculoskeletal pain chronic headache, obesity, and cardiopulmonary symptoms.⁹

Keeping children safe is one of the most important things to do as parents and caring adults. Primary prevention of CSA involves preventing the occurrence of sexually abusive acts by taking action before the abuse occurs. Parents and caretakers are often the first educators of children and thus are in the best position to foster primary prevention of CSA. Given the above facts this study aimed at evaluating the effectiveness of information package regarding child sexual abuse on the knowledge and attitude of parents.

Materials and Methods

An experimental, pre-test post-test control group design was adopted for the study. The present study was conducted in selected schools in Bangalore, Karnataka, India. The schools in Bangalore are divided into 3 zones, namely Bangalore North, South, and Rural. Bangalore South was selected randomly by the lottery method. From the south, 20 schools that gave permission were selected randomly by computer using a random number generator. The selected schools were designated as experimental (9 number) and control (9 number) schools randomly, using the lottery method. A total of 300 parents, 150 each in the experimental and control group were selected from the individual schools by simple random sampling technique using a

random number generator in the computer. The parents who met the inclusion criteria were selected for the study. The inclusion criteria were, parents who could read English or Kannada, and willing to participate in the study. The exclusion criteria included parents not available at the time of data collection

Variables and Study Tools

The independent variable was information package on CSA and the dependent variable was knowledge and attitude of parents.

Tool 1: Socio-Demographic Performance:

A structured self-administered questionnaire was developed to gather data regarding sample characteristics.

Tool 2: Knowledge questionnaire:

It questionnaire consisted of multiple choice questions under the domains namely; general aspects of CSA, characteristics of victims, characteristics of offender, types of CSA, consequences and treatment and prevention of CSA. One score was awarded for the correct and zero for the wrong response. The total attainable score was 32. To interpret the level of knowledge, the score was categorized arbitrarily as Poor: 0- 9(<30%), Average: 10-19(30-59%), Good: 20 & above(>60%).

Tool 3: Attitude Scale: A 5 point Likert scale was developed to assess attitude regarding CSA. It consisted of 30 statements under 5 domains, with 6 items each. To interpret the level of attitude, the score was arbitrarily categorized as unfavorable: 0-50(<33%), neutral: 51-100(34-67%), favorable attitude: ≥ 100 (>67%)

Validity and reliability

The tools was validated 16 subject experts. The calculated CVI (content validity index) for the knowledge questionnaire was 0.95 and the attitude scale was 0.9. Reliability using test-retest for testing

the stability was done. Reliability for internal consistency of the questionnaire was done using the split-half method calculating the Spearman prophecy formula. The reliability score was 0.8.

Study intervention

An Education intervention was developed as an information package, prepared based on the review of literature and suggestions of the subject experts. A video named KOMAL in English and Kannada language was used for the study. It is a video for 10 minutes prepared by the Child-Line organization available for use in 13 languages.

Data collection

Data collection was done from a period; July 2014 to February 2015, according to the dates given by the individual school. Parents were given intimation through the school circular to attend a parent-teacher meeting. Written consent was taken after explaining the purpose of the study. A pre-test questionnaire was administered followed by the educational program in the form of an information package on CSA. The Post-test was done after one week to evaluate the outcome. A similar process was followed for the control group but no intervention was done. A booklet and pamphlet containing information on CSA were distributed for the benefit of parents in the control group after the post test.

Statistical Analysis

The collected data was analyzed using descriptive and inferential statistics with the help of SPSS software 20.0. Descriptive statistics such as frequency, percentage, mean, SD, Inferential statistics included independent 't' test, ANCOVA, Pearson correlation, and chi-square test were done.

Results

Description of demographic variables of parents

In the experimental group, the majority 91(60.7%) were between the age group of 30-40 years. Most 108(72%) were females, 73(69.3%) parents had not given any CSA-related teaching to their child. In the control group, the majority 85(56.7%) were between the age group of 30-40 years, 101(67.3 %) were

females, 110(73.3%) parents had not given any CSA-related teaching to their child. The parents in both the groups were homogenous in terms of all demographic characteristics (p>0.05), except for the variable occupation as the p-value was found to be significant at 0.01 level. (Table 1)

Table 1: Frequency and percentage distribution of parents by demographic variables

(n=300)

Demographic Variable	Categories	Experimental group n=150		Control group n=150		Chi square	df	p value
		F	%	F	%			
Age	20-30	50	33.3	53	35.3	0.72	2	0.70
	31-40	91	60.7	85	56.7			
	above 40	9	6.0	12	8.0			
Gender	Male	42	28.0	49	32.7	0.78	2	0.379
	Female	108	72.0	101	67.3			
Religion	Hindu	135	90.0	142	94.7	3.24	1	0.198
	Muslim	8	5.3	6	4.0			
	Christian	7	4.7	2	1.3			
No. of Children	One	32	21.3	30	20.0	1.59	2	0.452
	Two	104	69.3	99	66.0			
	Three & more	14	9.3	21	14.0			
Educational qualification	Primary	48	32.0	34	22.7	4.43	3	0.218
	High school	63	42.0	66	44.0			
	PUC	32	21.3	44	29.3			
	Graduate & above	7	4.7	6	4.0			
occupation	Housewife	98	65.3	60	40.0	23.03	3	P<0.001**
	Govt Job	2	1.3	4	2.7			
	Private job	31	20.7	67	44.7			
	Business/others	19	12.7	19	12.7			

Cont... Table 1: Frequency and percentage distribution of parents by demographic variables**(n=300)**

Income	<10000	104	69.3	103	68.7	1.44	3	0.696
	10,001-20000	30	20.0	35	23.3			
	20,001-30,000	10	6.7	9	6.0			
	>30,000	6	4.0	3	2.0			
Type of Family	Nuclear	57	38.0	63	42.0	0.50	1	0.48
	Joint	93	62.0	87	58.0			
Previous Information on CSA	Yes	138	92.0	131	87.3	2.382	1	p>0.05
	No	12	8.0	18	12.0			
Source of Information	No Source Print Media	45	30.0	32	21.3	7.08	3	0.132
	Electronic Media	74	49.33	82	54.7			
	Friends/relatives	14	9.3	9	6.0			
	Multiple source	17	11.3	27	18.0			
Information on any CSA victim in family/friends	Yes	14	9.3	9	6.0	1.18	1	0.278
	No	136	90.7	141	94.0			
Teaching about CSA to own Child	Yes	46	30.7	40	26.7	.59	1	0.444
	No	104	69.3	110	73.3			

Level of knowledge and attitude of parents regarding CSA

In the pre-test majority of the parents in both the experimental 119(79.3%) and control group, 113(75.3 %) had an average level of knowledge regarding CSA. Around 4(2.7%) parents in the experimental and 35(23.3%) in the control group had poor knowledge level. (Figure 1).

Concerning attitude, the majority of the parents in both the experimental (76%) and control (90%) group, had a moderately favorable attitude for the prevention of CSA. (Figure 2)

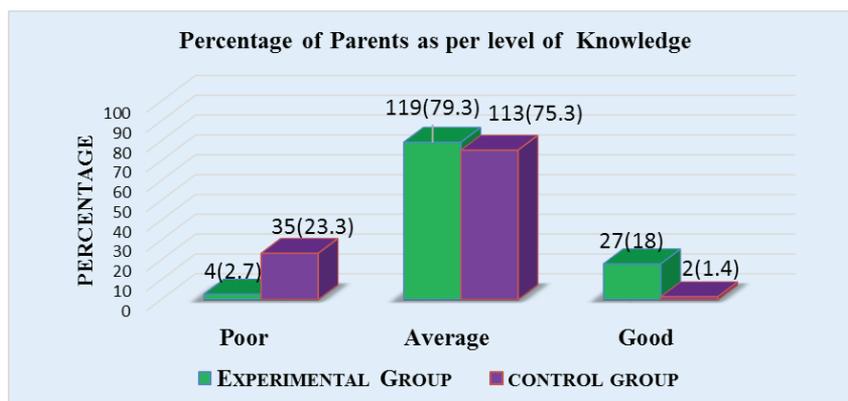


Figure 1: Pre-test level of knowledge of parents in experimental and control group

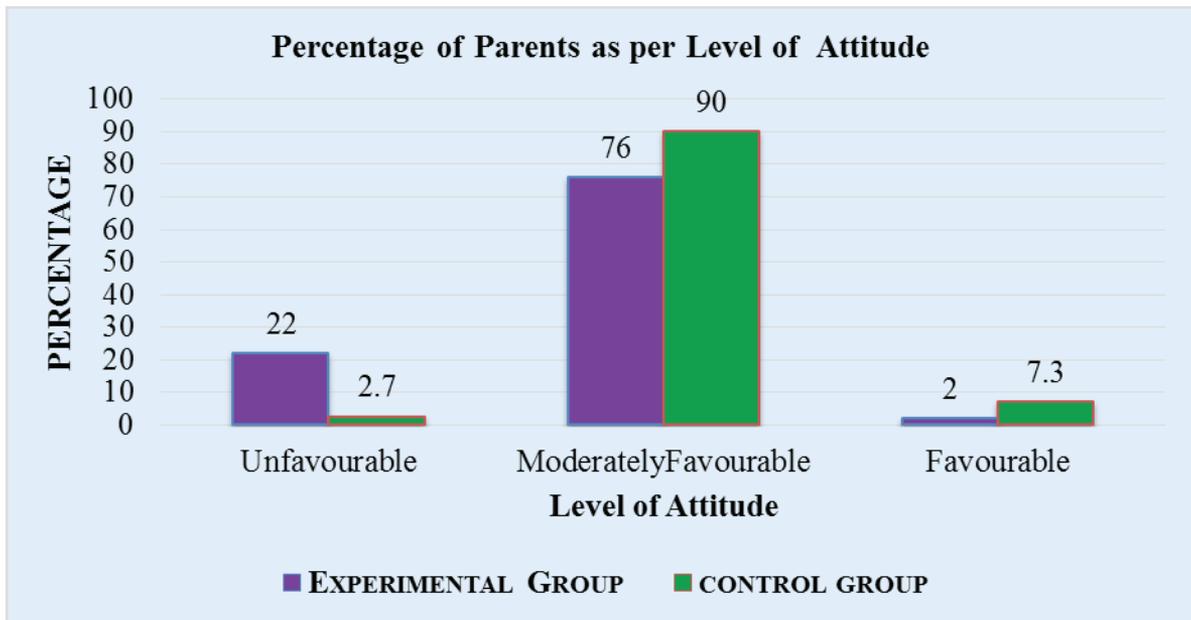


Figure 2: Pre-test level of attitude of parents in experimental and control group

Comparison of overall and area wise post-test knowledge and attitude scores of parents in the experimental and control group

The effectiveness of the information package regarding CSA on the knowledge and attitude of parents was determined by comparing the post-test scores in the experimental and control group by independent t-test and ANCOVA, for each pair of the measurement. Since the baseline mean pre-test knowledge of parents in the experimental (16.56) and control group (12.31) was different, the statistical analysis was adjusted while comparing the posttest mean. So ANCOVA was calculated which showed, the experimental group had higher mean knowledge (22.10±3.15 and 12.19±3.20; F = 101.33; p <0.001) and higher mean attitude scores than the parents in the control group (102.23±10.67; 84.23±15.18; F = 54.88; p<0.001). (Table 2). The domain wise analysis also showed that the mean post-test knowledge and attitude scores in the experimental group were higher than the control group which was statistically significant. (Table 3, 4)

Table 2: Overall Post-test knowledge and attitude scores of parents (n = 300)

Variables	Test	Experimental Group (n=150)		Control Group (n=150)		Ancova	
		M	SD	M	SD	F value	p value
Overall Knowledge	Post Test	22.01	3.15	12.19	3.20	101.33	<0.001**
Overall attitude	Post test	102.23	10.67	84.23	15.18	54.88	<0.001**

** Highly Significant at 0.001level

Table 3: Domain wise Mean, SD, and independent t value of post-test knowledge of parents regarding CSA. (n=300)

Knowledge Domains of CSA	Experimental Group(n=150)		Control Group (n=150)		Independent t-test	
	Post test		Post test			
	Mean	SD	Mean	SD	t value	p value
1.General aspects of CSA	5.53	1.19	2.79	1.17	20.13	<0.001**
2.Characteristics of victim	3.10	1.23	1.12	0.9	15.93	<0.001**
3. Characteristics of abuser	3.39	1.16	2.16	0.97	9.92	<0.001**
4. Types of CSA	1.69	1.04	1.09	1.02	5.09	<0.001**
5. Consequences of CSA	3.16	1.17	2.04	1.25	8.00	<0.001**
6. Treatment and prevention	5.14	1.23	3.00	1.41	13.98	<0.001**

** Highly Significant at 0.001 Level

Table 4: Domain wise Mean, SD, and Independent t value between post-test attitude of parents regarding CSA.

(n =300)

Attitude Domains of CSA	Experimental Group(n=150)		Control Group(n=150)		Independent t test	
	Post test		Post test			
	Mean	SD	Mean	SD	t value	p value
1. General aspects of CSA	20.01	3.39	16.61	3.84	8.10	<0.001**
2. Abuser	21.25	3.78	17.39	4.61	7.93	<0.001**
3. Victim	21.44	3.22	17.65	4.26	8.69	<0.001**
4. Parents	19.36	2.92	16.07	3.24	9.25	<0.001**
5.Prevention	20.17	3.22	16.50	3.62	9.28	<0.001**

** Highly Significant at 0.001level

Correlation between knowledge and attitude of parents

There was a low positive, but significant correlation between the knowledge and attitude of parents regarding CSA ($r=0.197$, $p< 0.01$) at 0.01 level of significance.

Association between knowledge and attitude of parents with their socio-demographic variables

There was an association of pre-test knowledge of parents with the variables, occupation ($\chi^2= 13.18$, $p< 0.040$) and teaching about CSA given to own child ($\chi^2=7.69$, $p< 0.021$). The pre-test attitude of parents and source of information on CSA ($\chi^2= 16.35$, $p=0.038$) was also found to have an association at 0.05 level of significance. The p-value of other variables was not significant.

Discussion

The focus of child care has been on health promotion and preventive services. Parental involvement in the personal safety education of their children is effective in several studies. Parents, when provided with developmentally appropriate materials for their children, can be very effective instructors of personal safety.¹⁰ Parents and caretakers are the first educators of children, thus are in the best position to foster primary prevention of CSA. This study attempted to develop and evaluate the effect of an information package regarding CSA.

The present study findings showed that the majority of the parents in both the experimental (79.3%) and control group (75.3%) had an average level of knowledge before the intervention. Around 2.7% of parents in the experimental and 23.3% in the control group had a poor level of knowledge. These findings are similar to the findings of the other studies^{11, 12, 13} wherein parents lacked the basic knowledge of CSA prevention. Contrary to that Mlekwa FM¹⁴

in Tanzania reported that the majority (95.6%) of mothers had high knowledge. Other similar studies^{15, 16, 17, 18}, also reported that the majority of mothers were knowledgeable about CSA and its prevention practices.

The present findings suggest that though parents lack knowledge, they are not completely unaware of CSA. They have an average level of knowledge regarding CSA. This can be explained by informal self-acquired information through mass media and printed materials. Parents must have a good level of knowledge. Hence strategies to increase their knowledge are important.

Concerning attitude regarding CSA, the majority of the parents in both the experimental (76%) and control (90%) group had a moderately favorable attitude before the educational intervention. These findings are congruent with the findings of Mohan S¹¹ and Khanjari S, et al¹⁷. Mlekwa FM¹⁴ reported 98.7% of respondents had positive attitudes on preventing CSA. In contrast, Pahantasing S,¹⁹ reported that more than half of the respondents had a negative attitude. Attitudes reflect opinions, thoughts, or feelings towards a concept or fact. Knowledge has an important role in influencing the attitude of an individual. The moderately favorable attitude towards the prevention of sexual abuse can be attributed to the average level of knowledge of parents.

In the current study the information package was found to be effective in increasing the knowledge of parents regarding CSA, as study findings showed a statistically significant difference in domain wise and overall post-test knowledge regarding CSA in the experimental group (22.01 ± 3.15) when compared to the control group (12.19 ± 3.20); $t=26.78$, $p<0.001$. Similar findings were observed in the study involving educational intervention for parents.²⁰⁻²⁵

The findings of the effectiveness of the information package on the attitude of parents revealed a statistically significant difference in the domain wise and overall mean post-test attitude scores of parents regarding CSA in the experimental group (102.23±10.67) when compared to the control group (84.23±15.18); $t=18.0$, $p<0.001$. These findings are consistent with that of other studies.^{22,23 24}

Parents are logically the ones from whom children may seek help. Educating them about abuse issues can be an important part of prevention. The advantages of prevention education for parents are: if parents learn to educate their children, the repetition of information from a trusted source can be more effective. It reduces the secrecy surrounding the topic, stimulates parent-child discussions on sexuality, may prevent abuse that begins early, make their home environment safer for children. Parents may easily identify the signs, if the abuse occurs and respond in more helpful ways to the discovery of abuse.²⁷ One-to-one parent-child communication is the most effective approach in empowering children for guarding their safety. The current study confirms that educational programs in terms of information package was very effective in increasing the knowledge and bringing a favorable change in the attitude regarding CSA and its prevention among parents.

In this study, the pretest knowledge of parents was associated with the variables occupation of parent and teaching about CSA given to child. This can be justified as majority of the mothers were housewives and had not given any teaching to children regarding CSA. A parent is deemed effective at protection if he or she has spoken about specific abusive behaviors such as inappropriate touching, perpetrator identities, and what to do in an abuse situation.²⁶ Hence inadequate knowledge may be one of the factors for lack of communication regarding the prevention of CSA between parent and child. Several studies on awareness

of parents on CSA have concluded that parents lack knowledge and recommended implementation of awareness and educational programs for empowering parents. The educational intervention in our study was found to be very effective.

Conclusion

This study was an attempt to assess the knowledge and attitude of parents and evaluating the effectiveness of educational intervention regarding CSA. Based on the findings, it is inferred that the information package as an educational intervention was effective in increasing the knowledge of parents and influencing a favorable change in the attitude of parents. Health care professionals must be involved in imparting CSA prevention education to parents. The validated information package of this study is recommended for teaching the parents about CSA and its prevention.

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Legal Certainty of Hospital Service Operations During Pandemic Corona Virus Disease 2019

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Abstract

Hospital is health service institution that provides complete individual health services that provide inpatient, outpatient, and emergency services. In operating services the hospital is required to have permit. Permit is granted if it meets the requirements and standards to ensure the safety of patients, hospitals and health workers. The quality of hospital services is tested through the accreditation process, it could not be done during pandemic. Therefore, many policies have been issued, including the Circular of Health Minister No. HK.02.01/MENKES/455/2020 Year 2020. This circular facilitates the permit extension but does not give detail about how to maintain the hospital quality and services. During pandemic, the surge in Covid patients is not comparable to the hospital capabilities that are different such as in the human resources and the facilities and infrastructure. Many hospitals are full because they have to treat Corona Virus Disease 2019 patients, a lot of equipment, rooms and beds must be added, but the health workers that work are limited so that health workers have to work extra to serve patients that increase every day. This situation can cause physical and mental fatigue which over time has impact on health services so that negligence can occur. So hospitals need to make protection standards for health workers in addition to legal responsibility for losses caused by health workers according to article 46 of the Hospital Law.

Keywords: hospital, Licensing, Health Workers, Covid-19

Introduction

Services of hospital as institution in the health sector during Corona Virus Disease 2019 (COVID-19) pandemic are still quite difficult. There are still many hospital functions and tasks that have not been carried

out optimally yet, including arrangement of separate entry flow for Covid-19 patients, providing negative pressure rooms, providing facilities and infrastructure such as adding rooms for Covid-19 patient services, laboratory support facilities and PPE for health workers. Pandemic impacts do not show improving indicators nationally where the number of COVID-19 patients until March 27, 2021 totals 1,492,022 people and the data of patients who died after being exposed to COVID-19 from March 26-27 were 198 patients. Thus, the death toll from Covid-19 has reached 40,364 people since the beginning of the pandemic ¹.

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Hospital is health service institution that provides complete individual health services that provide inpatient, outpatient, and emergency services. Regulations regarding hospitals are regulated in Law No. 44 of 2009 concerning Hospitals (hereinafter referred to as the Hospital Law)². Prior to the existence of Hospital Law, overall regulations regarding hospitals were regulated by sector through the Ministry of Health³. This health service institution has its own characteristics where the development of health science, the socio-economic life of community and technological advances have influence in improving quality services to achieve the highest degree of health.

During pandemic, the government has issued several policies, including Presidential Decree Number 11 of 2020 concerning the Determination of Public Health Emergency of Corona Virus Disease 2019 (COVID-19)⁴. Another decision related to Corona Virus Disease (COVID-19) is the Presidential Decree Number 12 of 2020 concerning the Determination of Non-Natural Disasters for the Spread of Corona Virus Disease 2019 (COVID-19) as National Disaster⁵, so that mitigation efforts must be carried out in accordance with the Circular Letter of the Minister of Health NUMBER HK 02.01/MENKES/455/2020 YEAR 20206. However, if we look at the policies above, it is not stated in detail by the government how a hospital maintains the quality of health services during pandemic. It is also not explained which institution should evaluate the hospital's performance. In the Minister of Health Regulation No. 3 of 2020⁶ it is stated that one of the requirements to obtain operational permit extension is accreditation certificate and thus every hospital that wishes to extend its operational permit must undergo accreditation. In accordance with article 40 of the Hospital Law which states that to improve quality, hospitals are required to carry out periodic accreditation at least once every 3 years which is

carried out by an independent institution appointed by the government both from within and from abroad based on applicable accreditation standards. This is intended so that the hospital service system can be measured and improved⁷. However, with the pandemic, the licensing process is made easier without going through the accreditation process. Is the with easier licensing process, the quality of hospital services can still be accounted for?

Hospitals have important role to optimally improve the community health status, so it is hoped that hospitals will be able to manage their activities by prioritizing the responsibilities of professionals, especially health workers in carrying out their assigned duties and authorities. Health workers as one of health resources elements also have obligation to carry out their service duties professionally. Given that these health workers are legal subjects, in essence the relationship that occurs between health workers, patients and health facilities is legal relationship. Legal relationship always producereciprocal rights and obligations where the rights of one party become the obligations of the other party and vice versa. As legal subjects who have carried out their service duties within the correct legal corridor, in essence, health workers are entitled to get legal protection from the applicable positive legal rules⁸. Article 28D paragraph (1) of the 1945 Constitution states that everyone has the right to fair recognition, guarantees, protection and legal certainty and equal treatment before the law⁹.

Many events have happened recently regarding hospitals during the pandemic, among others, hospitals accused make patients as COVID-19 patients, forced pick up of covid patient corpse, and different results for patient swab examinations results between hospitals which caused patients to feel mentally disadvantaged due to the status of covid-19. Disputes between patients and hospitals and health

workers become news focus in the mass media. Due to the large amount of information circulating about medical actions carried out by health workers which are considered not in accordance with the wishes of the community, causing the public confidence in health workers to be reduced.

High public need for health services during pandemic can cause legal problems for both health workers and hospitals. Many hospitals are full because they have to treat covid-19¹⁰ patients, many rooms and beds must be added but the health workers that work in these hospitals are limited, so health workers have to work extra to serve patients who increase every day¹¹. This situation can cause physical and mental fatigue which over time may allow for negligence to occur. Law No. 36 of 2009 concerning Health (hereinafter Health Law)¹² regulates matters relating to the health workers negligence. Article 29 of the Health Law states that in the event that health worker is suspected of negligence in carrying out his profession, the negligence must be resolved first through mediation. If mediation is not achieved, the hospital must be legally responsible for all losses caused by health workers negligence in accordance with article 46 of the Hospital Law.

The paper goal is to find out how the legal impact of health services operations due to the ease of hospitals licensing during pandemic and how the hospitals responsibility to protect health workers during pandemic.

Discussion

Hospital (Licensing during Pandemic)

In the period prior to pandemic, hospital licensing was actually regulated in Article 25 of the Hospital Law, which states that hospital operating permit consists of building permit and operational permit. Where for building permit given period of 2 years and can be extended for 1 year, while for operational

permit given period of 5 years and can be extended if it meets the requirements. Article 27 of the Hospital Law states that hospital permit can be revoked if several things are found, such as the expiration date, no longer meeting the requirements and standards, there is evidence of violating laws and regulations and or on court orders for law enforcement. One of the requirements to extend operational permit is to undergo accreditation process with the aim that the operation of health services in accordance with standards, can be measured and improved, all of which aim to ensure patient, hospital and health workers safety.

During pandemic, all procedures to extend the operational permits cannot be carried out as usual. For hospitals whose the permit expired during pandemic, they may be faced with problems such as delays in obtaining operational permit. This can cause legal problems because the hospital as legal entity operates their activities without permit and also sanctions for health workers who take action within the institution. Article 62 of the Hospital Law states that anyone who intentionally operates hospital without permit as referred to in Article 25 paragraph (1) shall be sentenced to maximum imprisonment of 2 (two) years and maximum fine of IDR. 5,000,000,000. (Five billion rupiah), then the government has just issued the Job Creation Law¹³ which discusses hospital licensing which reads "Anyone who intentionally operates hospital as referred to in article 25 paragraph (1) which results in casualties/damage to health, safety, and or environment shall be sentenced to maximum imprisonment of 2 (two) years and maximum fine of IDR. 7,000,000,000.00 (seven billion rupiah)". With the issuance of new regulations by the government in the Job Creation Law, hospitals can do several things, such as delaying the hospital licensing or not administer hospital permit at all because it seems that according to this law they cannot be punished if they do not result in casualties/damage to health, safety, and/or environment.

During pandemic, the government actually helped hospitals in the licensing process by issuing Circular Letter from the Minister of Health of the Republic of Indonesia Number HK.02.01/MENKES/455/2020 concerning Licensing and Accreditation of Health Service Facilities and Determination of Teaching Hospitals During the Corona Virus Disease 2019 Pandemic which adds hospital operating/operational permit whose validity period has expired but the permit extension process is constrained by the National Disaster or Corona Virus Disease 2019 Public Health Emergency. The operating/operational permit is declared to remain valid for 1 (one) year since the National Disaster status or Public Health Emergency of Corona Virus Disease 2019 (COVID-19) has been declared revoked by the Government, but the hospitals must make commitment in the form of attached letter whose format has been determined by the local government⁶. Furthermore, the hospital will still have to administer the hospital licensing as requirement for hospital operation as stated in Article 25 paragraph (1) of the Hospital Law. In the operational permit extension policy above, it does not require how the standard service quality and what indicators are used to measure the quality achievement as carried out or checked at the time of accreditation. Every 3 years the accreditation implementation in hospital ensures that all services provided to the community are according to standards. However, during pandemic, accreditation cannot be carried out because it is considered that the implementation process involves and gathers many people in one place. However, if accreditation is not carried out, it is likely that hospitals are “sleeping” or forgetting the standard procedures that have been set. This can lead to various quality services that may not meet the standards. The diversity of quality in services that do not have this standard can cause problems ranging from patient dissatisfaction to lawsuits because the procedures used may pose a threat to patient safety. If this situation occurs, there

is no standard procedure that can be used as legal protection.

Practice Permit of the Health Workers

Hospital is corporation in which there are health workers as the workers in it. In carrying out actions in hospitals, health workers must pay attention to several obligations, one of which is administer the practice permit including re-registration which can be done online during the pandemic. In Article 42 of the Medical Practice Law states that hospitals are prohibited from hiring doctors or dentists who do not have practice permit. In accordance with Article 80 of Law No. 29 of 2004 concerning Medical Practice, it is stated that any person who intentionally employs doctor or dentist as referred to in Article 42, shall be sentenced to maximum imprisonment of 10 years or maximum fine of IDR. 300,000,000.00 (three hundred million rupiah). It is stated in paragraph (2) that in the event that the criminal act as referred to in paragraph (1) is committed by a corporation, the punishment imposed is a fine as referred to in paragraph (1) plus a third or an additional penalty is permit revocation¹⁴. So it can be concluded that corporation is responsible for acts that are physically carried out by its employees.

Health Workers During Pandemic

Talking about the Hospital Law, of course, cannot be separated from the Health Law and the Health Worker Law. In General Provisions Article 1 point 1 of the Health Worker Law defines a health worker as every person who devotes himself to the health sector and has knowledge and or skills through education in the health sector which for certain types requires the authority to carry out health efforts while for the grouping of health workers stated in Article 11 paragraph 1.

During pandemic, health workers also at the forefront of handling covid 19 because they are dealing directly with patients suspected of / suffering

from covid 19. In this situation, health workers are very vulnerable infected by the virus when dealing with COVID 19¹⁵ patients. So here it is important to use Personal Protective Equipment (PPE) and understanding of standard procedures or regulations made in handling COVID-19 patients, especially for those who work in the COVID-19 isolation room.

According to Hila Halimatu who quoted from Rosario Baranco and Francesco Ventura in his article entitled Covid 19 And Infection In Health Care Workers An Emerging Problem, it was stated that the task of health workers in handling corona virus cases was not easy. The number of patients that continues to increase per day causes health workers to continue to work even beyond the provisions stipulated by Law¹⁶ as a result many health workers are exhausted from work¹⁷. Health workers can also experience burnout syndrome which has long-term effect on service quality because health workers can feel extreme fatigue, depression and even feel themselves less competent in carrying out their duties¹⁸. The psychological response of health workers in the COVID-19 pandemic is caused by feelings of anxiety about their own health. themselves due to the lack of PPE and the spread of the virus to their families¹⁹.

Hospital Responsibilities Toward Health Workers During Pandemic

The Hospital Law, the Health Worker Law and the Health Law are mutually supportive and closely related. This linkage regulates the standard of how hospital in providing health services to the community to achieve the highest degree of health including the responsibility arrangement, rights and obligations of either hospitals, health workers and patients. Although it has been regulated in the legislation, it turns out that its implementation is not as easy as the written rules.

During pandemic with so many workloads for

health workers such as working hours that exceed the legislation, physical and mental fatigue, anxiety, lack of PPE availability, lack of socialization of the Covid-19 handling flow and supporting facilities and infrastructure that are still inadequate can lead to negligence which can become a legal problem. The authors consider that four of the seven rights of health workers as stated in Article 57 of the Health Worker Law include 1) Obtaining legal protection when performing services in accordance with Professional Standards, Professional Service Standards, and Standard Operating Procedures; 2) Obtain complete and correct information from patients or their families; 3) Receive service fee; 4) Obtaining protection in work safety, based on human dignity, morals, decency, and religious values are rights that are often not fulfilled as they should be.²⁰

Talking about legal protection during pandemic, of course, cannot be separated from the rights and obligations of each patient and health worker. One of the health workers obligations as stated in Article 58 of the Health Worker Law is to provide health services in accordance with professional standards, professional service standards, standard operating procedures and professional ethics as well as the health needs of health service recipients. What about the rights of health workers during the pandemic, which is also the obligation of patients suspected being exposed to COVID-19, namely to provide honest information. If neglected, it is possible that health workers will not be protected from exposure to the COVID-19 virus. Based on data compiled by the IDI mitigation team from PDGI, PPNI, IBI, PATELKI and IAI from March to mid-January 2021, there were total of 647 medical and health workers died due to corona virus infection. Consisting of 289 doctors (16 professors) and 27 dentists (3 professors), 221 nurses, 84 midwives, 11 pharmacists, 15 medical lab personnel.

Sometimes hospitals are not able to prepare sufficient Personal Protective Equipment (PPE) as one of the hospital obligations to protect their health workers while on duty. PPE is the right of health workers that must be fulfilled so that they can work according to standards. Service standards for the patients care in the category of infectious disease outbreaks must be equipped with PPE in accordance with established standards. Occupational health and safety in hospitals need to be considered to prevent negative impacts that will arise for health workers who are at the forefront of COVID-19 services. Hospitals in Indonesia that are Covid referrals have different facilities and infrastructure. Based on the varying capabilities of each hospital, the government must make covid service standard which is the basis of reference for health workers in COVID-19 services. This standard is also used as legal protection for health workers in the event of complaint. The government, in this case the Ministry of Health, has also issued various guidelines in the COVID 19 management, one of which is the Technical Guide to Hospital Services During the Adaptation of New Habits which regulates the flow of Covid-19 patient care, the division of risk zones for COVID-19 transmission in hospitals, the application of the principles of PPI is in the period of adapting to new habits, developing health service innovation system and strengthening referrals in the new normal²¹.

In the event of a complaint, the Covid service standards can be used as benchmark for service by health workers. If there is no intentional act that results in losses, the hospital must provide legal protection in accordance with Law No. 44 of 2009. Health workers and hospitals have legal relationship that can be seen from the pattern of work relationships that occur. The hospital is a corporation that applies the principle of vicarious liability, this principle implies that responsibility arises due to negligence made by health workers²². In the Guidelines for the

Prevention and Control of Corona Virus Disease (Covid-19)²³. Hospitals need to make policies regarding the protection of health workers, such as: a) If health workers are sick, they are not allowed to work. b) Maximum working time of 40 hours a week with 7-8 hours per day and not exceeding 12 hours. c) Monitoring the health of workers through Acute Respiratory Infection /ARI surveillance. d) Monitoring the health status of health workers on a regular basis. e) Employability assessment for officers with comorbid and special conditions. f) If the officer who will work after sick will be reassessed. g) Ensuring health insurance and occupational accidents for officers in health facilities. h) Determination of the presence of Occupational Illness (PAK) in officers who are exposed to COVID-19 at work (in accordance with the Decree of the Minister of Health No. HK.01.07/Menkes/327/2020 concerning Determination of COVID-19. If there is a health worker who suffers property loss in effort to control the epidemic will be given compensation and awards for the risks borne in carrying out their duties as stated in Articles 8 and 9 of Law Number 4 of 1984 concerning Outbreaks of Infectious Diseases²⁴. Article 9 of the Law on Outbreaks of Infectious Diseases is truly fair and commensurate with the risks faced by health workers.

Conclusion

There are still many factors that affect the hospital operations during pandemic that must be considered and improved by the hospitals.

1. The ease to administer hospital permits cannot show any evidence in controlling the quality and services of hospitals because there is no evaluation from independent body so that legal problems can occur in the future. Hospital licensing administration during pandemic should still be carried out at least through online because hospitals as legal subjects have obligations, one of which must have hospital operating permit.

2. The hospitals responsibility to protect health workers during the pandemic is still not carried out optimally due to the limitations of each hospital are different so it is necessary to make regulations including how to apply them based on laws and regulations and various guidelines and manuals issued by the Ministry of Health.

Ethical Clearance : Nil

Conflict of Interest: Nil

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Abstract

The Difference of EPDS Examination Results before and after Delivery in High-Risk Pregnant Woman at Unair Hospital

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Abstract

Background: Pregnancy is an exceptional condition for a woman who will become a mother. The prospective mother will experience a phase to continue the offspring, and depression is a complication that generally does not occur in childbirth. **Objective:** To determine the difference in the prevalence of depression through the results of the EPDS examination before and after delivery in high-risk pregnant women at Unair Hospital. **Method:** This research will use an observational analytic study design with a one-group pretest-posttest design strategy—data retrieval using primary and secondary data, namely by questionnaires and medical records at Unair Hospital. The sample in this study consisted of 23 multigravida pregnant women and one primigravida mother who sought treatment at the Outpatient Installation of Unair Hospital in March 2021 - May 2021. **Results:** The prevalence of pregnant women with a tendency to postpartum depression on the EPDS scores before and after delivery are 8% and 17%, respectively. There is a very weak positive correlation between pre-delivery EPDS scores and post-delivery EPDS scores. **Conclusion:** There is no significant difference between the scores before and after delivery in a high-risk pregnant woman at Unair Hospital.

Keywords: EPDS Score, High-Risk Pregnant Woman, Pre and Post Partum.

Introduction

Perinatal mental disorders refer to disorders that are common during pregnancy and one year after delivery. The postpartum time frame is debatable as most investigators use a period ranging from 4 weeks to 3 months postpartum. In addition, disorders that occur before pregnancy, or recur with disorders during pregnancy or in the postpartum period, are all considered perinatal mental illnesses¹. Depression postpartum generally does not occur in childbirth

but is quite often reported in women. Women have twice the risk of depression as men, and it is often associated with severe symptoms². Studies show that the factors associated with postpartum depression can be classified into four broad categories: risk factors for psychiatric, obstetrics, social factors, and lifestyle factors. This mix of factors makes women vulnerable to postpartum depression³.

There is study estimated that one in seven women globally has experienced postpartum depression. However, depression is often underdiagnosed and untreated⁴. The recorded prevalence of postpartum depression ranges from as low as 0.5% to 60% globally. The prevalence for countries in Asia ranges

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from 3.5% to 63.3%, with Malaysia as the lowest and Pakistan occupying the highest position⁵. In Indonesia, the prevalence of postpartum depression is estimated at 22.3%⁶. There is a study that says that the prevalence of antenatal depression risk in Surabaya reaches 18.95%. This value beats infection, which is the third cause of death (11%) in childbirth⁷.

Postnatal depression can be detected as early as possible through the Edinburgh Postnatal Depression Scale (EPDS). The use of routine screening aims to identify symptoms of depression effectively, simply, and economically in women at risk for postpartum depression. In the postpartum period, the EPDS has become the most widely used instrument to identify postpartum depression, and the results of this screening can be used as an additional clinical test⁸. The purpose of this study was to carry out early detection to find out the symptoms of depression in pregnant women on the front lines and to determine the difference in the results of the EPDS examination before and after delivery in high-risk pregnant women.

Materials and Methods

In this research design, the data used primary and secondary data. Primary data was obtained by filling out the Edinburgh Postnatal Depression Scale (EPDS) to measure the level of depression before and after childbirth. In contrast, secondary data was obtained through medical records as a requirement for the sample used in this study. The women will be given a pre-test or initial test to determine the development of the mother during pregnancy. After the subject has passed the labor for two weeks, a post-test or final test will be given to determine the extent of the mother's development after labor. The data processing is done through editing, coding, tabulating and data entry stages using SPSS 26.0 software. A statistical test was carried out to find out the difference in EPDS scores before and after labor in high-risk pregnant women.

The sampling will be carried out through consecutive sampling techniques. It means all women must meet the inclusion and exclusion criteria and will be included in the study. The inclusion criteria are 36–42 weeks gestation, have a high and very high-risk pregnancy, the baby is alive, have a married status and living husband, live in the same house, the prominent family does not face financial problems, the primary family does not have work problems, does not have mental disorders, and not feeling grief in the last three months.

One of the most widely used in clinical practice is the Edinburgh Postnatal Depression Scale (EPDS) score. It showed a sensitivity of 92% and a specificity of 72% for a cut-off of 8 as well as a sensitivity of 81% and a specificity of 88% for a cut-off of 11⁹. The Edinburgh Postnatal Depression Scale (EPDS) was initially developed in the UK. The EPDS is one of the commonly used screening instruments to assess the symptoms of Perinatal Common Mental Disorder (PCMD) in depression and anxiety¹⁰. EPDS is used to assess the level of depression of postpartum women from the side of the race, ethnicity, and socioeconomic background of the risk of postpartum depression. The EPDS can be used for approximately seven days postpartum to six weeks and includes ten questions¹¹. The use of routine screening aims to identify symptoms of depression effectively, simply, and economically in women at risk for postpartum depression; the results of this screening can be used as an additional clinical test¹⁰.

Scores ranging from 0-30 suggest using 12/13 as the cut-off score can be used for clinical screening, but also recommend a lower cut-off score (9/10) for use in community samples¹². A lower cut-off was found in other EPDS whose population does not speak English, i.e., Cut-off the optimal 8/9 off in Myanmar when combining the probability of major and minor depression¹³, the 8/9 cut-off was also performed in

Greek, Japanese, and Croatian^{14,15,16}, and in the United Kingdom for EPDS English and Punjabi or Urdu also use a cut-off of 8¹⁷. In this study, we use an 8/9 cut-off point on the EPDS, which has been translated and validated in Bahasa Indonesia

Result and Discussion

Table 1 Results of the Wilcoxon Signed Rank Test Difference Test on EPDS Examination Results Before and After Labor in High-Risk Pregnant Women

	N (Total)	Median	p Score
		(Minimum-Maximum)	
EPDS Before Labor	24	2.50 (0 - 8)	0.295
EPDS After Labor	24	2.00 (0 – 9)	

Wilcoxon Signed Rank Test; 12 sample scores decreased, 7 remained, and 5 increased

A statistical test was carried out to find out the difference in EPDS scores before and after delivery in pregnant women. The normality test of EPDS score before and after labor using the Saphiro-Wilk test was not normally distributed ($p < 0.05$). Furthermore, a different test will be carried out using the Wilcoxon Signed Rank Test (Table 1). However, the difference test also did not show a significant difference between scores before and after delivery ($p= 0.295$)

Table 2 Data frequency of research variable

Variables	Category	Total	%
Age	≤ 16 years	0	0
	17-35 years	14	58
	≥ 36 years	10	42
Last Education	Primary School	7	29
	Junior High School	5	21
	High School	9	38
	Higher Education	3	12
Profession	Housewife	17	71
	Employed	7	29
Types of Delivery Methods	C-Section	17	71
	Vaginal Delivery	7	29
Baby Weight	≤ 2999 gram	8	33
	3000 – 3499 gram	10	42
	≥ 3500 gram	6	25
Baby Length	≤ 49 cm	17	71
	≥ 50 cm	7	29
EPDS Score Before Labor	≥ 8	2	
	< 8	22	
EPDS Score After Labor	≥ 8	4	
	< 8	24	

Based on Table 2, 24 total pregnant women met the inclusion criteria determined during the study, which sought treatment at the Outpatient Installation of Unair Hospital in March-May 2021. EPDS results can detect depression in pregnant women without a significant difference in diagnostic value with the postpartum population⁹. This study tried to compare the EPDS screening scores performed before and after delivery. A study in Iran with a population of women giving birth with a history of high-risk pregnancies even reported a higher prevalence of depressive episodes, namely 68.0% consisting of 15.5% mild depression, 23.5% moderate depression, and 29% major depression¹⁸. The prevalence of depressive episodes, both major and minor, reaches 6.5 – 12.9% during pregnancy and 19% of postpartum mothers experience major depressive episodes in the three months postpartum¹⁹; This can be a rationale for screening for postpartum depression in the perinatal period, including before delivery. This study found that on examination of the EPDS score before delivery, the prevalence depression of high-risk pregnant women reached 8.33%. Previous studies have unfortunately reported the prevalence of postpartum depression in normal pregnancies. However, when looking at the data on normal pregnancies, this is slightly lower than the previous systematic review reports that reported the prevalence of depression reaching 20% in teenage pregnancies and 10-25% in adult pregnancies^{20,21}.

The results showed no statistically significant difference between the EPDS score before delivery and the EPDS score after delivery. These results can be understood that an increase in the EPDS score before delivery may predict an increase in the EPDS score after delivery. The score represents the possibility of postpartum depression. The current study reported the prevalence of postnatal depression tendencies in high-risk pregnant women using the EPDS score and found that 16.67% of high-risk pregnant women had a predisposition to postpartum depression (EPDS \geq 8),

its increase two times compared to the pre-tested prevalence of depression before labor. Its similar to a study in Ethiopia with 308 subjects also reported a prevalence of postpartum depression of 15.6%²².

The increasing prevalence of depression tendencies is a possible phenomenon, especially for mothers with high-risk pregnancies. A history of previous depressive episodes is one of the main risk factors that increase the risk of postpartum depression^{23,24}. A systematic review reported that mental disorders such as depressive episodes occurring during pregnancy are strong predictors of postpartum depression²⁵. This may explain the increasing prevalence of depression in high-risk pregnant women before and after delivery, coupled with the risk factors for the pregnancy itself, which can also predispose to the emergence of postpartum depression³. This is supported by the findings in a systematic review and meta-analysis, which found that cesarean section procedures increased the risk of postpartum depression 1.15 – 1.36 times²⁶. The same thing was also reported in 2017, which showed that there was an increased risk of postpartum depression in patients with cesarean section²⁷.

Meanwhile, the observed decrease in EPDS scores can also be explained by the same approach. A study of pregnant women's perceptions of cesarean section in Ghana showed that subjects had positive perceptions of cesarean section and its role in reducing perinatal and neonatal mortality²⁸. This actually provides an alternative explanation that is different from the previous review because cesarean section can act as a reliever factor from the triggers of depression and anxiety that he experienced before the delivery process. Furthermore, similar to the approach used to explain the increased risk of postpartum depression, acceptance of the newborn, adequate self-esteem and parenting skills, good coping mechanisms for pregnant women, positive perception

of cesarean section, exemplary implementation of exclusive breastfeeding, Adequate nutrition, adequate socioeconomic support and empathy from family and the surrounding environment, a good lifestyle such as eating balanced nutritious foods and regular exercise according to ability can have a positive impact and reduce the risk of postpartum depression³.

Each pregnant woman has very different predisposing factors, especially in relation to the complexity of social life in Indonesia as a developing country. The factors such as maternal insight, self-esteem, coping mechanisms, social and environmental support are different that can explain the findings of increased EPDS scores, persistent EPDS scores, or decreased EPDS scores in high-risk pregnant women with cesarean section.

Conclusion & Acknowledgment

In conclusion, This study showed that the prevalence of pregnant women with a tendency to postpartum depression on the EPDS scores before and after delivery were 8% and 17%, respectively. In this study, there was a fragile positive relationship between the EPDS scores before delivery and the EPDS after delivery. Then the statistical tests found that the data were not normally distributed. The results of the different tests did not show any significant difference between scores before and after delivery. Various efforts and preventive measures can be prepared and carried out so that postpartum depression does not occur with minimal severity and does not interfere with daily activities.

Conflict of Interest: There was no conflict of interest in this study

Ethical Clearance: This study had been approved by the Research Ethics Committee of Airlangga University Hospital, Surabaya, Indonesia.

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The Analysis of Personal Protective Equipment Covid-19 in The Hospital Sallelwangan, Maros Regency 2020

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Abstract

Purpose: to analyze of the use of Covid-19 Personal Protective Equipment on health workers at Salewangang Hospital, Maros Regency.

Methods: The type of research used is the analytical survey method of research conducted without intervention to the research subject. Data collection regarding free and dependent variables was carried out online using Google Form and the results of Google Form data collection

Results: Availability of personal protective equipment has a significant effect on the behavior of using personal protective equipment Covid-19, Knowledge of health workers has a significant effect on behavior of using personal protective equipment Covid-19, Attitudes of health workers have a significant effect on behavior of using personal protective equipment Covid-19 in health workers at the Salewangang Hospital, Maros Regency with a sig. 0.000

Conclusion: there is a relation between personal equipment on helath workers at the sallewangan hospital at maros regency

Keywords: *covid-19, Actitute, Behavior*

Introduction

Based on data from the International Labor Organization (ILO) in 2013 it is known that every year 2.34 million people die from work-related illnesses and accidents and around 2.02 million cases die due to work-related diseases. In Indonesia, the current picture of occupational diseases, such as the "Peak of the Iceberg" phenomenon, occupational diseases that are known and reported are still very limited and partial based on research results so that they do not

describe the magnitude of occupational safety and health problems in Indonesia. This is because human resources capable of diagnosing occupational diseases are still lacking so that services for occupational diseases are not optimal.¹

Corona Virus Disease 2019 (COVID-19) due to work as an occupational disease that is specific to a particular job is a Corona Virus Disease 2019 (COVID-19) disease that is suffered or causes death to a worker whose duties/work processes are directly related to exposure The SARS-COV-2 coronavirus is quite high. In an outbreak condition and the establishment of a Public Health Emergency, health services and efforts to handle COVID-19 areHealth workers can protect themselves when caring for

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patients by adhering to infection prevention and control practices, which include administrative, environmental and engineering controls as well as the proper use of Personal Protective Equipment (PPE) (i.e. proper selection of the appropriate type of PPE, how to use it, how to remove it and how to remove it). method of disposal or washing of PPE). Health workers need to be reminded that the use of PPE is only one aspect of infection prevention and control measures. In treating COVID-19 patients, health workers are very vulnerable to infection, so the PPE used is standard PPE based on a risk assessment ^{2,3}.

Personal protective equipment (PPE) is a device that is designed as a barrier against the penetration of substances, solid particles, liquids, or air to protect the wearer from injury or the spread of infection or disease. When used properly, PPE acts as a barrier between infectious materials (e.g. viruses and bacteria) and the skin, mouth, nose, or eyes (mucous membranes) of healthcare workers and patients^{4,5}. The barrier has the potential to block transmission of contaminants from blood, body fluids, or respiratory secretions. In addition, other infection control practices such as washing hands, using alcohol-based hand sanitizer, and covering the nose and mouth when coughing and sneezing with the inside of the upper arm or a tissue, can minimize the spread of infection from one person to another. The effective use of PPE includes the proper transfer and/or disposal of contaminated PPE to prevent exposure of the wearer and others to infectious materials ^{6,7,8}

Materials and Methods

The method used in this research is an analytic survey, namely research conducted without intervention on the research subject. Based on the

approach, this study uses a Cross Sectional approach because the cause and effect variables that occur in the object of research are measured or collected at the same time and carried out at the same situation.

To collect data in this study using an online questionnaire (Google Form). Before answering/filling out the Google Form, respondents will be asked to read and sign the Informed Consent electronically (by clicking on the option "Yes, I agree, I agree". Next, provide some information in the form of age, gender, education level, length of work (in years), the type of health worker (for example, doctors, nurses, pharmacists, etc.), the location of health services, and the length of work in a day (in hours), then answer/fill in an online questionnaire about the analysis of determinants of the use of COVID-19 PPE for health workers Salewangang Hospital, Maros Regency.

Result

Validity and Reliability Test Results

Validation and reliability tests were carried out by testing the results of the google form. The 30 initial respondents in the google form contained 12 questions that were tested using the SPSS program. The 20 tests were needed to ensure that the google form used in the study was able to measure research variables properly and the results of the validity and reliability tests were carried out. can be seen in the attachment. Based on the validity and reliability test results in the appendix, it can be seen that all questions are valid where all Corrected Item-Total Correlation values of each question have a value above the r table value of 0.361. While the results of the reliability test of each question are very good with Cronbach's Alpha value greater than 0.60.

Table 1: Characteristics Respondent for Health Workers in The Hospital

The Characteristic	n	%
≥ 30Years	45	22
31– 40Years	98	49
≤ 41Years	56	28
sex		
Man	61	30
Women	138	69
Education		
SMP/SMA	38	19
Diploma/S1	156	78
S2/S3	5	2

Based on the table. 1 characteristic of respondents, it is known that from 199 respondents, most were 31-40 years old (49.%). 138 people (69%) and the least male 61 people (30%). The educational level with the most diploma / S1 is 156 people (78%) and at least 5 people are S2 / S3 (2.5%). The maximum length of work is 6-10 years with 126 people (63%) and at least 10 years (7.5%).

Table 2 Characteristics of Type of Work Profession In the hospital

Location of work	n	%
Poliklinik	2	1
IGD	10	5
Laboratorium	10	5
Nutrition	10	5
asoka's treatment room	21	11
Teratai A treatment room	13	6.5
Obygn/KB	21	11
Isolation Covid	8	4
Operation theater/OK	5	2.5
ICU	10	5
Administraton	17	8.5
Pharmacy	15	7.5
Radiology	8	4
Fisioterapy	8	4
BPJSregistration	10	5
IPRS	25	13
Ambulance	6	3

Table 3: Research Variable Bivariate Test Results

Research variable	Personal Protective Equipment				Sign
	Good		Little		
	n	%	N	%	
personal protective equipment					
Good	174	92	15	8	0,000
little	10	100	0	0	
Knowledge					
Good	180	92	15	8	0,000
Litlee	4	100	0	0	
Actitude					
Good	166	94	10	6	0,000
Little	18	78	5	22	

Based on Table 3, it shows that respondents who have sufficient knowledge of good behavior in using PPE Covid-19 are 180 (92%) and less behavior in using PPE Covid-19 are 15 (8%). Respondents who had sufficiently good attitudes in the use of Covid-19 PPE were 166 (94.3%) and 10 (6%) had less behavior.

Discussion

First-level health workers such as doctors, nurses, pharmacists who provide health services are recommended to wear work clothes, gloves and a three-layer surgical mask. Second-level health workers are doctors, nurses, and analysts who work in treatment rooms, sampling, or virus testing laboratories. Health workers in this category must wear PPE in the form of gowns (surgical gowns), head coverings, disposable rubber gloves, three-layer surgical masks, and goggles

or eye protection. Third-level health workers are those at high risk, namely doctors and nurses who have direct contact with patients suspected of or confirmed positive for Covid-19. Third-level health workers are required to wear hazmat clothing or coveralls that cover all parts of the body, headgear, sterile surgical gloves, N95 masks, boots and face shields⁹.

Availability of PPE Covid-19 in the form of surgical masks (surgical/facemask), N95 masks, face shields (face shields), eye protection (goggles), gowns/gowns (disposable dresses and reusable gowns), aprons (apron), Gloves, protective headgear and protective shoes according to health care settings.

The increasing socio-economic level, knowledge and awareness of the community about their rights as the use of health services and progress in the field

of hospitalization, spur hospitals to further improve services. Quality of service that meets standards is an absolute must. For this reason, the Salewangang Hospital continued to change itself and at the end of 2017 again participated in the accreditation assessment survey process which was assessed by the Hospital Accreditation Committee on 06 - 09 December 2017. The improvement efforts that had been carried out finally produced results that did not disappoint Salewangang Hospital was declared accredited with the title plenary session^{4,6}.

Attitude is a form of evaluation or reaction of feelings, a person's attitude towards an object is a feeling of support or partiality (favorable) or feelings of being unsupportive or unfavorable to the object. Among the various factors that influence the formation of attitudes are personal experience, culture, other people who are considered important, mass media, educational institutions and religious institutions, as well as emotional factors within the individual¹⁰. At SalewangangMaros Hospital, in providing services, providing facilities needed by suspected and confirmed Covid-19 patients, always carrying out various health protocols when treating Covid-19 patients by protecting themselves as much as possible with PPE according to standards, having sufficient knowledge to handle Covid-19 patients. , following strict requirements to take off official clothes, leave the hospital or meet with family/community, treat with sincerity, even fight for life to treat Covid-19 patients¹¹.

Conclution

1. The availability of personal protective equipment has a significant effect on the behavior of using Covid-19 personal protective equipment on health workers at the Salewangang Hospital, Maros Regency with a sig. 0.000 0.05 and the OR (Odds Ratio) value is 11.6. This shows that the availability of PPE has an 11.6-fold effect on the behavior of

using personal protective equipment Covid 19

2. The knowledge of health workers has a significant effect on the behavior of using Covid-19 personal protective equipment at the Salewangang Hospital, Maros Regency with a sig. 0.000 0.05 and the OR (Odds Ratio) value is 12. This shows that knowledge has a 12-fold effect on the behavior of using personal protective equipment Covid 19.

3. The attitude of health workers has a significant effect on the behavior of using Covid-19 personal protective equipment on health workers at the Salewangang Hospital, Maros Regency with a sig. 0.000 0.05 and the value of OR

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Ethical Clearance- taken from Comitee ethical Universitas Muslim of Indonesia Makassar

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Sensorineural Hearing Loss and Cochlear Outer Hair Cell Function Nasopharyngeal Carcinoma Due to Influence of Cisplatin

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Abstract

Background: Nasopharyngeal carcinoma is a type of tumor sensitive to chemotherapy and radiotherapy. One of the various chemotherapy drugs is cisplatin. However, the cisplatin effects on sensorineural hearing loss and cochlear outer hair cell dysfunction in patients with nasopharyngeal carcinoma have not been evidently discovered. **Objective:** This research aims to prove the cisplatin effects on sensorineural hearing loss and cochlear outer hair cell dysfunction in patients with nasopharyngeal carcinoma. **Materials and Methods:** This research adopted analytical observation by employing a prospective cohort study approach. In addition, the sampling technique implemented consecutive sampling. This research was conducted at the ENT-HN Outpatient Unit (URJ) of the Neuro-otology Division of Dr. Soetomo Public Hospital during September-November 2020 period. The auditory test was executed by Pure-Tone Audiometry (ANM) and Distortion Product Otoacoustic Emission (DPOAE). Meanwhile, the statistical analysis was assessed by the Wilcoxon and McNemar test. **Results:** This research involved 22 samples. The cumulative dose of cisplatin up to chemotherapy series III ranged from 260-270 mg with an average of 265.45±5.10 mg. The results of the ANM test before and after chemotherapy series III employing Wilcoxon test indicated significant differences in frequency of 500 Hz (p-value = 0.014), 6000 Hz (p-value = 0.011), 8000 Hz (p-value = 0.019), 10000 Hz (p-value = 0.000), and 12500 Hz (p-value = 0.002). The frequency of 125 Hz with a p-value = 0.343, the frequency of 250 Hz with a p-value = 0.690, the frequency of 1000 Hz with a p-value = 0.179, the frequency of 2000 Hz with a p-value = 0.459, and the frequency of 4000 Hz with a p-value = 0.125 indicated no significant difference with a p-value greater than 0.05. Meanwhile, the DPOAE test results before and after chemotherapy series III utilizing the McNemar test demonstrated the frequency of 1000 Hz (p-value = 1.000), 2,000 Hz (p-value = 0.453), 4000 Hz (p-value = 1.000), 6000 Hz (p-value = 0.388), 8000 Hz (p-value = 0.754), and 10000 Hz (p-value = 1.000). The comparative analysis of the DPOAE test results before and after chemotherapy Series 3 suggested no significant difference, with a p-value greater than 0.05 at all frequencies. **Conclusion:** There were cisplatin effects on sensorineural hearing loss in patients with nasopharyngeal carcinoma after chemotherapy series 3 based on ANM test at the frequencies of 500 Hz, 6000 Hz, 8000 Hz, 10000 Hz, and 12500 Hz. There were no cisplatin effects on cochlear outer hair cell dysfunction in patients with nasopharyngeal carcinoma after chemotherapy series 3.

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Keywords: cisplatin, sensorineural hearing loss, cochlear outer hair cell dysfunction, nasopharyngeal carcinoma

Introduction

High-frequency sensorineural hearing loss and cochlear outer hair cell dysfunction are some of the clinical manifestations of ototoxicity after cisplatin administration.¹Ototoxicity is a condition where there is damage to the cochlea or the vestibular apparatus, caused by exposure to chemicals, including drugs.²Hearing loss may occur at lower frequencies if the chemotherapy is continued. One of the causes of hearing loss severity is the cumulative dosage of cisplatin. Cisplatin administered at a high dose of 100-120 mg/m² based on body surface area, can cause progressive, irreversible, and bilateral sensorineural hearing loss starting at a frequency of 8000 Hz.³

Cisplatin damages the cochlea outer hair cells progressively from the base to the apex, which causes sensorineural hearing loss at high frequencies. Ototoxicity occurs through necrosis, apoptosis, or a combination of both. Cisplatin causes an increase in reactive oxygen species (ROS), which will trigger apoptosis. Apoptosis causes the death of the cochlea outer hair cells, resulting in sensorineural hearing loss.³

Research conducted in Tamil Nadu, India, in 2018, reported that 63% of nasopharyngeal carcinoma patients experienced hearing loss due to cisplatin use, but 37% of patients did not experience hearing loss. An audiometry test was performed after administration of chemotherapy series 3 and series 6.³ Another study in India reported that after receiving cisplatin administration, 22% of the patients experienced hearing loss at a frequency of 4000 Hz to 6000 Hz and 71% of the patients experienced it at a frequency of more than 8000 Hz.⁴

Auditory tests to detect the cisplatin effects on sensorineural hearing and cochlear outer hair cell dysfunction employs pure tone audiometry (ANM) and distortion product otoacoustic emission

(DPOAE). The degree of hearing loss was determined by calculating the hearing threshold of air conduction (AC) on pure tone audiometry. Distortion product otoacoustic emission could evaluate the cochlear response at high frequency, a sensitive frequency for detecting ototoxicity.⁵

This study aims to prove the effect of cisplatin on sensorineural hearing loss and identify the function of outer hair cells in patients with nasopharyngeal carcinoma.

Materials and Methods

The research was done on an analytic observational basis. The research design utilized a prospective cohort study. The study population included nasopharyngeal carcinoma patients, who received cisplatin chemotherapy up to series 3 at the "Teratai" Surgical Inpatient Care Unit of ENT-HN Polyclinic at RSUD, headed by Dr. Soetomo Surabaya from September until November 2020. The research sample covered all accessible populations that met the inclusion and exclusion criteria. The inclusion criteria required patients aged from 18 to 60 years old, while the exclusion criteria included patients with nasopharyngeal carcinoma with hypertension and diabetes mellitus and patients with nasopharyngeal carcinoma who had received radiotherapy. Additionally, the criteria for dropout patients included the replacement of the cisplatin chemotherapy regimen by another platinum-based group (carboplatin). Sampling was conducted by consecutive sampling.

This research has fulfilled ethical clearance (Ref. No: 0112/LOE/301.4.2/IX/2020). The variables analyzed included sensorineural hearing loss and the functions of outer hair cells. The procedure of this study encompassed recording and examination of ANM and DPOAE before and after cisplatin chemotherapy series 3. The cumulative dose of

cisplatin until the chemotherapy series 3 in patients with nasopharyngeal carcinoma in this study ranged from 260 to 270 mg with a mean value of 265.45 + 5.10 mg.

Sensorineural hearing loss is a hearing loss in one ear caused by damage to outer hair cells in the cochlea. The examination involved pure tone audiometry at each frequency. The audiometer employed in this study was Astera II of Madsen, produced in Denmark in 2017. Audiogram of AC and BC values reached more than 25 dB which coincide (negative air-bone gap). The ANM examination resulted in an audiogram with AC and BC curves. AC examination was performed at the frequencies of 125 Hz, 250 Hz, 500 Hz, 1000 Hz, 2000 Hz, 4000 Hz, 6000 Hz, 8000 Hz, 10000 Hz, and 12500 Hz, while BC examination was at the frequencies of 250 Hz, 500 Hz, 1000 Hz, 2000 Hz, and 4000 Hz. The PTA examination was performed before receiving chemotherapy series 1 and after chemotherapy with cisplatin series 3.

Cochlear outer hair cell dysfunction is a disorder

of the function of transmitting sound waves from the inner ear to the auditory nerve cells. The cochlear outer hair cell dysfunction was detected through the DPOAE examination. The DPOAE examination in this study was performed at six frequencies, namely 1000 Hz, 2000 Hz, 4000 Hz, 6000 Hz, 8000 Hz, and 10000 Hz. The criteria for the examination results were "pass" and "refer."

All data collected in data collection sheets were then arranged in a tabular form and then analyzed statistically. Analysis of the effect of cisplatin on sensorineural hearing loss and impaired cochlear outer hair cell function in patients with nasopharyngeal carcinoma utilized Wilcoxon and McNemar statistical tests. The results of this study were determined by a significance level (α) of 0.05.

Results and Discussion

The results indicated more male patients than females with a ratio of 4.5:1 (Table 1). The age distribution of the samples displayed that most of the patients belonged to the 40-50 year age group.

Table 1. Age and Sex distribution of the Karsinoma Nasofaring patients

Age	n	%
<20	1	4.55
>20 - 30	1	4.55
>30 - 40	2	9.10
>40 - 50	12	54.55
>50 - 60	6	27.25
Total	22	100
Gender		
Male	18	81.82
Female	4	18.18
Total	22	100

Table 2. Sensorineural hearing loss before and after chemotherapy series 3

Before chemotherapy	After chemotherapy		Total	Percentage (%)
	Normal	SNHL		
Normal	1	8	9	40.91
SNHL	0	13	13	59.09
Total	1	21	22	100.00
Percentage (%)	4.55	95.45	100.00	

Sensorineural hearing loss before chemotherapy was detected in 13 patients (59.09%), and normal hearing was detected in 9 patients (40.91%). Sensorineural hearing loss before chemotherapy series 3 was detected in 21 patients (95.45%), and normal hearing was detected in 1 patient (4.55%).

Table 3. Comparison of PTA examination results before and after chemotherapy Series 3

Frequency (Hz)	Shapiro-Wilk		Wilcoxon (p)
	Before chemotherapy (p)	After chemotherapy series 3 (p)	
125	0.000	0.000	0.343
250	0.002	0.000	0.690
500	0.000	0.002	0.014*
1000	0.003	0.092	0.179
2000	0.057	0.001	0.459
4000	0.049	0.166	0.125
6000	0.043	0.443	0.011*
8.000	0.017	0.006	0.019*
10000	0.000	0.004	0.000*
12500	0.000	0.000	0.002*

Comparative analysis of PTA examination results before and after chemotherapy series 3 using the Wilcoxon test indicated significant differences at frequencies of 500 Hz ($p = 0.014$), 6000 Hz (p -value = 0.011), 8000 Hz (p -value = 0.019), 10000 Hz (p -value = 0.000) and 12500 Hz (p -value = 0.002). The frequency of 125 Hz with a p -value of 0.343, the frequency of 250 Hz with a p -value of 0.690, the frequency of 1000 Hz with a p -value of 0.179, the frequency of 2000 Hz with a p -value of 0.459, and the frequency of 4000 Hz with a p -value of 0.125 indicated no significant difference, where the p -value of was greater than 0.05.

The age of the patients as the samples ranged from 18-60 years. The minimum age was determined based on the lowest age for adult patients, while the maximum age of 60 years was applied to avoid bias from presbycusis. Presbycusis is a hearing loss due to a degeneration process at 65 years or more, characterized by decreased hearing sensitivity in both ear.⁶

The most affected age group ranged > 40-50 years. This age group was the initial age for exposure to carcinogenic agents. That is because it takes several decades to develop malignant cells of nasopharyngeal carcinoma until they appear clinically. Therefore, exposure to carcinogens has a significant effect on the incidence of malignancy. Old age affects the incidence of head and neck malignant tumors, related to a decrease in physiological capacity and a reduced ability to deal with environmental stress. Thus, they are easily exposed to oncogenic viruses, carcinogenic substances, and other environmental factors.⁷

The cumulative dosage of cisplatin to chemotherapy series 3 in patients with nasopharyngeal carcinoma in this study ranged from 260 to 270 mg. The cumulative dosage of cisplatin given was an important factor in chemotherapy. The cumulative dosage of cisplatin providing chemotherapy effects

to nasopharyngeal carcinoma was an average of 200 mg/m Cisplatin administered at a high dosage of 100-120 mg/m² based on body surface area could cause progressive, irreversible, and bilateral sensorineural hearing loss starting at a frequency of 8000 Hz.³ The cumulative dosage of cisplatin also affected the severity of the hearing loss. An audiometry test was performed after administration of chemotherapy series 3 and series 6.³

An audiometry test was performed after administration of chemotherapy series 3 and series 6.³ It was in accordance with the literature stating that high-frequency sensorineural hearing loss and cochlear outer hair cell function were one of the clinical manifestations of ototoxicity after cisplatin administration.^{1,3}

High-frequency hearing loss is synonymous with SNHL because of damage to the cochlear hair cells. Acoustic energy entered the cochlea through the footplate of the stapes at the foramen ovale and was amplified in the perilymph of the scala vestibuli. Sound waves traveled along the basilar membrane from the base to the apex. The High-frequency sound waves (10000 Hz) traveled maximally at the base and did not reach the apex. However, the low-frequency sound waves (125 Hz) could travel up to the apex. Sound waves travel caused deflection of the stereocilia, causing ion channels at the ends of the stereocilia to open and close. This explained that high-frequency sounds were sensitive at the base of the cochlea and low frequencies at the cochlear apex. It caused hearing loss which usually occurs at high-frequency first and then the low-frequency.⁸

The analysis of the DPOAE examination result before and after cisplatin chemotherapy series 3 utilized the McNemar test on all frequencies indicated no significant difference. This result contrasts with the study of Teotia et al. reported that 90.00% of patients had ototoxic on DPOAE examination after cisplatin

chemotherapy series 3. The study of Eiamprapai et al. suggested that the administration of cumulative dose in laboratory animals causes cochlear outer hair cells loss on cochlea basal turn. Therefore, it more apparent at high frequency.^{9,10} These differences could be attributable to the dissimilarity in the sample and utilized tools size in this study. Many things could be the factor of induced ototoxicity, namely cumulative dose more than 400mg, long-term administration of greater than or equal to 6 months, administration techniques, individual variations in susceptibility, extreme age, previous hearing loss, anemia, history of radiation exposure, and other ototoxic medication use.¹¹

Conclusion

There were cisplatin effects on sensorineural hearing loss in patients with nasopharyngeal carcinoma after chemotherapy series 3 based on ANM test at the frequencies of 500 Hz, 6000 Hz, 8000 Hz, 10000 Hz, and 12500 Hz. There were no cisplatin effects on cochlear outer hair cell dysfunction in patients with nasopharyngeal carcinoma after chemotherapy series 3.

Conflict of Interest: No conflict of interest.

Ethical Approval: All procedures performed in this study that involved human participants are in accordance with the ethical committee standards of Dr. Soetomo Hospital, Surabaya, with ethical number 0112/LOE/301.4.2/IX/2020.

Informed Consent

Informed consent was obtained from all samples of individuals included in this study.

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Enucleation of Scrotal Epidermoid Cyst: Case Report

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Abstract

Background: Epidermoid cyst is one of the most common forms of blockage in the pilosebaceous gland. The surgical procedure for excision of the cyst aims to prevent recurrence, however, several reports suggest that enucleation can be performed with certain considerations.

Case: A 22 year old man who lives in Wonogiri, came to Dermatology and Venereology Outpatient clinic of Dr. Moewardi General Hospital Surakarta, with the chief complaint of a lump in the scrotum. The lumps appeared since 3 years ago, the size of a pea seed and accompanied by itching. The results of the examination of the dermatological status of the scrotal region showed several nodules of the skin color, felt dense and rubbery without any signs of inflammation. Histopathology shows a basket wave type parakeratosis at the stratum corneum, while the epidermal layer shows cysts filled with keratin and surrounded by squamous cells. Based on the results of the history, physical examination and histopathology, the diagnosis of the patient was a scrotal epidermoid cyst, then we performed two enucleation measures and found improvement.

Conclusion: Epidermoid cyst is a form of benign epithelial cyst that can occur in the scrotal area. The main treatment to prevent recurrences is surgery, where the enucleation technique can be performed with several considerations such as small size of the cyst without signs of inflammation, preventing dystrophic calcification and capsule routine.

Key words: enucleation, epidermoid cyst, scrotal, pilosebaceous gland, histopathology

Introduction

Epidermoid cyst or also known as epithelial cyst is one of the most common forms of blockage in the pilosebaceous gland. The clinical picture of epidermoid cysts is generally in the form of nodules

in the dermis or subepidermis layer containing keratin material with a punctum in the middle.¹ Epidermoid cysts are generally found in the head, face and back of the body, but in some cases can occur in the testes and scrotal skin.² The prevalence of scrotal epidermoid cysts worldwide is still very rare, but Kawai, et al in Japan reported that to date there have been 12 cases of scrotal epidermoid cysts.³

The pathogenesis of epidermoid cysts is still not clearly understood. Tanaka, et al stated that there are three hypotheses that underlie the mechanism of epidermoid cysts, 1) lesions arise from ectopic skin

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tissue due to tissue dislocation to the surrounding area, 2) teratoma consisting of a single layer of germ cells and 3) due to trauma to the epidermal tissue in the dermis and subcutis layer.⁴ The diagnosis of epidermoid cyst can be made based on the results of clinical examination, histopathology and ultrasonography to rule out cutaneous calcinosis, steatocystoma or testicular malignancy.⁵

The surgical procedure in the form of excision of the cyst aims to prevent recurrence, but Heidenreich, et al stated that enucleation can be performed in cases of benign multiple scrotal epidermoid cysts based on the results of histopathological and ultrasonographic examinations.⁶ Kawai, et al also stated that scrotal epidermoid cysts that did not expand to the testes and were small in size could be enucleated by removing the cyst capsule.³ Based on this description, we report a case of scrotal epidermoid cyst that received enucleation therapy so that it can increase the insight and skills of a dermatologist in the management of scrotal epidermoid cysts.

Case

A 22-year-old man who lives in Wonogiri, came for treatment at the Dermatology and Venereology Polyclinic of the Regional General Hospital (RSUD) Dr. Moewardi Surakarta with the chief complaint of a lump in the scrotum. The results of the autoanamnesis of the history of the present disease, since 3 years ago the patient complained of a lump in the scrotum the size of a pea, the color of the skin accompanied by itching. The patient then went to a general practitioner and received topical medication (the patient forgot the name of the medicine) but the complaints were slightly reduced. Two weeks later, similar skin complaints appeared which were increasing in number and felt very itchy, so the patient decided to seek treatment at the Dermatology and Venereology Polyclinic, RSUD Dr. Moewardi Surakarta for further treatment. The results of the autoanamnesis of previous medical

history, the patient had never experienced similar skin complaints before, while based on the patient's family history of disease stated no family members had similar skin complaints.

The results of physical examination of vital signs were within normal limits, while on examination of the dermatological status of the scrotal region, several skin-colored nodules were seen measuring 0.3 cm to 0.8 cm, palpable firm and supple without any signs of inflammation in the skin of the scrotal area (**Figure 1**). Based on the results of autoanamnesis and physical examination, the differential diagnosis of this case is epidermoid cyst and scrotal calcinosis cutis.

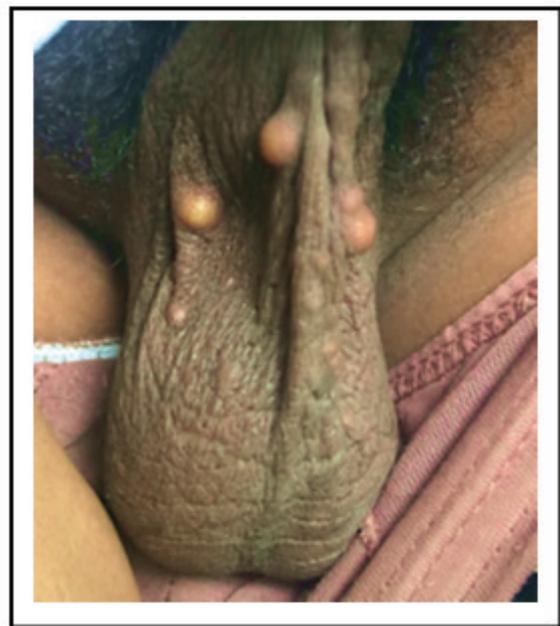


Figure 1. In the scrotal region, multiple skin-colored nodules are seen, which feel firm and firm without any signs of inflammation.

We performed histopathological examination to establish and rule out the differential diagnosis in this case. Cyst tissue from the scrotum was removed using the enucleation technique and then stained with Hematoxylin and Eosin (HE). The results of histopathological examination showed a basket wave-type parakeratosis on the stratum corneum, while in the epidermis layer there were cysts filled with keratin material

and surrounded by squamous cells (**Figure 2**). Based on the results of autoanamnesis, physical examination and histopathology, the patient's diagnosis was epidermoid kitsascrotalis.

We perform stepwise enucleation which is done once a week and twice. The steps of the enucleation procedure that we carried out were as follows (**Figure 3**):

1. Antiseptic action on the area to be enucleated using 10% povidone iodine solution by turning it outwards until it is outside the action area.
2. Perform local anesthesia by intradermal infiltration using Pehacain® solution (lidocaine 20 mg and epinephrine 0.0125 mg/ml) in 1 cc

3. Make a 3-4 mm long incision in the skin over the cyst.
4. Insert the tip of the hemostat into the incision hole, then a blunt dissection is performed and free the cyst capsule from the surrounding tissue.
5. After being free from the surrounding tissue, the cyst capsule was enucleated with an incision using a sharp curette tip.
6. After the cyst capsule is removed, an antibiotic ointment is applied and a pressure dressing is applied using sterile gauze to prevent the formation of a hematoma.

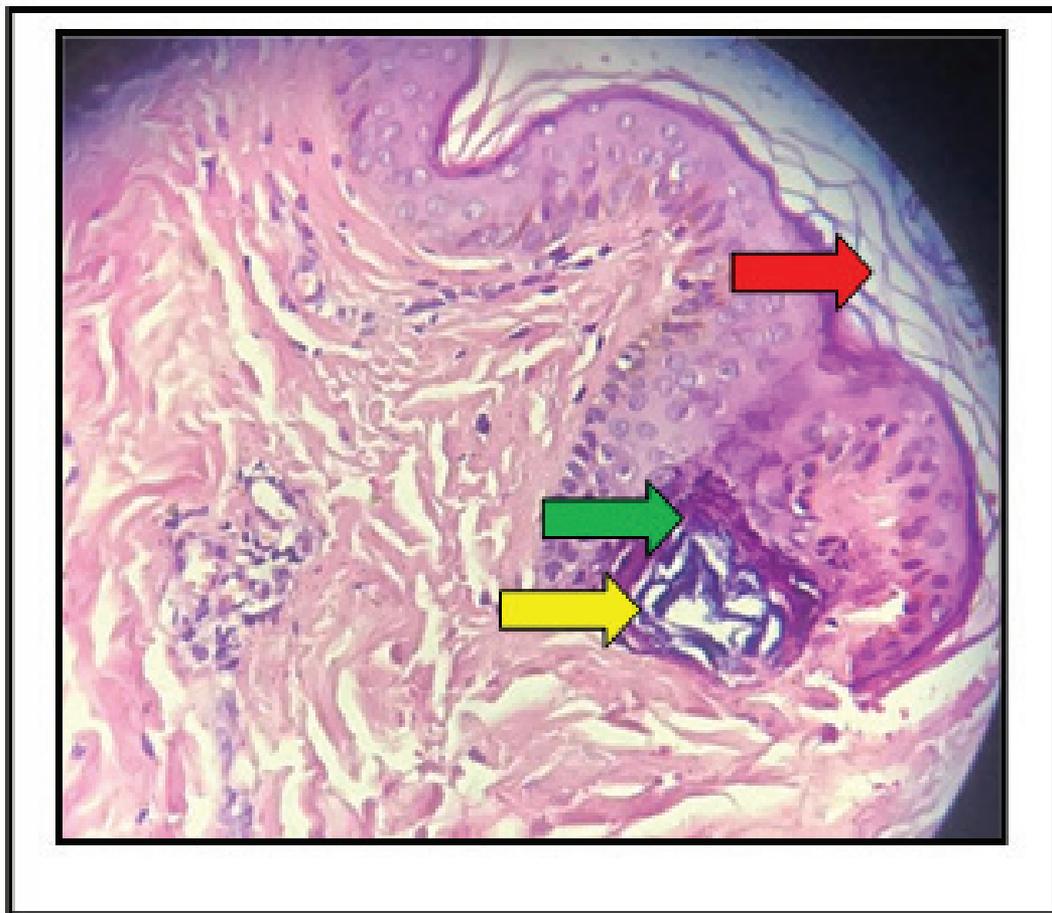


Figure 2. In the stratum corneum, basket wave-type parakeratosis appears (red arrows), while in the epidermis layer, cysts containing keratin material (yellow arrows) appear with surrounding squamous epithelium (green arrows). (HE 10x).

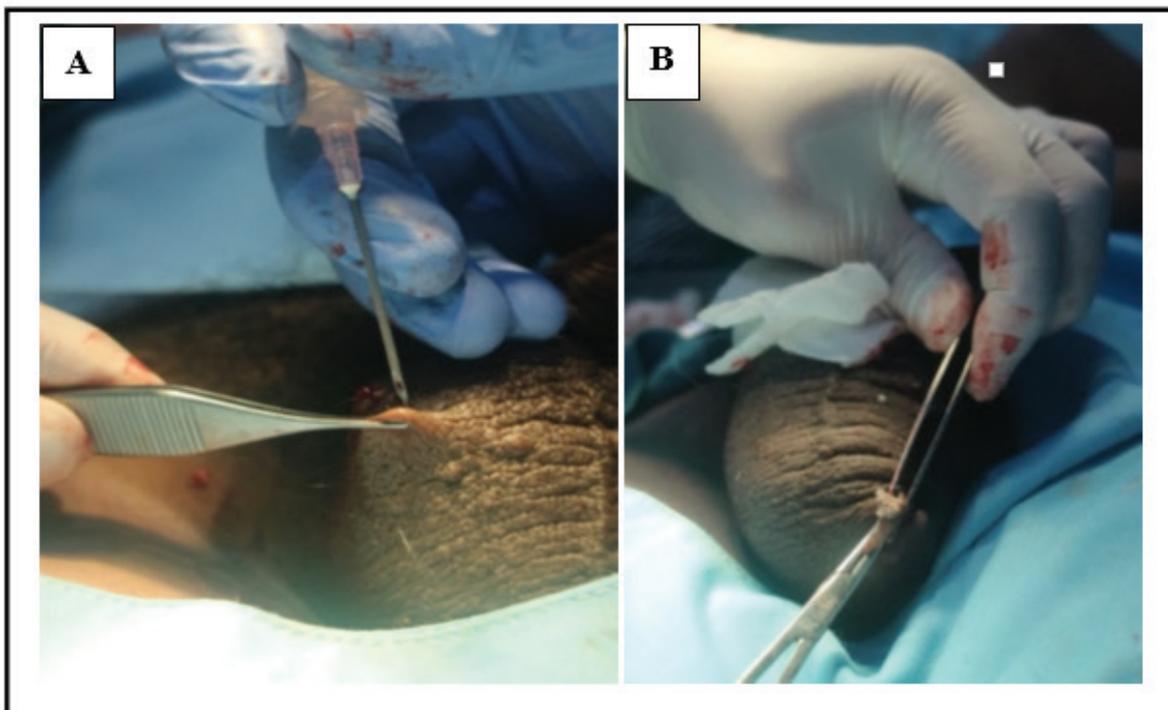


Figure 3. Enucleation procedure. (A) Local anesthetic technique (B) Enucleation of the cyst capsule.

Discussion

Epidermoid cyst is one of the most common forms of benign epithelial cysts. Most cases of lesions often occur in the head and body area, but in certain cases can be found in the scrotal area.⁷ The prevalence of scrotal epidermoid cysts is still unknown, but Trinh et al stated that the incidence is more common after puberty and is more common after puberty. found in men.⁸ Clinical manifestations of scrotal epidermoid cysts are characterized by solitary or multiple, skin-colored nodules of varying size without signs of inflammation in the skin of the scrotal area. Most cases are generally asymptomatic, but in some case reports it can be accompanied by itching.^{9,10}

In this case, the patient was a 22 year old male who complained of several lumps in the scrotum. Based on the history of the disease, the lump was initially the size of a pea, but within two weeks the lump had increased in size and was accompanied by

itching. The results of physical examination showed skin-colored nodules that were palpable and firm, and there were no signs of inflammation in the scrotal skin, so based on the results of the history and physical examination, the diagnosis of scrotal epidermoid cyst was supported.

Epidermoid cysts are the most common type of benign epithelial cysts found without the risk of developing into malignancy, so histopathological examination is needed to be sure. Histopathological features of epidermoid cysts are characterized by cysts lined by stratified epithelial cell walls resembling squamous epithelium, while the lumen contains keratin and a layer of stratum granulosum. In cysts that occur infection will show a picture of inflammatory cell infiltration around the cyst wall.^{11,12} The results of histopathological examination in this case showed a basket wave-type parakeratosis on the stratum corneum, while in the epidermis layer

there were cysts filled with keratin material and surrounded by squamous cells. The conclusion of the histopathological examination of this case supports the diagnosis of an epidermoid cyst.

The differential diagnosis of the case is scrotal calcinosis cutis, which is a benign skin disorder characterized by solitary or multiple nodules or papules which are generally asymptomatic. Nodules contain mostly calcium and phosphorus which can enlarge and expand progressively.¹³ The prevalence of the disease is still unknown, but it is often found in adolescents to adults.¹⁴ Clinical manifestations of scrotal calcinosis cutis in the form of nodules measuring 0.5-1.5 cm that are palpable solid, yellowish white and in some cases the skin of the scrotal area looks erythematous. Histopathological picture shows basophilic globules with calcium deposition in the epidermis or upper dermis layer.^{15,16} The main treatment for scrotal calcinosis cutis is elliptical surgical excision and complete excision which gives good cosmetic results.¹⁷

The results of the physical examination of the dermatological status in the scrotal area showed multiple skin-colored nodules without any signs of inflammation of the scrotal skin. The results of histopathological examination didn't reveal basophilic globules containing calcium deposition in the dermis and upper dermis, so that the differential diagnosis of scrotal calcinosis in this case could be ruled out.

Treatment of scrotal epidermoid cysts is generally complete surgical excision, but some reports suggest enucleation techniques in certain cases. Tela and Ibrahim stated that the technique of enucleation of scrotal epidermoid cysts can be performed on small lesions, whereas large lesions that spread throughout the scrotum are preferable to complete surgical excision up to scrotal reconstruction.¹⁸ Feinstein, et al stated that traumatized epidermoid cysts minor can cause dystrophic calcification or rupture, so

to prevent this, enucleation can be considered in cases of epidermoid cysts.¹⁹ Chhabra, et al stated that a modified enucleation technique in cases of epidermoid cysts can be performed for small lesions. The modified enucleation technique is performed by aspirating the contents of the cyst and then removing the capsule, which has been shown to be effective in preventing recurrence.²⁰

In this case, the epidermoid cyst was small between 0.3 cm and 0.8 cm without any signs of inflammation, so we decided to gradually enucleate it. The initial stage of enucleation is by infiltrating the skin around the treatment area using local anesthetic in the form of a Pehacain[®] solution (lidocaine 20 mg and epinephrine 0.0125 mg/ml) in a 1 cc syringe. The needle is inserted into the cyst cavity through the hair follicle hole, then the follicle orifice is gradually widened using a needle and other instruments. The cyst wall is pressed against the dilated hair follicle with the help of bent tweezers and removed using arterial clamps until the capsule is released. After the cyst capsule came out, an antibiotic ointment was applied and a pressure dressing was applied using sterile gauze.

Conclusion

Epidermoid cyst is a form of benign epithelial cyst that can occur in the scrotal area. The main treatment to prevent recurrence is surgery, where enucleation technique can be done with several considerations such as small cyst size without signs of inflammation, preventing dystrophic calcification and capsule rupture.

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Ethical Clearance: This study did not use ethical clearance

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Role of Fine Needle Aspiration Cytology in Evaluation of Cervical Lymphadenopathy

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Abstract

Introduction: Cervical lymphadenopathy is one of the commonest clinical presentations of patients of head and neck pathologies. Metastatic cervical lymphadenopathy is a common presentation in head and neck oncology. The aim of this study is to evaluate the effectiveness of FNAC as a primary investigation tool.

Aims and Objectives: The aim of the study was to evaluate the effectiveness of FNAC in diagnosing cervical lymphadenopathy.

Material and Methods: A study was conducted in the Department of Pathology and E.N.T., SIMS, Hapur, U.P., India from January 2019 to February 2020 which included 52 patients with cervical lymphadenopathy presenting to outpatient clinics. FNAC was done in patients of cervical lymphadenopathy before surgery or incision/excision biopsy and the results of FNAC was correlated with histopathological evaluation.

Results: In our study 88.47.% of sensitivity and specificity of 92.03% was found on FNAC of cervical lymph nodes.

Conclusion: FNAC remains a safe, cost effective and appropriate first line investigation though the results have to be confirmed with HPE for proper evaluation and management.

Keywords: *Fine Needle Aspiration Cytology, Cervical Lymphadenopathy*

Introduction

Most head and neck tumors may present to the clinician as neck nodes. Depending on the primary site, up to 80% of patients with upper aero

digestive mucosal malignancy will have cervical nodal metastasis at presentation. The occurrence of nodal metastasis has a profound effect on the management and prognosis of these patients. Cervical Lymphadenopathy is one of the commonest clinical presentations of patients, attending the outdoor clinics in most hospitals. The aetiology varies from an inflammatory process to a malignant condition¹. Fine needle aspiration cytology is a simple, quick and inexpensive method that is used to sample superficial

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masses like those found in the neck and is usually performed in the outpatient clinic. It causes minimal trauma to the patient and carries virtually no risk of complications. Masses located within the region of the head and neck can be readily diagnosed using this technique^{2, 3}.

FNAC is both diagnostic and therapeutic in a cystic swelling⁴. It is an initial and well established technique for the management of patients presenting with head and neck lumps.⁵⁻⁸ FNAC is helpful in differentiating between a malignant and a benign tumour with over 90% accuracy⁹. It is helpful in the work-up of cervical masses and nodules because biopsy of cervical adenopathy should be avoided unless all other diagnostic modalities have failed to establish a diagnosis¹⁰.

FNAC does not give the same architectural detail as histology but it can provide cells from the entire lesion as many passes through the lesion can be made while aspirating¹¹. Limitation of FNAC lies in the fact that it does not allow evaluation of the morphology of the lesion.

False-negative and false-positive results are known to occur in FNAC due to which a definitive diagnosis is necessary for cases with clinical suspicion.

The purpose of our study was to ascertain the reliability of FNAC in the diagnosis of cervical lymphadenopathy.

Materials and Method

This is a prospective study carried out at Department of Pathology and Otolaryngology, Saraswathi Institute of Medical Sciences, Hapur, Ghaziabad, Uttar Pradesh, from January 2019 to February 2020 which included 52 patients with cervical lymphadenopathy presenting to the outpatient clinics. FNAC procedures were done in all the patients before surgery after a thorough physical examination.

Palpable neck masses were aspirated using 23G needle and 20ml syringe. A negative pressure was applied to the syringe by pulling the syringe plunger and at least two dry specimens and two alcohol fixed specimens were taken. The alcohol fixed smears were immediately submerged in 95% ethyl alcohol. All the slides preparations were made by the trained staff of Pathology Department. Alcohol fixed smears were subsequently stained with Haematoxylin and Eosin stains.

The results of the FNAC were evaluated into inflammatory process, tubercular and malignant disorder.

Comparisons were made when patients were subjected to surgery and biopsies were sent to the Department of Pathology for evaluating the mass. After correlating the specimens with available histological findings obtained by surgery and from the patient's records, accuracy, sensitivity, specificity were calculated.

Results

The study included 52 patients with cervical lymphadenopathies. There were 32 female and 20 male patients with an age range of 14-68 years.

Out of 52 patients who underwent FNAC for cervical lymphadenopathy 34 (65.3%) came out to be inflammatory, 10 (19.2%) tubercular, 6 (11.5%) malignancy, and 2 (3.8%) came out inconclusive or as blood aspirate.

All the patients who underwent FNAC in the study group were subjected to excision/incision biopsy to confirm then diagnosis. Out of

34 inflammatory nodes reported in FNAC, 8 came out to be other lesions on HPE. The sensitivity for inflammatory nodes was found to be 90.9% while specificity was 84.61%. Sensitivity for tubercular lymphadenitis was found to be 88.8% and

specificity 95.65%, and for malignant nodes sensitivity was 85.71% and sensitivity 95.83%.

The overall sensitivity was 88.47% and specificity was 92.03%.

Discussion

Cervical lymphadenopathy often pose a challenging diagnostic problem to the clinician. Surgical biopsy is the commonest method of tissue diagnosis, FNAC is in practice since the 1930s. This method has become popular as a diagnostic step in the evaluation of a head and neck mass.¹²

In our study the cases of FNAC revealed 88.47% of sensitivity and specificity of 92.03%. less than 100% sensitivity of the cervical lymphadenopathies demonstrates the fact that we cannot always rule out disease by depending exclusively on FNAC. Sensitivity for tubercular lymphadenitis was found to be 88.8% and specificity 95.65%, and for malignant nodes sensitivity was 85.71% and sensitivity 95.83%.

According to a study the diagnostic accuracy of FNAC in metastatic disease varies from 87% to 97.9%¹³ and for lymphomas is 82%¹⁴. The sensitivity of FNAC for metastatic lesions to lymph nodes has varied from 97.9% to 100%, whereas the specificity has been found to be 100%¹⁵

For the lymphomas the sensitivity has been found to be 80% and specificity 100%¹⁵

Although the majority of procedures were performed by the pathologists, there were no statistical differences in the results of FNACs performed by an attending or by residents in the second half of their

residency. In other words, the learning period does not require years of experience and procedure is easy to master. Moreover, it can be performed in outpatient clinics, as was done in our study.

In about 75% of patients, a single FNAC was needed for evaluation and no further aspirate was required making this tool highly efficient for diagnosing masses in the head and neck region.

FNAC is only one step in the overall evaluation of patients with cervical lymphadenopathy, and one should not rely exclusively on FNAC results. Negative FNACs results in patients with a high index of suspicion for malignancy were further investigated by removing the suspicious mass surgically.

FNAC is a safer choice for initial evaluation of masses suspicious of malignancy as the frequency of needle seeding of tumor cells in the procedure is reported as low as 0.003-0.009%.¹⁶ Hence, considering this range, tumor risk in FNAC is almost nonexistent in comparison to excision biopsy.

To avoid or minimize false positive results various factors including regenerative changes, metaplasia and others should be taken into considerations while reporting.

False negative results may be due to cystic change, necrotic and hemorrhagic areas revealing no diagnostic cellular yield.¹⁷ With increasing cost of medical facilities, any technique which speeds up the process of diagnosis, limits the physical and psychological trauma to the patient, and saves the expenditure of hospitalization, will be of tremendous value.¹⁸

Table 1: result of FNAC and HPE

	No. of patients in FNAC	No. of patients in HPE
Inflammatory	34	32
Tubercular	10	11
malignancy	8	9

Conclusion

It was concluded that FNAC is an easy and suitable investigation tool for the primary assessment of patients with cervical lymphadenopathy on OPD basis. Its diagnostic accuracy is variable and depends on multiple

Ethical Clearance: Taken from Institutional Committee

Source of Funding: Self

Conflict of Interest: No

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Effect of Tamsulosin on Biomarkers after Kidney Stones Lithotripsy

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Abstract

The disease of kidney stones is one of the urinary system's oldest and most common issues. Children with kidney stones have also become more popular, In recent years. Kidney stones are also very painful, and in some people, they can continue to occur. Kidney stone attacks result in over 2 million visits to health care facilities . People tend to get stones in the middle of life. During midlife, family and work commitments are at their height, which makes kidney stones costly. The diagnosis, treatment and prevention of kidney stones and the time away from work due to stones cost approximately \$5.3 billion a year. To understand the effect renal some biomarkers in patients with kidney stones, before and after Lithotripsy and to evaluate the effect of Tamsulosin on these biomarkers in patients. Forty patients and twenty healthy persons were participate in this case-control study, Bl.urea, level of ALP, Level of KIM-1, and level of Na c were investigate in serum with kidney stones patients and control healthy persons. The results were shown significant elevation in the levels of KIM-1, and serum urea were decreased significantly (p value <0.05) while the levels of ALP and serum sodium show an improvement when compared to patients who were not used tamsulosin but it's results were statistically not significant. the results were given an evidence for the crucial role of some biomarker in serum in patients with kidney stones, also the effects of using tamsulosin for 14 days in patients with kidney stones who underwent lithotripsy compared with those patients who were not used tamsulosin.

Key words: kidney stones, lithotripsy, tamsulosin

Introduction

In 5% to 13% of the adult population, kidney stones and chronic kidney disease (CKD) have been identified. CKD is a complication of kidney stones as a result of unusual genetic conditions. Kidney stones can be associated with complications, such as inflammation, acute renal failure (due to obstructive uropathy) and chronic kidney damage.

[1]. Extracorporeal shock wave lithotripsy (ESWL) was a safe and approved treatment for urinary tract stones since its launch in the early 1980s and has been essentially restricted to the management of renal and proximal ureteric stones. Improved ESWL technology and developments in lithotripsy design and fluoroscopic imaging have currently allowed middle and lower ureter calculi to be effectively identified and in situ treated. [2]. Obstructive uropathy of the kidneys and chronic injury. The prevalence of ESRD (i.e. the end-stage renal disease) due to kidney stones was approximately 3.2 percent among patients beginning maintenance hemodialysis. Infection stones are the most common one of the causes of the

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urolithiasis-related ESRD, particularly in the bilateral development of the configuration of stag horn stone. [3]. Extensive stone formation has been identified with uric acid, calcium oxalate or cystine stones. And with well-known obstructive and infectious mechanisms of kidney injury crystal deposition in the tubules and interstitium in both kidneys, the precise mechanism of progressive renal failure has not yet been thoroughly accepted. [4].

Materials and Methods

Patients:

Totally 20 patients (aged between 25 and 60) that have kidney stones, who have been admitted in Al-Yarmok teaching hospital in Baghdad, Iraq, they have been admitted for undergoing the ESWL have been approached and the ones with the kidney stones have been chosen. The study sample included 30 participants that have been enrolled based on specific description, and after that sub-divided to 3 groups:

Group1: which includes ten patients who have kidney stones and take tamsulosin for a 14-day period and have been treated afterwards by the lithotripsy.

Group2: which includes ten patients who have kidney stones and do not take any medications and have been treated afterwards by the lithotripsy.

Group3: which includes ten normal individuals for the purpose of comparing their data to the patients as the control group.

Those sub-divisions have been made following the completion of the process of matching for every one of the groups with one another with respect to: the age, absence of the existing chronic diseases and stone sites. The drug has been administered for a 14-day period, immediately succeeded by the extra-corporeal fatty stones for every one of the sample groups, and there have not been any missing values between study samples.

Diagnostic approaches

The patients have been evaluated with:

- 1- Urine microscopy and analysis.
- 2- X-ray kidney ureter and urinary bladder (KUB).
- 3- Tests of the renal function.
- 4- Ultra-sonography (USG).
- 5- Intra-venous urography
- 5- Computed tomography of urinary tract, carried out besides the ultra-sonography. [5,6]..

Design of the Study:

A Modulith SLX-F-2 machine that has been supplied by a cylindrical source of the electromagnetic shock wave (Storz Medical, Switzerland) has been utilized for performing the lithotripsy. Each one of the 24 patients had received 3,000 shocks in 30min. with a 7kV energy level that has been increased in a gradual manner to 9kV in 500 initial shocks and an average 1.50Hz frequency with variable focus.

Parameters of the Outcomes and Follow-Ups:

The renal bio-markers must be measured prior to the starting therapy utilizing the Omnic® (Astellase) for a 14-day period, and after that, the patients will undergo the ESWL, succeeded with the 2nd renal bio-markers' measurement following the completion of the ESWL. The fundamental follow-up approaches have been diagnostic tests (i.e. Alkaline phosphatase, Sodium, Serum urea, and Kidney injury molecules No1).

Methodology

Kits and Chemicals:

The diagnostic kits below have been utilized in this research, which have been listed with their

suppliers in table1.

Instruments:

The fundamental devices, instruments and tools which have been utilized throughout the research have been listed with the suppliers in table2.

Table1: Diagnostic Kit or chemicals

Chemical or Diagnostic Kits	Suppliers	Origins
Kit of Alkaline Phosphatase Reflotron ®	Roche	U.S.
Enzymatic Kit of Serum Sodium	Stanbio Lab	U.S.
Enzymatic Kit of Serum Urea	Linear chemicals	Spain
Kidney Injury Molecule no1 ELISA Kit	Cusabio	China

Table2: Tools

Instrument	Supplier	Origins
Water bath	KK	Malaysia
Ultra-violet – visible spectro-photometer	Apel DP303	Japan
Centrifuge	KK	Malaysia
Reflotron	Roche	U.S.

Blood Samples:

There have been 5ml of the samples of the venous blood have been obtained from every one of the patients prior to beginning with the therapy and following the ESWL. The samples of the blood have been obtained from the healthy persons and the patients. Those samples have been transferred to clean plain testing tubes, kept at the temperature of the room (25oC) a minimum of 15min to clot, after that, they have been centrifuged and serum has been obtained for the purpose of being utilized for the bio-chemical

evaluations. The parameters that have been measured include: alkaline phosphatase, serum sodium, serum urea, and kidney injury molecules no1 level.

Statistical Analysis

For the encoding and analyses of the data; the SPSS v. 16.0 has been utilized and the significance association test has been performed with the use of the one way ANOVA test and the significance cutoff point has been (less than 0.05) P value.

Results

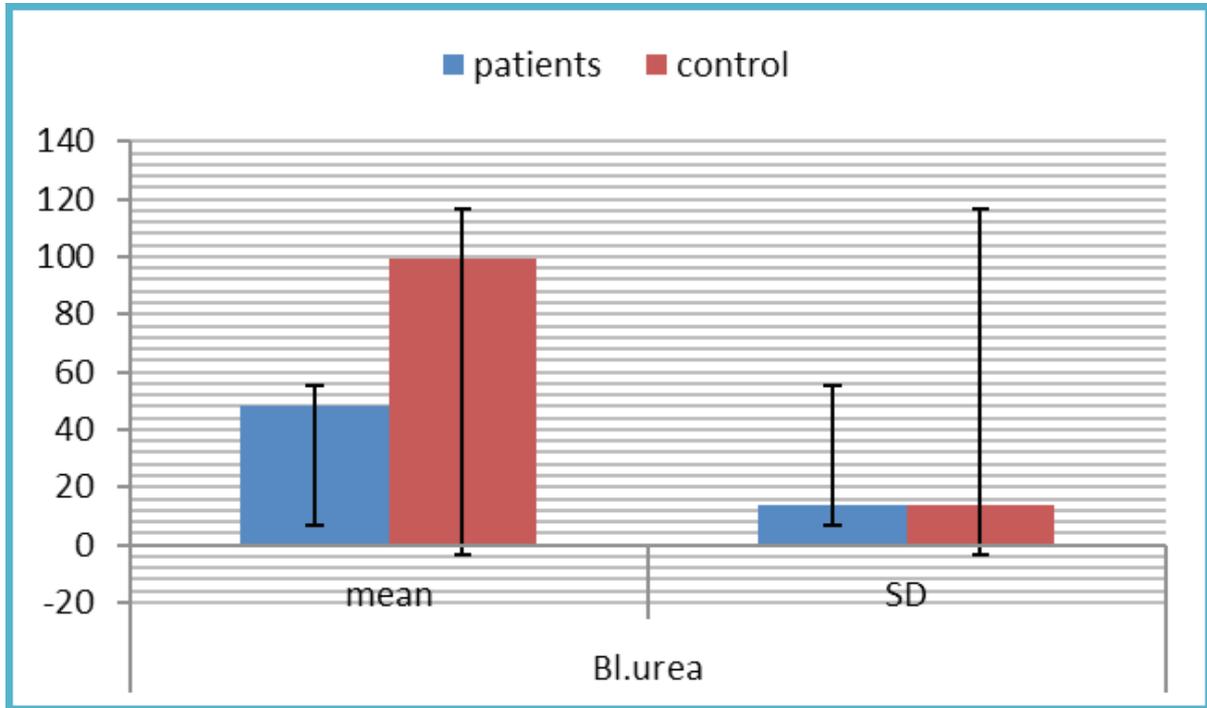


Figure (1): Measurement (Bl.urea) in patients and healthy persons, Mean and standard deviation for every one of the variables amongst the study sample.

Bl.urea= blood urea mg/Dl.

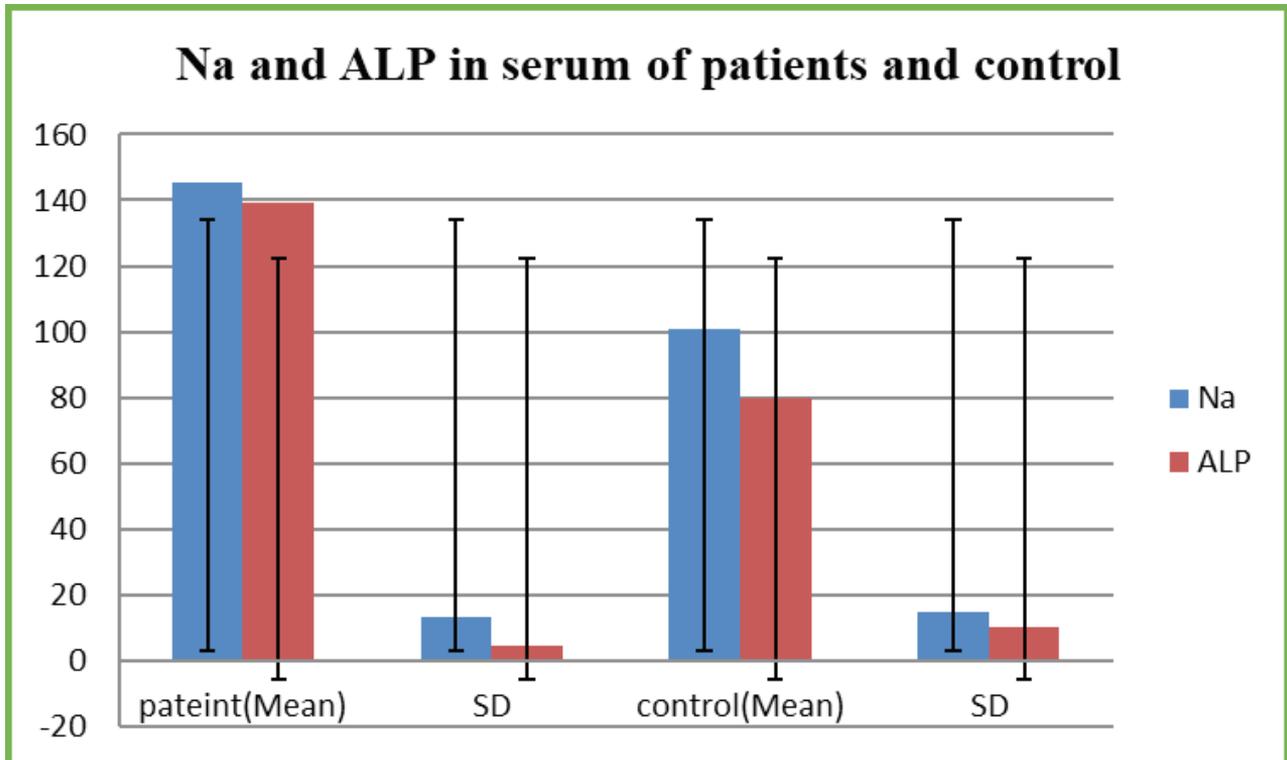


Figure (2): Measurement (Na) &(ALP) in patients and healthy persons, Means and standard deviation for each variable among study sample.

Na= Sodium mmol/L

ALP= alkaline phosphatase U/L

Table (3): Distribution of study samples depending on personal characteristics

Groups		Patient without any treatment	Patient with treatment
Age		(36.5±5.25)*	(37.3±6.35)*
Gender	Male	49%	51%
	Female	55%	45%
Stone size (mm)		(15.45±1.66)*	(14.8±1.93)*
Smoking state		60%	55%

* Significant at P value <0.05

Table (4): measurement (Bl.urea), (ALP), (Na), and (KIM-1) in patients with use treatment (after 14 days) and without use treatment

Groups	Kidney stone (after use treatment)	Kidney stone (before use treatment)
Bl.urea	39.05±12.3	48.1±14.9
ALP	92.1±17.4	100.9±14.7
Na	130.5±9.2	145.5±13.2
KIM-1	2.02±0.69	2.1±0.64

* Significant at P value <0.05

Discussions

Our Figure (1) shows the distribution of patients and controls according to measurement (Bl.urea) in patients with kidney stone, who do not take any medications and are treated by the lithotripsy and healthy persons, The results of the present study showed decrease in the (Bl.urea) in serum of patients

who have kidney stones compared to the healthy persons (mean ± SD) respectively and significant difference P <0.05.^[7]

Also the results of the present study in figure (2) showed decrease in the serum (sodium Na) in patients with kidney stones that do not take any medications

and undergo the treatment by the lithotripsy and healthy persons in the serum of patients who have kidney stones compared to the healthy persons (mean \pm SD) respectively and significant difference $P < 0.05$. This study was agreement with the study conducted for the purpose of investigating the risk factors and prevalence of the kidney failure Nephritis and exhibited that the urinary stones prevalence has been estimated to be approximately 2%-3%. The likelihood of the fact that a white individual under the age of 70 experience a minimum of 1 renal stone is 1/8. [8].

The figure (2) in the results of the present study also showed an increase in the (ALP) in patients with kidney stone who do not take any medications and undergo the treatment by the lithotripsy and healthy persons in serum of patients with kidney stones compared to the healthy persons (mean \pm SD) respectively and significant difference $P < 0.05$. this study agreement with study that showed parameters on continuous scale between research and control groups. Pvalue less than 0.05 considered as significant. [9].

Our study in table (3) showed distribution of study samples depending on personal characteristics such as (age, gender, stone size ,smoking state) between patient they aren't use any treatment and the patient they are use treatment, this study agreement with the study that appeared Of 200 patients, 70 cases (i.e. 35%) have been females and 130 (i.e. 65%) have been males, and comparisons of measured blood markers in the patients according to the stone type, have shown as well the type of the stone (in the case where the evaluation has been possible) has been mixed (11.50%). [10].

Also our study appeared in table (4) the measurement (Bl.urea), (ALP), (Na), and (KIM-1) in patients without use treatment tamsulosin that showed increase in (Bl.urea), (ALP), (Na) but decrease in (KIM-1) after lithotripsy, P value < 0.05 considered as significant. While our study appeared in table (5) and

figure (5) the measurement (Bl.urea), (ALP), (Na), and (KIM-1) in patients with use treatment tamsulosin after lithotripsy that showed decrease in (Bl.urea), (ALP), (Na) but increase in (KIM-1) P value < 0.05 considered as significant. [11].

That appeared ALP is a hydrolytic enzyme that works to remove various phosphate molecules and works more effectively in alkalinity environment. ALP is ubiquitous in the human body, but it is particularly concentrated in the bones and liver, Placenta, leukocytes and kidneys. ALP is produced by the bone in the bone tissue in response to low calcium Levels have a significant impact on the bone mineralization via the decomposition of pyrophosphate in the extracellular medium. [12].

Conclusions

The results were given an evidence for the crucial role of some biomarker in serum in patients with kidney stones, also the effects of the use of the tamsulosin for a 14-day period in the patients who have kidney stones and have undergone the lithotripsy in comparison to the ones who haven't utilized the tamsulosin.

Ethical Clearance: Taken from College of Pharmacy, Al- Mustansiriyah University

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Conflict of Interest: The author declares no conflict of interests.

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Stimulation of Growth and Development of Pandalungan Toddler in Indonesia

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Abstract

Background: The delay in growth and development is still a serious problem for both developed and developing countries in the world. One of the factors that cause delays in growth and development in toddlers is the lack of early stimulation of toddler development. Solving the problem of child growth and development cannot be separated from the existence of cultural diversity.

Method: The qualitative research methods through a phenomenological approach was chosen as the approach because this study tried to explore parents' behavior in fulfilling the growth and development stimulation of the Pandalungan ethnic group. The sampling technique in this study used purposive sampling. Participants in this study were 15 participants with in-depth interviews used a semi-structured interview.

Results: The results of the research get three main themes, and each theme has a sub-theme. The theme of stimulation from the family (Interaction with family, Parent's Education, Parent's Economic and Technology). The theme stimulation from social (Interaction with neighbors and government regulation). The theme Stimulation from culture and value (Cultural factors and values in society and Religious factors).

Conclusion: The role of the family is needed in stimulating the growth and development of children because it will affect the growth and development of children. Social influences and government policies affect parents in stimulating growth and development. Culture and values will affect the growth and development of children because children are raised in that environment, so that they must follow the rules in the social environment.

Keywords : *Growth; Development; Toddler; Pandalungan*

Introduction

The toddler period is important in the growth and development of children because, at this time, basic growth will influence and determine the next development of children. The realm of development

growth can be seen from the weight, height, and head circumference, while the visible development of motor skills, social and emotional, language, and cognitive skills. Every child will go through growth and development according to the stages of his age, but many factors influence it. In this case, if the process does not go well, it does not rule out the possibility of delays in growth and development in toddlers.¹

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The delay in growth and development is still a serious problem for both developed and developing countries in the world. One of the factors that cause delays in growth and development in toddlers is the lack of early stimulation of toddler development. Indeed, every child has their speed of growth and development. This is what often makes parents neglect and don't realize when their child has developmental delays. Problems that often arise during a child's growth and development include impaired physical, motor, language, emotional, and behavioral growth. A child can experience developmental delays in one domain, or it can also be in more than one developmental domain, which is called global developmental delay.²

The problem of child growth and development in Indonesia is still not resolved and requires special attention. This is indicated by the prevalence of delayed growth and general development of children, which is still quite high, ranging from 5-10%. The Ministry of Health of the Republic of Indonesia stated that as many as 0.4 million (16%) toddlers in Indonesia experienced developmental disorders in gross and fine motor development, hearing, intelligence, and speech delays.³ More than 14 million people in Indonesia are children with social and emotional development disorders.⁴ This figure is comparable to 1.7 per 1000 population. Data on the incidence of child developmental delays, in general, are not known for certain. Still, it is estimated that 1-3% of children under 5 years experience general developmental delays. Indonesia's strategic development plan for 2015-2019, states that 11.5% of children under five in Indonesia have growth and development disorders.⁵

Problem-solving in health services cannot be separated from the existence of cultural diversity. Several social trends that can affect health, especially in child growth and development, are lifestyle changes, changes in family composition and lifestyle,

increases in household income, and improvements in the definition and quality of health care. Based on this, it is necessary to understand the culture adopted by the client so that the assessment of health problems can be carried out properly and completely. In addition, culture can also be a bridge to interact and communicate well with clients and their environment.⁶

Pandalungan is a term applied to the mixed Javanese and Madurese people who live in the Horseshoe area, East Java, which includes the Jember, Bondowoso, and Lumajang areas. The term mixed in this case is not only biological but also cultural. This suggests that the region is a multicultural area that has so far succeeded in applying the foundation of multiculturalism intensely in everyday life. The Pandalungan people in their daily life are very open and adaptable. In making decisions, they tend to follow figures who are used as role models in the region. The culture of Pandalungan local wisdom in family parenting patterns will underlie the structure and function of the family in providing fulfillment of children's growth and development.⁷

The role of nurses in transcultural nursing is to bridge between the care system carried out by the community and the professional care system through nursing care. In this case, nurses provide nursing care related to the fulfillment of the growth and development of children in the Pandalungan community while maintaining, negotiating, and reconstructing the Pandalungan culture.⁸ The pattern of family parenting in its implementation is adjusted to the culture of the local community. The value of local wisdom in the Pandalungan family can be optimized in child care. Child parenting is based on local wisdom values, including stimulating children's growth and development.

Materials and Methodology

Study Design and Participant

The qualitative research method was chosen as the approach because this study tried to explore parents' behavior in fulfilling the growth and development stimulation of the Pandalungan ethnic group. Parents' behavior in fulfilling the stimulation of growth and development in the Pandalungan ethnic group is an experience that will be explored using qualitative research methods through a phenomenological approach. In-depth exploration will be used in nursing interventions for families with children under five. This research was conducted in Besuki residency which includes Jember, Bodowoso, and Banyuwangi. This research was conducted in January-April 2021. The sampling technique in this study used purposive sampling, namely the technique of determining the data sample by including special considerations determined by the researcher. In this study, the criteria for participants were parents who had children under five, parents who could speak Indonesian, and those who agreed to the Informed Consent. Participants in this study were 15 participants with in-depth interviews used a semi-structured interview.

Data Collection

In-depth and semi-structured interviews were used for data collection. Participants were asked open-ended questions in the interviews. These were based on an interview guide, which was formulated based on a critical review of the literature. Each participant interview was recorded and transcribed verbatim. The researcher refrained from using judgmental, condoning and negatory statements and attitudes during the interviews. All of these interviews were carried in participant home.

RESULT

The median age of 15 participants was found 26 years, 86,7 % of participants were female.

1. Stimulation from family

The role of the family is needed in stimulating the growth and development of children because it will affect the growth and development of children. Most of the participants reported that the child's growth and development were influenced by several things. In this theme, it is known that there are several sub-themes found: Interaction with family, Parent education, Parents' economy And Technology.

a. Interaction with family

Children imitate parents in the family more so that parents become examples of behavior in everyday life.

“Children do not need to be ordered to play, often they play alone with their neighbors outside the house. if there are no friends outside the house he plays with his mother inside the house “

“ When I invite to play outside the house I often introduce my child to my child's peers “

“Parents must teach and set an example How to behave to people around because that is very important”

b. Parent education

Parents in educating their children usually learn from social media such as Facebook, YouTube, and Google.

“ about children's growth and development I usually often discuss with my friend related to how to educate children “

“I learn to educate my children often with friends in neighboring groups or I usually see how to educate through Google YouTube and Facebook”

c. Parents' economy

The family's economic situation does not affect the child's growth and development.

“our economic needs do not affect children's

growth and development all children's needs are met"

"we always meet children's needs when it can be used for personal development of children we will always try even though at that time we lack the economy"

d. Technology

Technology affects the growth and development of children besides parents learning from social media, which comes from technology children also learn from social media when given by parents.

" at this time children learn more from YouTube so that it causes addiction when playing gadgets, children often get angry by hitting and crying When parents don't give gadgets to watch Youtube"

"technology now that there is good and bad children are more intelligent when watching and learning from the internet and being supervised by parents, the bad impact that often occurs is that children play excessively from morning to night and I am afraid that children will be exposed to radiation from their gadgets"

"Children often learn from gadgets and I think children can show something good. good with like learning to sing learning to play and I think it stimulates the child's brain"

2. Stimulation from social

Social influences and government policies significantly affect parents in stimulating growth and development. In this theme, it is known that there are several sub-themes found: Interaction with neighbors and government regulation.

a. Interaction with neighbors

Children often interact with the surrounding environment so that they grow and develop well.

"Children imitate parents when interacting with other people in my child, yes, he imitates my father and grandfather's behavior"

" obeying fathers because they are more afraid so that when daughters always obey the words of their fathers to respect others."

b. Government regulation

Government regulations will affect how health workers assist parents in stimulating children's growth and development.

"now it's a pandemic so we are limited to going to the Medical Center at the Medical Center we are told how to stimulate children's growth and development and besides that we are also given vitamins and immunizations"

"Now we follow the government's advice not to leave the house so that information about child development at the Medical Center not facilitation well but we got info still from the internet"

3. Stimulation from culture and value

Culture and values will affect the growth and development of children because children are raised in that environment, so that they must follow the rules in the social environment. In this theme, it is known that there are several sub-themes found: Cultural factors and values in society and Religious factor.

a. Cultural factors and values in society

The myths that exist in society will affect parents in educating children so that these myths affect the growth and development of children.

"There are many myths that exist in society, these myths can affect children's growth and development, such as when children are fussy when the baby is less than six months old, children are given bananas so they are not hungry. If this is not in accordance with

what is said at the Medical Center”

“myths What is often felt is that the child’s legs should not be stretched out when carried, they must be straight and sideways because when they grow up the child cannot walk properly and straight”

“Another myth in society when a baby is a child there is a ceremony to eliminate the startled reflex in babies by being often surprised. I don’t think this is true because babies are shocked when they fit immediately reflex shock “

b. Religious factor

Children follow the worship done by parents so that children learn the religion from their parents

“children are always taught how to worship according to the religion believed by their parents.”

“Children always imitate what their parents do in worship according to religion.”

Discussion

One of the successes in stimulating children’s growth and development is environmental factors, both from the family environment to the social environment. The family is the first known social environment and the most frequent contact with children.⁹The formation of character and the process of child growth and development first starts from the family. Parents who are the core part of the family have a role and involvement in supporting the development of children. The active role of parents is needed in stimulating the development of their children, especially when the child is in toddlerhood. This is so that toddlers can reach the optimal level of development as expected. This process can be obtained as early as possible, depending on the environment in which the child lives.¹⁰

Social and moral development in children cannot be separated. These two aspects are interrelated in

influencing early childhood development. Social and moral are closely related to building relationships in society and the environment around children. The social development of children in question is how early childhood interacts with peers, adults, and the wider community to adjust well according to what is expected by the nation and state.¹¹ There is a close relationship between social skills and happy childhood—the child’s ability to adapt to the environment. Acceptance of the environment and other positive experiences during social activities is a very important basic capital for a successful and enjoyable life in the future. The development of children’s social behavior is marked by an interest in friends’ activities, increases a strong desire to be accepted as a group member, and is not satisfied when not with friends.¹²

The role of the social environment in the formation of early childhood character states that the social environment, be it the family environment, school, peers, social community, and the physical environment has a very important role in the development of children. Children, especially in the formation of character. All aspects of the environment must support each other in fostering character development in children. From this description, the researcher concludes that the environment can affect the growth and development of children while still paying attention to environmental aspects, which are a single unit so that the character that grows in children is from good potentials.¹³

Education is an effort given to the community to implement behaviors that aim to maintain, solve problems, and improve their health. Parents are the main subject in providing care to children, so parents’ education, especially mothers, is very influential on the development of children. A low level of education will make it difficult for parents to understand and understand the needs of children in supporting growth

and development according to their age stages. In contrast, parents with a high level of education are easier to understand how they should support the stages of child development.¹⁴

The factors that influence the growth and development of toddler-age children, which states that parental education actors have a significant relationship to children's growth and development. That is because a parent with a good education will be able to receive a variety of information, especially about raising a good son, keeping the child's health, education and so on so that children can grow and Air flowers with optimal. The level of education of parents, especially mothers, influences their knowledge, which means that the higher the level of education of the mother, the higher the knowledge about child growth and development so that it has an impact on the better growth and development of children. Meanwhile, a mother with low education has a risk of experiencing problems or delays in child development because she does not understand how to stimulate growth and development in her child. From this description, the researcher concludes that the level of education influences the growth and development of children. However, it is possible that parents with higher education can support their child's growth and development well. This is also influenced by other factors such as family attachments and so on.¹⁵

Policies or regulations are everything that affects individual activities in providing cross-cultural nursing care. A policy is made to be followed and implemented by a person or group of actors to solve problems. Policy To achieve health goals, including child development, good cross-sectoral cooperation between clients and stakeholders is also needed so that efforts run optimally and sustainably. Indonesia, as a country with a fairly large number of children under five, needs serious attention in seeking the quality of growth and development, namely by fulfilling good

nutrition, stimulation by growth and development, as well as the reach of quality health services in terms of early detection and intervention of growth and development deviations. A comprehensive and coordinated effort to stimulate, detect, and early intervention must be carried out with cooperation between clients, community leaders, professionals, and policies that support implementing programs to improve the quality of child growth and development. Because of this, government policies greatly affect the process of child growth and development by uniting the movements of development actors in the health sector. However, a policy will not work without the active role of the community and policy implementers. So that both parents and the community must always build communication with related parties so that the implementation of policies runs effectively to improve the quality of children's growth and development.¹⁶

Fact or economy is one of the things that can affect the growth and development of children. In general, if a child comes from a family with high economic status, it will be easier to meet nutritional needs when compared to a child from a family with a low economic status. A child who comes from a family with a low economic background usually has problems with food shortages, poor environmental health, and ignorance of growth and development. This will indirectly inhibit the growth and development of children.¹⁷

Low-income family economic conditions lead to food shortages, poor environmental health, and limited knowledge of parents will hinder children's growth, parenting environment, mother and child interaction. This, of course, greatly affects the development of children. Stimulation of children's growth and development includes the provision of game tools, socialization. Parental involvement is very supportive of children's growth and development so that if this is not fulfilled or disturbed, it will affect the development of children. Children's thinking ability is influenced

by the nutrition provided. Suppose nutrition is not met or tends to be chronically deficient as a result of the poor economic situation of the family. This results in a child's IQ tends to decrease. The average decline in children's thinking power is indeed due to a decrease in the child's amount of quality nutrition for consumption. This reflects that the economic status of a family can affect the quality of children in terms of nutrition, IQ, and body growth of the child. The welfare of the family can be seen from the size of the income in one household. So with a high income, of course, it will support various adequate health care facilities, decent housing, and the fulfillment of various nutrients that can maintain and improve the quality of this early childhood.¹⁸

Culture is something that has become a habit and is difficult to change. The culture applied in the family environment is very influential on the development of children. The development that has grown within a person will continue to grow so that every society will pass on values from one generation to the next, and that is how civilization takes place. Culture plays an important role in the process of child growth and development. Therefore parents must be more selective in choosing and sorting out the culture introduced to their children.¹⁹

Cultural factors in an environment will affect how people view their daily health needs, including how to apply feeding patterns to their toddlers. This is in line with the theory of culture-based nursing or transcultural nursing, which states that culture, values, beliefs will affect a person's health behavior. Cultural aspects contribute greatly to infant feeding practices, so it is important to explore cultural-based factors that influence infant feeding practices to support optimal growth and development.²⁰

Religion is one of the factors that can affect children during their growth and development. Religion is closely related to a child's moral development in the

future, where this is very important so that children do not fall into things that are deviant and detrimental to themselves and others. Children's religious growth cannot appear automatically by itself, but a strong and repeated stimulus arises from outside the child. The first is the child's hearing, which is stimulated time and again by religious values. The second is vision, which is stimulated by repetitive attitudes and behaviors. Third, some facilities are available as a trigger for children to carry out the process of imitation/imitation of religious behavior.²¹

Children get value from their environment, especially their parents, because parents are the closest environment and the first to come into direct contact with children. In this case, the role of parents is very important. In developing children's moral values, parents' attitudes that need to be considered include the appreciation and experience of the religion adopted. Parents are role models for children, including about religion. Based on this description, it can be concluded that the cultivation of religious values influences the growth and development of children, wherein in this case, parents must play an active role in guiding and guiding their children to instill religious teachings so that children's development will run as expected.²²

The impact of information and technology has now been felt by almost all levels of society, especially among children, both from positive and negative impacts. This makes technological factors can influence children's growth and development. The use of technology can indeed positively influence children, among others, to make it easier for children to hone their creativity and intelligence. The many features offered, such as learning to read, coloring, and so on, will, of course, have a good influence on children's brain development. However, the convenience offered by technology can also have a negative influence on children's development. Not infrequently, children will be lazy to do activities

because they prefer to enjoy the world of technology, such as gadgets. This behavior certainly hurts the health and development of children, especially the brain and psychology. In addition, spending more time in front of the device will adversely affect children's social skills.²²

The effect of using gadgets on the psychosocial development of preschoolers stated that there was an influence between the use of gadgets on the psychosocial development of preschoolers in Immanuel Christian Kindergarten. The description can be concluded that technology affects the growth and development of children, both positive and negative influences. The impact obtained depends on how children use the technology, and the role of parents is needed in supervising children when using technology. But it would be better if families did not rely on technology too much, especially gadgets, to accompany their children.²³

Conclusion

The role of the family is needed in stimulating the growth and development of children because it will affect the growth and development of children. Most of the participants reported that the child's growth and development were influenced by several things. Social influences and government policies significantly affect parents in stimulating growth and development. Culture and values will affect the growth and development of children because children are raised in that environment, so that they must follow the rules in the social environment.

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Social Status and Oral Hygiene with Quality of Life in Patients with Primary Hypertension

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Abstract

Background: The main problem in primary hypertension is that most people who have been diagnosed with primary hypertension do not know the etiology clearly. To find out the relation between social status and oral hygiene with quality of life in primary hypertension. **Methods:** The type of research used was an observational in a cross-sectional study design. The study was conducted in February-May 2021. The number of sampling was 61 people. The sampling method uses purposive sampling technique. The results of data collection were tested by using the Path Analysis test. **Results:** Research conducted at Padongko Public Health Center in Barru Regency. Based on the results of the analysis of the path analysis of social status tests on primary hypertension, the results obtained $p_{\text{value}} 0.188 > \alpha 0.05$, oral hygiene against primary hypertension results obtained $p_{\text{value}} 0.914 > \alpha 0.05$, this H_a obtained means that there is a the relation but not significant, while social status on quality of life results are obtained $p_{\text{value}} 0.837 > \alpha 0.05$, oral hygiene against quality of life results obtained $p_{\text{value}} 0.227 > \alpha 0.05$, this H_a obtained means there is the relation but not significant, while the the relation of primary hypertension to quality of life has a significant the relation where the results of $p_{\text{value}} 0.012 < \alpha 0.05$. **Conclusion:** There is the relation between social status and oral hygiene with quality of life in patients with primary hypertension but does not appear significantly.

Keywords: Knowledge, Social Status, Oral Hygiene, Quality of Life and Primary Hypertension

Introduction

Hypertension or famously known as the silent killer is a condition where the increase of blood pressure above normal. Increased age is one factor causing the occurrence of hypertension, this is due

to the increasing age of organ function decreased marked by decreased elasticity of the arteries and stiffness occurs blood vessels so vulnerable to an increase in blood pressure. Hypertension is defined as persistent blood pressure where the systolic pressure is above 140 mmHg and diastolic over 90 mmHg. [1],[2],[3] One of the major risk factors of hypertension is stroke, heart failure, chronic kidney disease, visual impairment, and hypertension is often called the silent killer. Hypertension is a condition when a person experiences a rise in blood pressure either slowly. [4],[5],[6],[7]

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Hypertension or famously known as the silent killer is a condition where the increase of blood pressure above normal. Increased age is one factor causing the occurrence of hypertension, this is due to the increasing age of organ function decreased marked by decreased elasticity of the arteries and stiffness occurs blood vessels so vulnerable to an increase in blood pressure. Hypertension is defined as persistent blood pressure where the systolic pressure is above 140 mmHg and diastolic over 90 mmHg.^{[8],[9],[10]}

One of the major risk factors of hypertension is stroke, heart failure, chronic kidney disease, visual impairment, and hypertension is often called the silent killer. Hypertension is a condition when a person experiences a rise in blood pressure either slowly. Hypertension or high blood pressure is a disease characterized by an increase in blood pressure that exceeds normal. Blood pressure is measured in millimeters of mercury (mmHg (millimeter Hydrargyrum)) and recorded as two different values namely systolic blood pressure and diastolic blood pressure.^{[11],[12],[13]}

Health-related quality of life (HRQOL) includes physical, psychological and social aspects of the health field that are influenced by one's personal experience of trust, hope and perception. The issue of quality of life in adults is receiving enough attention, because the management of the disease is expected to not only eliminate symptoms but also improve quality of life. This treatment is expected not only to focus on the lives and health of sufferers, but also care must be able to conduct supervision and learning in the form of education about the importance of maintaining oral

hygiene that can affect social factors and quality of life.^{[14],[15]}

Material

The type of research used was an observational method in a cross-sectional study design. The study was conducted in February-May 2021. The number of sampling was 61 people. The sampling method uses purposive sampling technique. Inclusion criteria were adult patients aged 18-40 years who had been diagnosed with hypertension clinically, adult patients who had systolic blood pressure measurements > 140 mmHg and diastolic > 90 mmHg, adult patients who were willing to fill out informed consent and questionnaires. Exclusion criteria are patients diagnosed with hypertension in pregnancy or using dentures, patients who are deaf, blind and illiterate, adult patients who are diagnosed with hypertension with systemic disease or complications. The results of data collection were tested by using the Path Analysis test.

Result

The subjects of the study conducted at Padongko Public Health Center in Barru District were 61 people who went to Padongko Public Health Center with a diagnosis from a primary hypertension doctor and the sampling was adjusted according to inclusion criteria. Data collection for quantitative approaches uses questionnaires and direct observation while data collection for qualitative approaches uses interview techniques from both the direct respondent and the respondent's family.

Table 1. Coefficient Value of Social Status and Oral Hygiene with respect to Primary hypertension

Variable	Analysis	Significant
Social Status (X1)	,173	,188
Oral Hygiene (X2)	-,014	,914

a. Predictors: (Constant), Oral Hygiene (X2), Social Status (X1) Primary Hypertension (Z)

Table 2. The value of Regression Model I Social Status, Oral Hygiene is Primary Hypertension

Model	R Square
Model I Regression	,031

a.

Dependent Variable: Z

Table 3. Coefficient Value of Social Status and Oral Hygiene on Quality of Life

Variabel	Analysis	Significant
Social Status (X1)	-,026	,837
Oral Hygiene (X2)	-,153	,227
Primary hypertension	,300	,021

b. Dependent Variable: Quality of Life (Y)

Table 4. Value of the results of Regression Model I Social Status, Oral Hygiene there is Quality of Life

Model	R Square
Regression Model 2	,113

Discussion

In essence, education is one way for someone to gain knowledge and knowledge in school. Education is one of the important aspects in people’s lives which has a role in improving the quality of life. This is in line with research by Rebecca, et al (2015) found that the higher the level of formal education, the better the knowledge and behavior of healthy living, otherwise low education will hinder the development of one’s attitude towards new values that are known.^{[16],[17],[18]}

Extensive knowledge is easy to understand, digest and understand information that can be obtained either from social media or from information obtained from the closest people. Respondents with primary hypertension who are not handled properly according to doctor’s recommendations will continue to become

resistant hypertension or secondary hypertension and new disease problems can arise, therefore sufficient education and knowledge is very necessary to support the individual’s understanding of the seriousness of a particular disease of hypertensionprimary. Her study also said that when someone is at a higher level of knowledge, attention to oral health will be higher, and vice versa when someone has less knowledge, dental attention and care is also low.^[19]

People with moderate incomes are able to provide better health services for themselves and their families. People with inadequate income capacity will find it difficult to meet their basic needs, so it will be difficult to provide health services for their families. This is in line with Jumriani’s research which says some people with medium and low incomes tend to

think about visiting and treating dental and oral health conditions, even though he knows that examining and treating dental care is very important to maintain health teeth and mouth.^[20]

In contrast to people who have high incomes, they tend to be very concerned about the condition of their teeth and mouth because for them oral health is very important. This is also supported by research from Monica et al in 2017 which says that in low-income groups, the situation is far from satisfying and is a problem that is often overlooked because not everyone sees a tooth disorder as a disease that needs treatment. People who have adequate income will make it possible to provide better health services. People with economic ability will have difficulty meeting their basic needs so it will be difficult to provide health services for their children.^[21]

In addition to the variable level of education and income, the level of work in this study there is also a relationship that is social status as seen from work has a relationship to the occurrence of hypertension. The results of this study are in line with the research of M. Hasan Azhari (2017) which says that more work is seen from the possibility of special exposure and the level or degree of exposure as well as the magnitude of risk according to the nature of work, environment and socioeconomic nature of certain jobs. Work also has a close relationship with socioeconomic status, while various types of diseases that arise in the family are often related to the type of work that affects family income.^[22]

Work is also a factor affecting oral hygiene and quality of life. A person's work will affect the life of his personal life, the work occupied by each person is different, the difference will cause differences in the level of low income to a high level of income, depending on the work occupied by it. This is supported which cites research from Cristiono and Rama who said that oral hygiene with work status is

because people from the upper middle class consider it important to maintain dental health and expect teeth to function optimally in the mouth.^[23]

It can be concluded that the data in research conducted at Padongko Public Health Center in Barru Regency which explains the social status of primary hypertension has 0.188 greater than alpha 0.05 or it can be said that social status is $0.188 > 0.05$ so that it can be concluded that social status has a direct influence on primary hypertension but not significant. The analysis of social status variables studied explains the social status of quality of life 0.837 greater than alpha 0.05 or it can be said that social status is $0.837 > 0.05$ so that it can be concluded that social status has a direct effect on social status variables on quality of life but is not significant. While the analysis of the influence of social status on quality of life through primary hypertension is known to be the direct effect of social status on quality of life of -0.026. While the indirect effect of social status on quality of life through primary hypertension is the result of the multiplication of social status beta scores on quality of life with beta values of hypertension on life quality: $0.173 \times 0.300 = 0.05$. Then the total influence given to social status on quality of life is a direct effect added (+) with an indirect effect, namely: $-0.026 + (0.05) = 0.02$. Based on the results of the previous analysis, it can be explained that the direct effect is -0.026 and the indirect effect is 0.05, which means that the direct effect is smaller than the indirect effect. This shows that the indirect effect of social status variables on quality of life through hypertension appears to be significantly.

In this study, to measure the level of cleanliness of respondents' oral cavity using the Oral Hygiene Index Symplified (OHI-S) score by screening using a sonde and mouth glass, after which a score calculation is based on the OHI-S level distribution. Human oral cavity is never free of plaque. Plaque plays an

important role in the formation of debris and calculus. The attachment of calculus begins with forming dental plaque and the surface of calculus itself is always covered by plaque. Oral hygiene that is not maintained properly will cause disease in the oral cavity. Periodontal diseases (such as gingivitis and periodontitis) and dental caries are the result of poor oral hygiene. Oral hygiene also has the most important role for the problem of one important component of healthy life.

This study gets results related to the results of the educational characteristics of respondents in Padongko Public Health Center where the results of this study indicate the level of education has a relationship to the oral hygiene index in primary hypertension sufferers, because in this study it is known that the oral hygiene index is the best. at the last senior high school level and the worst oral hygiene index was at the last non-school education level.

The previous study said that education and knowledge are two inseparable things, but in this study these two things did not guarantee that respondents who had a higher education and extensive knowledge were able to maintain their oral cavity to stay clean and healthy or vice versa. Knowledge of respondents must be reviewed in terms of adequate electronic media and health services that have entered the world of television so that it can facilitate the public to understand and understand about health issues, especially dental and oral health. Respondents also said that dental and oral health knowledge was still lacking because some health services promoted through counseling conducted either from health centers or dental health students.

Knowledge obtained from respondents is also still considered trivial for respondents who tend not to apply in daily life to maintain oral and dental hygiene, so that the relationship of knowledge to oral hygiene does not have a very large relationship. Research

conducted at Padongko Public Health Center shows that the relationship there is a relationship between primary hypertension patients with oral hygiene, based on this study there is a relationship that can occur if primary hypertension patients do not maintain oral hygiene or oral hygiene.

It can be concluded that the data in a study conducted at Padongko Public Health Center in Barru Regency where oral hygiene variables under study explained oral hygiene against primary hypertension has 0.914 greater than alpha 0.05 or it can be said oral hygiene is $0.914 > 0.05$ so it can be concluded oral cavity hygiene, which is oral cavity hygiene, there is a direct effect on oral hygiene variables on primary hypertension, but not significantly. Analysis of oral cavity hygiene variables studied explains oral cavity hygiene to quality of life has a value 0.227 greater than alpha 0.05 or it can be said that X_1 is $0.227 > 0.05$ so that it can be concluded that oral cavity hygiene has a direct effect on oral cavity cleanliness but not significant. While the analysis of the effect of oral hygiene on quality of life through primary hypertension is known to be the direct effect of oral hygiene given on quality of life by -0.153. While the indirect effect of oral hygiene on quality of life through primary hypertension is the result of the multiplication of the oral hygiene beta value of primary hypertension with beta value of primary hypertension on quality of life: $-0.014 \times 0.300 = -0.04$. Then the total effect given by oral hygiene on quality of life is a direct influence plus (+)

The indirect effect is: $-0,153 + (-0,004) = 0,157$. Based on the results of the previous analysis it can be explained that the direct effect of -0.004 and the indirect effect of 0.05 which means that the indirect effect is smaller than the direct effect. This shows that the indirect effect of oral hygiene variables on quality of life through primary hypertension does not appear significantly.

In this research, it can be seen that the quality of life of a person can only be described by that person personally. Even this opinion is supported by the existence of a previous study by Sri Santiya which said the picture of quality of life, a person can only be described by the person himself subjectively and cannot be defined exactly. Hypertension is a chronic disease caused by multifactorial and has implications for many things in the lives of sufferers. Hypertension has a relationship with quality of life. Besides the implications for organs, hypertension can have effect on socio-economic life and quality of life of someone. This is because hypertension has a bad influence on vitality, social function, mental health, and psychological function. Therefore, it is important to measure the quality of life with hypertension. It is very important to measure hypertension so that optimal management can be performed.^[24]

Research supported by previous research, Kaliyaperumal, et al 2016 in India, found that there was a relationship between hypertension and quality of life because hypertension significantly disrupted quality of life both in terms of physical and mental health. The problem of quality of life of patients today receives more attention because the management of the disease is expected to not only eliminate symptoms but also improve quality of life, this is due to hypertension giving a bad influence on vitality, social function, mental health, and psychological function.^{[25],[26]}

Based on the analysis of research conducted at Padongko Public Health Center in Barru Regency, it was shown that primary hypertension on Quality of Life obtained a significance value of primary hypertension of $0.021 < 0.05$, so it can be concluded that there is a direct influence of primary hypertension variables on quality of life significantly. This proves that in the research conducted there is a relationship of primary hypertension to the quality of life

of respondents. Research that sees a very close relationship of quality of life to hypertension, one of which is the results of research conducted by Yung, French and Leung who say that relaxation training in the form of muscle relaxation and cognitive imagery can reduce blood pressure in people with hypertension. When there is a decrease in blood pressure in people with hypertension will have an impact on improving their quality of life both physically, psychologically, socially and comfortably to feeling therapy in general.^[27]

Conclusion

There is the relation between social status and oral hygiene with quality of life in patients with primary hypertension but does not appear significantly.

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Ethical Considerations: Ethical clearance was obtained from Universitas Muslim Indonesia, Makassar; with number "519/A/KEPK-UMI/II/2021. Just before the interview, written (or thumb impression) consent was obtained from each participant in Universitas Muslim Indonesia, Makassar guidelines.

Conflicts of Interest: The authors alone are responsible for the views expressed in this article and they do not necessarily represent the views, decisions, or policies of the institutions with which they are affiliated.

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Diagnostic Problems in Leptospirosis Patients : A Case Report

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Abstract

Leptospirosis is a zoonosis infection found in almost every part of the world but is mostly endemic in tropical and subtropical countries. In 2013 there were 640 new cases reported with 60 death cases (CFR 9.37%). An increase in new cases had caused an outbreak in Sampang, Madura due to a flood following a high rainfall. A male patient aged 45 years old with Leptospirosis. The diagnosis was made based on the epidemiology, clinical manifestations, and Microscopic Agglutination Test (MAT) examination data. In the early treatment, the examination of IgM and IgG *Leptospira* showed negative results a confirmation with MAT examination was done subsequently. Diagnosis using MAT also provides information about an outbreak in a region. Upon receiving antibiotic and symptomatic treatments and clinical recovery, the patient's condition continued to improve.

Keywords : *Leptospira, Leptospirosis, Microscopic Agglutination Test*

Introduction

Leptospirosis is an infectious disease affecting humans and animals. Precisely, this disease is a zoonosis disease that could affect humans, one of the most common zoonoses in the world. Leptospirosis is also known as flood fever, for it appears primarily because of a flood. The most common reservoir of this bacteria is rodents and mice⁽²⁾. In Indonesia, this disease is considered a re-emerging disease, meaning a new case could appear sporadically with the potential to cause an outbreak.

In 2013 there were 640 new cases with 60 death cases (CFR 9.37%) reported. The increased cases were due to an outbreak in Sampang, Madura. Whereas until October 2014, there were 411 new cases with 56 death cases (CFR 13.63%) reported. The increased cases were due to an outbreak in DKI Jakarta and Central Java due to a flood following a high rainfall⁽³⁾.

The diagnosis was made based on the isolation of the bacteria from blood or urine. Dark-field microscope examination often gives false value, while rapid quantitative examination to detect IgM and IgG *leptospira* is commonly used as a screening tool, although it frequently shows negative results, especially in the early phase of leptospirosis. The gold standard of diagnosis is the Microscopic Agglutination Test (MAT) serology examination. The current development in molecular research has contributed to the characteristic correlation study amongst pathogen strains and outbreak investigation epidemiologically

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in a region⁽²⁾.

Case Description

Mr. A.S, 45 years old, resided in Sampang, Madura. The patient was referred from Sampang Hospital with calf muscle pain ten days before hospital admittance accompanied by fever, headache, dizziness, vomiting dark-colored blood, stomachache, and nausea. The patient also complained of reddened eyes since three days before hospital admittance that changed into yellowish, shortness of breath not affected by activity, with no cough and chest pain observed. The patient also complained of tea-colored urine with a reduced volume of approximately 500 mL/24 hrs since three days before hospital admittance, with no black stools observed. The patient had a history of contact with rodents and history of flood in his residence. The IgG and IgM *Leptospira* examinations from Sampang Hospital showed negative results.

From the physical examination, the patient showed a weak general condition with GCS of 4-5-6. The vital signs examinations showed blood pressure of 100/70 mmHg, heart rate of 80 x/min, respiratory rate of 20 x/min, and axillary temperature of 36.7⁰C. Head and neck examinations showed icteric sclera and conjunctival suffusion. Thorax inspection showed petechiae in the thoracic region. On heart auscultation we obtained single S1 and S2 heart sounds, no murmur, gallop, or friction. Lungs examination showed normal results. Upper extremities examination showed dry reddish warm hands, petechiae on the left shoulder, no edema, and no palmar erythema. Lower extremities examination showed dry reddish warm foot and no edema.

Laboratory examinations showed hemoglobin of 12.2 g/dl, leucocyte of 11,700/mm³, thrombocyte level of 34,000/mm³, HCT of 37.2%, neutrophil of 84.6%, aPTT of 31/22.8, PPT of 11.3/12.1, BUN of 104 mg/dl, SK of 7.52 mg/dl, albumin of 2.25 g/dl,

blood sugar level 71 mg/dl, direct bilirubin 11.51 mg/dl, total bilirubin 12.96 mg/dl, SGOT 29 U/L, SGPT 27 U/L, Na 133 mmol, K 3.5 mmol, Cl 101 mmol, CRP 108.91 mg/dl, non-reactive HbsAg, IgM *Leptospira* (+), IgG *Leptospira* (-), alkaline phosphatase 108 U/L, and non-reactive HCV. MAT examination with icterohaemorrhagic serogroup result showed a titer of 1:640. From urinalysis it was obtained results as followed: Glucose (-), Bil (+3), Ket (-), SG 1.010, BLD +/- intact, pH 5.5, Prot (+), Uro 3.2, Nit (-), yellow in color, normal clarity, Eryt -2/HPF, Leuko -2/HPF, Low epithelial cells, Crystals (-), Cylinder (-). Chest X-ray and electrocardiogram showed normal results.

The patient was diagnosed with Leptospirosis. The patient was given soft diets high in carbohydrates and protein of 1.900 kcal, D5:NS(1:2) IV fluid 1.500 ml/24hrs, Ceftriaxone 1 gr I.V. BID, Omeprazole 40 mg I.V. BID, Sucralfate suspension 1 tablespoon QID. On the ninth days of hospitalization, the patient's condition continued to improve; no fever, no icteric on the sclera, no blood vomiting, no calf pain, and the urine production was 2.000 cc, clear yellow in color.

Discussion

Leptospirosis is a zoonosis infection found in almost every part of the world but is generally endemic in tropical and subtropical countries. There is more than 250 serovar of *Leptospira* genus known to have caused broad spectrum of various diseases, ranging from mild to life threatening conditions⁽⁴⁾. The incidence of Leptospirosis could be affected by several factors, including culture and social backgrounds, occupancy, behavior, and environment. Humans are at risk of contracting this disease in correlation to their occupancies; farmers, stock farmers, miners, gutter cleaners, soldiers, and other jobs exposed to *Leptospira* contaminated water are at high risk of contracting this disease^(2,5).

The clinical manifestations of this disease are varied, ranging from mild fever to the icterohaemorrhagic form with complications in several organs, including the brain, kidney, and liver⁽¹⁾. The incubation period ranging from 2-20 days with 7-10 days on average. The onset of the disease is sudden. It began with high fever, and one third of patients reported prodromal signs, such as fatigue and headache⁽⁷⁾. The clinical manifestations of Leptospirosis is biphasic, the acute phase last for approximately one week, followed by the immune phase accompanied by antibody production and expression of *Leptospira* in urine. Most complications of Leptospirosis are associated with the location of *Leptospira* in the tissues during the immune phase on the second week of the disease progression^(6,7).

Most Leptospirosis infections are subclinical or mild. Few cases display unspecific manifestations, such as sudden fever, headache, myalgia, stomachache, red-eye, photophobia, nausea, and vomiting⁽⁷⁾. The ocular manifestations are usually seen in severe Leptospirosis while conjunctival suffusion could be seen in most cases. Conjunctival suffusion and icteric sclera are thought to be pathognomonic of Weil's disease. Anterior uveitis, whether unilateral or bilateral could develop after the resolution of the acute phase in few cases⁽⁶⁾. It could be followed by manifestations of organ damage, including liver, kidney, muscle tissues, and other organs. In severe cases, they could be accompanied by anemia, loss of consciousness, continued fever, icteric, and bleeding. Hemolysis could also contributed to the severity of icteric⁽²⁾.

Kidney involvement is a crucial manifestation of severe Leptospirosis. Two mechanisms have been suggested in the development of kidney failure in Leptospirosis: (1) direct nephrotoxic due to several endotoxins or endotoxin-like substances, and (2) the anoxic effect due to kidney circulation disturbances⁽⁸⁾.

In mild cases, several urine sediments are found, including albuminuria, microscopic hematuria, pyuria, and granular cast. Oliguria and anuria could develop in severe cases. Renal insufficiency usually developed simultaneously with icteric on days 3 to 4, followed by increased urea and creatinine levels that often need renal replacement therapy⁽⁶⁾. Polyuria could develop on days 10-18, and creatinine levels usually begin to decrease at the end of the second week and reached a normal level on weeks 3 to 5 after the onset of the disease, where kidney injury due to Leptospirosis is mostly not permanent⁽²⁾. Complications of severe Leptospirosis could affect several organs. Acute Kidney Injury (AKI) is reported in 16% - 40% cases, and it is crucial to distinguish between pre-renal azotemia (non-AKI) and AKI. Patients with pre-renal azotemia respond well with rehydration therapy, hence dialysis could be postponed until 72 hours. While in patients with AKI, oliguria is a predictor of mortality⁽⁹⁾.

On direct bacteriology examination of blood and urine using a dark-field microscope, the rate of false-positive results is high since protein filaments are frequently found in the samples and highly resemble *Leptospira*. *Leptospira* could be isolated directly from the blood, urine, tissues, and culture. Culture results could be used for diagnosis⁽¹⁰⁾. Laboratory diagnosis of Leptospirosis is primarily based on serology examinations. Microscopic Agglutination Test (MAT), Enzyme-Linked Immunosorbent Assay (ELISA), and Immuno-fluorescent Antibody (IFA) test are the most frequently used serology examinations⁽¹⁰⁾.

MAT is the main referenced test and is commonly used as the gold standard of serology test in evaluating new potential Leptospirosis diagnostic tests due to its high sensitivity. MAT could detect antibody in the serovar level so that it could detect different strains of *Leptospira*⁽²⁾. Using MAT, we could determine

the agglutination of antibodies found in the patient's serum by mixing it with *Leptospira*. Anti-*Leptospira* antibodies found in the serum will cause the *Leptospira* to adhere to each other and form clumps. This process is called agglutination and could be observed under a dark-field microscope. MAT could not differentiate antibodies agglutination of current, recent, or past infection. Ideally, like other serology tests, two samples must be examined subsequently to determine seroconversion or increased titer four-fold or more⁽⁵⁾.

General treatment of Leptospirosis includes symptomatic and supportive therapies based on the severity of the signs and symptoms. Bed rest is recommended for 1 to 2 weeks for the mild disease and 2 to 4 weeks for the severe disease⁽¹¹⁾. *Leptospira* are sensitive to several antibiotics, including Penicillin, Cefepim, Aminoglycosides, Tetracycline, and Macrolides. A study suggested that Penicillin and Cefepim have the lowest Minimal Inhibitory Concentrations (MIC) against *Leptospira*. Penicillin could eliminate *Leptospira* on the logarithmic growth phase but not on the stationary phase. Streptomycin demonstrates its ability to eliminate *Leptospira* both on the logarithmic growth and stationary phases. Tetracycline shows leptospirocidal effect only on a high concentration. Gentamycin, Tobramycin, and Isepamicin exhibit significant bactericidal effect on both the logarithmic growth and stationary phases⁽⁴⁾. Due to the biphasic nature of Leptospirosis, the effect of antibiotic agents given on the immune phase is doubtful⁽²⁾.

The prognosis of Leptospirosis depends on the severity and complications of the disease. Anicteric Leptospirosis generally has a good prognosis, without jaundice, this disease is never fatal, although Pulmonary hemorrhage and myocarditis are reported in anicteric cases occasionally. The mortality rate of Weil's disease ranged between 15% - 40% and higher

in elderly patients aged more than 60 years old⁽¹²⁾.

Conclusion

It has been reported a male patient, aged 45 years old, with Leptospirosis. The diagnosis was made based on the epidemiology, clinical manifestations, and Microscopic Agglutination Test data. During early hospitalization, the IgM and IgG examinations showed negative results. MAT examination was done subsequently to confirm the diagnosis. MAT examination could also give information regarding an outbreak in a specific region. Symptomatic treatment and antibiotic agents were given to the patient, the patient's condition continued to improve.

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Ethical Clearance: Not required for a case report.

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Serum Levels of Interleukin-2 and Interleukin-6 among *Helicobacter Pylori* Positive Patients in Relation to Prognosis of Gastro-duodenal Disease

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Abstract

Background: *Helicobacter pylori* is the most widespread pathogenic associated with gastro-duodenal Disease. Interleukin 2 and interleukin 6 are the main cytokines involved in host immune response to *H.pylori* infection. The aim of the present study are to assess the serum level of IL-6 and IL-2 in *H.pylori* infected patients and to study their association with gastric endoscopy findings. **Methods:** One hundred and seven suspected patients and 19 healthy were recruited. The confirmed positivity of *H.pylori* detection was based on a rapid urease test (R.U.T) and stool antigen test. Serum concentrations of IL-2 and IL-6 were measuring by ELISA. **Results:** Seventy-five (70%) patients were positive for *H.pylori*. Both interleukins were found at higher levels in patients than in healthy (p-value <0.05). Interestingly, the level of IL-2 was lower in patients infected with *H.pylori* (43.40 pg/ml) than those who were not infected (85.2 pg/ml), while the level of IL-6 was higher in patients infected with *H.pylori* (117 pg/ml) than those not infected (40 pg/ml), p-value <0.05. Furthermore, the increasing levels of both interleukins were correlated with disease progress.

Keywords: Gastritis; Gastric cancer; *Helicobacter pylori*; IL-6, IL-2; Peptic ulcer.

Introduction

H.pylori infection induces strong immune responses but the host is still unable to clear the organism from the mucosa. The first-line of defense against *H.pylori* infection are Gastric epithelial cells, these cell express a Toll-Like Receptor¹, and the Nucleotide-binding oligomerization domain NOD-like receptor family members². Recognition of *H.pylori* by these molecular on gastric cells leads to

the activation of intracellular signaling pathways that culminate in the induction of various genes involved in host defense including the production of interleukins (IL-8), (IL-6), (IL-1 β), chemokine, and antigen presenting molecules³. Many of these molecules act as a chemotactic factor for neutrophils and lymphocytes, and a proliferative response with a dense infiltrate of these cells in the gastric mucosa, resulting in a chronic active gastritis. *H.pylori* also induces gastric mucosal infiltration by dendritic cells and T and B cells, and stimulates secretion tumor necrosis factor (TNF)- α , IL-12, IL-10, transforming growth factor (TGF)- β , and interferon (IFN)- γ ⁴. Gastro duodenal diseases caused by *H.pylori* lead to different pathological

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outcomes in relation to host's immune response. One of these outcomes is changing cytokine serum levels⁵. T- helper cells (Th1) secrete (IL-2), and (IFN- γ) that increase proinflammatory cytokines, and promote both neutrophil recruitment and macrophage activation⁶. Activated Th1 produce high levels of IL-2 when properly stimulated through both the TCR and the CD28 costimulatory molecule. *H.pylori* also stimulate the production of IL-2 in the gastric immune response⁷. *H.pylori* cytotoxin Vacuolating (VacA) inhibits Th1 proliferation by inducing arrest of the G1 / S cell cycle through interference with the cell receptor/IL-2 signaling pathway. The (VacA) prevents the activation of CD4 + cells. IL-6 is a multifunctional cytokine produced by monocytes, [Th2], macrophages and intestinal epithelial cells. It synthesized upon the stimulation of TLRs by *H.pylori* lipopolysaccharide or upon stimulation of cells by (TNF)⁸. IL-6 act as a messenger between innate and adaptive systems in the mechanisms of host defense by stimulating (Th2) to produce IFN- γ and activates B cells to secret immunoglobulin^{9,10}. The aim of the present study was to assess the serum level of IL-6 and IL-2 in *H.pylori* infected patients and to study their association with gastric endoscopy findings.

Materials and Methods

Study population

The study was conducted in the Endoscopy Unit of a specialist center for the digestive system and liver at Al Sadr Teaching Hospital, Basrah – Iraq on 107 patients with Gastro-duodenal Diseases (suspected *H.pylori* infection) and 19 healthy individuals. The eligible patients had been confirmed to have *H.pylori* with chronic gastritis or peptic ulcer through clinical and laboratory examinations, and histopathologically confirmed to have gastric cancer by endoscopic biopsy. Informed consent was obtained from all participants, and a questionnaire regarding age, job, marital status, residential address, number of

endoscopic, smoking, symptoms, and family history of Gastro-duodenal Diseases. Exclusion criteria were as follows: taking antibiotics in past 4 weeks, proton pump inhibitors in past 2 weeks or H2-blocker agents in past one week, taking immunosuppressive agents, active gastrointestinal bleeding, pregnancy, breast-feeding and history of gastrostomy. All individuals were tested for *H.pylori* fecal antigen and all patients tested positive *H.pylori* stool antigen were confirmed by R.U.T.

Assessment of serum IL-2 and IL-6

Serum concentrations of IL-2 and IL-6 were measured by commercial enzyme linked immunosorbent assay (ELISA) kit (KOMABIOTECH, KORA, IL-2 Lot No:42223, IL-6 Lot No:46223). The tests were performed according to the manufacturer's instructions. Briefly, standards and samples were pipetted into the 96 wells plate, coated with antibody specific for Human IL-2 and IL-6. IL-2 and IL-6 present in samples were bound to the wells by the immobilized antibody. Then, the wells were washed, and biotinylated anti-Human IL-2 and IL-6 antibody was added. After washing away unbound antibodies, HRP conjugated streptavidin was pipetted into the wells. The wells were washed and a TMB substrate solution was added to the wells. The intensity of the color was measured at 450nm. The results were calculated according to standard curve by reducing the data using ELISA reader's computer software capable of generating standard curve-fit.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 25 was used. P-value of <0.05 was considered a clue for the presence of significance.

Results

One hundred and seven patients with Gastro-duodenal Diseases (suspected *H.pylori* infection) and

19 healthy were recruited. The confirmed positivity of *H.pylori* detection was based on a R.U.T and stool antigen test; 75 (70.1%) patients were positive for *H.pylori*. Age group ≤ 30 years (39.3%), females (65.1%), and O blood group (58.7%) showed higher rate *H.pylori* infection (table 1).

Both interleukins (IL-2 & IL-6) were found at higher levels in patients than in healthy with

significant differences (p-value <0.05) (table2&3). Interestingly, the level of IL-2 was lower in patients infected with *H.pylori* (43.40 pg/ml) than those not infected (85.2 pg/ml), whilst the level of IL-6 was higher in patients infected with *H.pylori* (117 pg/ml) than those not infected (40 pg/ml), p-value <0.05 (table2). Furthermore, the increasing level of both interleukins was correlated with disease progress (Table2&3).

Table 1. Distribution of *H.pylori* status according to blood group

Blood group	H.pylori status			p-value
	HP +	HP-	Total	
A	19 (25.3%)	7 (21.9%)	26	0.015*
B	10 (13.3%)	13 (40.6%)	23	
O	44 (58.7%)	11 (34.4%)	55	
AB	2 (2.7%)	1 (3.1%)	3	
Total	75 (100%)	32 (100%)	107	

Table 2. Serum level of IL-2 in relation *H.pylori* infection and gastric endoscopy findings.

Category	IL-2 Level (median) pg/ml	Kruskal-Wallis Test	P-value
Study populations			
Patients	43.6	134.5	0.0001
Healthy people	31.1		
H.pylori status			
H.pylori Pos+	43.40	34.96	0.0001
H.pylori Neg-	85.2		
Gastric endoscopy findings			
Gastritis	40	22.54	0.001
Peptic ulcer	52		
Gastric cancer	103.9		

Table 3. Serum level of IL-6 in relation *H.pylori* infection and gastric endoscopy findings.

Category	IL-6 Level (median) pg/ml	Kruskal-Wallis Test	P-value
Study populations			
Patients	107.5	3.500	0.001
Healthy people	10.0		
<i>H.pylori</i> status			
<i>H.pylori</i> Positive	117	56.5	0.0001
<i>H.pylori</i> Negative	40		
Gastric endoscopy findings			
Gastritis	104	30.56	0.001
Peptic ulcer	167		
Gastric cancer	495		

Discussion

In the present study, we found that the prevalence of infection with *H. pylori* was 70.1% of patients with gastro-duodenal disorders. This finding was higher than that found in previous study Iraq (55.8%)¹¹, and in neighboring countries, e.g., 46.5% Saudi Arabia¹², 83.5% Iranian population¹³, 49.7% in Kuwait¹⁴, but lower than those found in Jordan (88.6%)¹⁵ and in Turkey (66.3%)¹⁶. In Egypt was (70%)¹⁷. The prevalence of *H.pylori* infection in china 62%, Korea 66.9%, Pakistan 74.4%¹⁸. These variations in the prevalence rates of *H.pylori* across the world might be attributed to different factors such as living standards, socioeconomic status, geographical location and ethnicity. In addition to the variability in the (*H.pylori*) detection methods, size of the study and exclusion of prior used of antibiotic¹⁹. Although there was no statistically significant difference observed among the studied age groups of patients,

the occurrence of the disease was higher in people aged ≤ 30 (39%). These findings agreed with other studies which reported that young people are the most effected age group who suffer from gastroduodenal disorders²⁰. However, many studies showed high prevalence in young as well as old age²¹. No statistically significant difference associated between sex and gastro-duodenal a disorder was observed in our study, though the occurrence of gastrointestinal disorders was higher in females (56.1%) compared to males (43.9%). These results corresponded with numerous previous research and studies²¹. In contrast, other studies reported that the occurrence of *H.pylori* infections is higher in males than in females^{23,24}. The present results revealed a relationship between ABO blood type and *H.pylori*, we found that the patients with blood group O (58.7%) were more prone to *H.pylori* infection than others groups (P=0.012). Similar studies demonstrated that the distribution

of ABO blood groups in *H.pylori* positive patients were A=31.4%, B=15.4%, AB=25.0% and O=53.7%, with a statistically significant link for blood group O ($p=0.05$)²⁵. The association of *H.pylori* with blood group antigens may be related to the Lewis blood group system (Lewis b antigen) which acts as a receptor for *H.pylori*. This antigen is most frequently found on blood group O, that people with blood group O have more *H.pylori* adhesions and have a higher density of colonized *H.pylori*²⁶. In our study we found that the IL-2 level was significantly down regulated in the serum of the *H.pylori* pos+ patients when compared with the *H.pylori* neg- patients, which is similar with those obtained by Dlugovitzky et al.⁵. Interleukin-2 secreted mainly by T helper cells (Th1) and other immune cells⁶. The *H.pylori* vacuolating cytotoxin A (VacA) protein can interact with lymphocytes, resulting in blockage of IL-2-mediated T cell proliferation²⁷. By its ability to induce vacuolization of epithelial cells, has also been revealed as an inhibitor of T cells signaling and proliferation by inducing a G1/S cell cycle arrest through the interference with the T cell receptor/IL2 signaling pathway²⁸. On other hand, IL-6 levels in patients infected with *H.pylori* were higher than in uninfected patients. The association between serum IL-6 levels and *H.pylori* found in the current study could play an important role epidemiologically and clinically. The association observed in this study is consistent with the result obtained by previous study²⁹, which showed that *H.pylori*-positive Japanese had a higher level of IL-6. Our findings suggest that a strong immune response to *H.pylori* enhanced the systemic inflammation, which was reflected in an increased level of serum IL-6. In addition, the increasing levels of IL-6 in peripheral blood of the patients is likely to be associated with ulceration inflammation, macrophage stimulation and active secretion by the neutrophils and vascular endothelial cells. Once attached to the gastric epithelial cells,

H.pylori incites an immune response characterized by activated inflammatory and immunologically competent cells such as neutrophils, lymphocytes and monocytes release IL-6, IL-8 and IFN-gamma. As a result, the serum levels of IL-6 increase³⁰. In this study we describe the level of IL-2 and IL-6 in the serum with relation to the gastric endoscopic findings in gastro-duodenal disease associated with *H.pylori*. We found IL-2; IL-6 levels significantly rise with the progress of gastric endoscopy findings. The results obtained in the present study regarding the level of IL-2 is comparable with those obtained by Sugimoto et al.⁹, who found that the serum levels of IL-2 were higher in Gastric cancer patients⁹. Also, in our findings of IL-6 level were similar with a study carried by Wu et al.³⁰, who found that the IL-6 levels in the serum of gastric cancer patients were significantly elevated³¹. Sugimoto et al., also found that IL-6 controls the development of chronic inflammatory diseases and the Serum levels of IL-6 are higher in patients with gastric cancer than gastritis⁹. Other studies also showed that the overproduction of IL-6 was responsible for the pathogenesis of various inflammatory diseases³⁰. IL-2 and IL-6 play an active role in the pathogenesis of gastro-duodenal disease. Several studies observed the correlation of IL-2 and IL-6 levels in the patient serum with the severity of gastro-duodenal disease¹⁰.

Conclusion

Serum IL-2 and IL-6 were markedly higher in *H.pylori* infected patients and the levels of the both interleukins were significantly higher in patients with peptic ulcer(s) and gastric cancer. However, IL-2 level was lower in *H.pylori* positive patients than *H.pylori* negative patients.

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Transnasal Esophagoscopy Procedure in Outpatient Clinic at Dr. Soetomo General Academic Hospital

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Abstract

Background: Transnasal esophagoscopy is a small caliber flexible esophagoscopy technique that has the same as diagnostic accuracy as conventional esophagoscopy. In addition, this technique is safer than any other techniques and has patient tolerance. **Aims:** This study aims to analyze and evaluate the transnasal esophagoscopy (TNE) procedure in patients. **Method:** retrospectively approach was used in this study by taking data from the activity register of the broncho-esophagology division of outpatient unit of ORL-HNS Dr. Soetomo General Academic Hospital. TNE report book and patient medical records were collected to have the data. The observation period was 2013 to 2017. **Result:** patients who met the inclusion and exclusion criteria was 99 patients, with a male to female ratio of 2.3: 1. Most endoscopic findings were normal esophagus (56.57%). Existing abnormal findings included esophageal stenosis (18.18%), esophageal mucosal lesions (14.14%), and esophageal tumors (11.11%). TNE examination complications were found to be 1%. **Conclusion:** Transnasal esophagoscopy is widely used in patients aged 51-60 years with dysphagia, reflux or globus as the most common indications. TNE is quite safe to do in an outpatient hospital setting.

Keywords: Esophagoscopy, Transnasal, Outpatient, Esophagus Disease, Dysphagia, Health Policy.

Introduction

Transnasal esophagoscopy (TNE) is a small caliber flexible esophagoscopy technique performed transnasally to evaluate the esophageal lumen. This technique aims to observe the entire upper aero digestive tract, from the vestibule of the nose to the gastroesophageal junction, and to examine the actions

of water flushing, water insufflation, suction and biopsy of the esophagus.¹⁻³

The TNE diagnostic procedure objectify to evaluate patients with dysphagia, esophageal stricture, laryngopharyngeal reflux, esophageal foreign body and other esophageal disorders. The patient is treated in a sitting position, where the scope is inserted through the nose under topical anesthesia without the use of sedation. This examination can be carried out easily, safely and effectively^{4,5}. In contrary, conventional (rigid) esophagoscopy requires routine general anesthesia and carries a risk of aspiration, hypoventilation and cardiopulmonary complications. The rigid esophagoscopy procedure also needs

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intensive care and monitoring both before and after the procedure^{6,7}.

On the other hand, TNE has as the same diagnostic accuracy as conventional esophagoscopy, however it has better safety and patient tolerance. TNE is performed without sedation, hence the side effects and risks of sedation can be eliminated which allows the patient to carry out routine activities immediately after the procedure.^{8,9}.

This study aims to analyze and evaluate the description of TNE results in outpatients at Dr. General Academic Soetomo Hospital.

Methods

The study was conducted retrospectively by taking data from the activity register of the bronchoesophagology division of ORL-HNS outpatient clinic Dr. Soetomo General Academic Hospital, including TNE report book and patient medical records. The population were all patients who underwent TNE examinations at the bronchoesophagology division of outpatient unit of ENT-KL RSUD Dr. Soetomo hospital from January 2013 to December 2017.

Sample of Research

The study sample was patients who qualify to the inclusion criteria, namely patients who underwent TNE from January 2013 to December 2017. Meanwhile, the exclusion criteria included incomplete medical record data and patients who failed to perform TNE. A total of 99 patients met the criteria of the study sample. All patients signed an informed consent form prior to the TNE procedure and were provided with an overview of the goals and benefits of undergoing this procedure.

Examination Procedure

Transnasal esophagoscopy was performed using topical anesthesia without sedation. Topical anesthesia was administered to the nasal cavity bilaterally using 2% lidocaine - ephedrine at least 10 minutes before the TNE procedure. The objective of topical anesthetics and decongestants was to have a vasoconstrictive effect and reduce pain. Then they gave a 10% xylocaine spray on the oropharynx and hypopharynx to reduce cough reflex and reduce pain.

A flexible esophagoscope with a scope diameter of 6.8 mm using the Olympus Evis Excera II camera system is used as a research instrument. The device has a water flushing and air insufflation system, as well as suction through the available working channels. The tip of the scope is lubricated with lidocaine gel before it inserted into the nose into the pharynx. The scope entered to the esophagus via cricopharynx. The patient was in a sitting position and asked to swallow at the same time when the scope was inserted into the esophagus. The scope is entered to the lumen until it reached the gastroesophageal junction.

Analysis Data

All data collected from the patient's medical record were recorded in the data collection sheet and then tabulated. The results are presented descriptively in the form of tables, diagrams and narrative.

Result

Data Demography

Demographic data in this study were divided based on age and gender. The mean age of the patients in the study was 52 years, with an age range of 16-77 years. Most patients were in the age range 51-60 years as many as 34 people (34.35%). Most of the patients were male (69.69%) with a male to female ratio of 2.3: 1 (Table 1).

Table 1. Patient characteristics

Age	Total (n)		Total Percentage (%)
	Men	Women	
< 11 Years	0	0	0%
11-20 Years	5	1	6.06%
21-30 Years	2	3	5.05%
31-40 Years	6	2	8.08%
41-50 Years	9	5	14.14%
51-60 Years	24	10	34.35%
61-70 Years	18	3	21.21%
71-80 Years	9	2	11.11%
> 80 Years	0	0	0%
Total	73	26	100%

Clinical Data

Clinical data in this study include indications of examination, endoscopic findings and complications. The indications for TNE examination consist of dysphagia, reflux or globus, screening for head and neck malignancies, evaluation of esophageal foreign bodies and evaluation of tracheoesophageal fistula. Endoscopic findings consist of normal esophagus, esophageal stenosis, esophageal mucosal lesions and esophageal tumors. The most indications for TNE were dysphagia, reflux or globus (46.47%), followed by

screening for head and neck malignancies (38.38%), evaluation of esophageal foreign bodies (13.13%) and evaluation of tracheoesophageal fistulas which were the least indicators obtained (2.02%) (Table 2).

The most indications for TNE examination in the form of dysphagia, reflux or globus are in the age range 51-60 years. Screening for head and neck malignancies is mostly in the age range 51-60 years. Most evaluations of esophageal foreign bodies were in the 51-60 years age range (Figure 2).

Table 2. Patient characteristics based on TNE indications

Inspection indication	Total (n)	Percentage (%)
Dysphagia, reflux / globus	46	46.47%
Head and neck malignancy screening	38	38.38%
Esophageal foreign body Evaluation	13	13.13%
Tracheoesophageal fistula Evaluation	2	2.02%
Total	99	100%

Based on gender, TNE examination in the form of dysphagia, reflux or globus were found in 25 male patients and 21 female patients. Screening for head and neck malignancies were 36 male patients and 2 female patients. Evaluation of esophageal foreign bodies in 7 male patients and 6 female patients. Tracheoesophageal fistula evaluated 1 male patient and 1 female patient (Figure 3).

Most endoscopic findings were normal esophagus (56.57%). Other findings recorded included esophageal stenosis (18.18%), esophageal mucosal lesions (14.14%), and esophageal tumors (11.11%).

Endoscopic findings for dysphagia, reflux or globus indications were normal esophagus in 15 patients, esophageal stenosis in 18 patients, esophageal mucosal lesions in 3 patients and esophageal tumor in 10 patients. In the indication for head and neck malignancy screening, the results showed normal esophagus in 37 patients and esophageal tumor in 1 patient. The indication for the evaluation of esophageal foreign bodies obtained normal esophageal results in 2 patients and esophageal mucosal lesions in 11 patients. The indication for the evaluation of tracheoesophageal fistula obtained normal esophageal results in 2 patients (Table 3 and 4).

Table 3. Patient characteristics based on TNE endoscopic findings

	Total (n)	Percentage (%)
Endoscopic findings		
Normal	56	56.57
Esophageal stenosis	18	18.18
Esophageal mucosal lesions	14	14.14
Esophageal tumor	11	11.11

In this study, the indication for TNE examination of dysphagia, reflux or globus was the highest in esophageal stenosis. The most indications for screening for head and neck malignancies are found in normal esophagus. The indication for the evaluation

of esophageal foreign bodies has the highest results in esophageal mucosal lesions. The most indication for tracheoesophageal fistula evaluation was found in normal esophagus (Figure 3). TNE examination in this study, as many as 99 patients, found no complications.

Table 4. Patient characteristics based on TNE indications

Inspection indication	Normal	Stenosis	Lesions	Tumor
Dysphagia, reflux / Globus	15	18	3	10
Head and neck malignancy screening	37	0	0	1
Esophageal foreign body Evaluation	2	0	11	0
Tracheoesophageal fistula Evaluation	2	0	0	0

Dicussion

Based on the age category, TNE examinations were found mostly in the age range 51-60 years. The youngest patient was 16 years old and the oldest patient was 77 years old. The mean age of the patients was 52 years. This is consistent with a study conducted by Chung et al (2014) in Korea involving 137 patients with an average patient age of 55 years. The youngest age of the study was 19 years and the oldest 78 years⁹. This equation can be caused by the similarity of the indications.

Based on gender, the researchers were found that most of patients were male. The ratio of male to female is 2.3: 1. The incidence of head and neck malignancies is mostly found in males, hence it explained why the participants of this study were dominated by males. Sombuntham, et al., (2015) reported a retrospective use of TNE in Thailand as many as 58 patients, showing the same results where there were more men than women with a ratio of 2.8:1¹¹. Our study found that the most research samples were head and neck malignancies as much as 60%. Another study in England on 257 patients, showed different results where it was found that men were equal to women with a ratio of 1: 1¹⁰. There were not many patients with head and neck malignancies in the study sample which led to balance proportion of the ratio of men and women.

The most indication for TNE examination was the evaluation of dysphagia as much as 46.47%. Dysphagia patients in this study were found to be more male than female as much as 54%. Research by Hoy, et al (2013) reported 96 patients, showing the same results where the most indication of TNE was an evaluation of dysphagia as much as 79%.¹¹ Another study reported that out of 100 patients with dysphagia the majority were male as much as 58%¹².

The patient characteristics of this study were

dominated by the age range 51-60 years. This shows that dysphagia is common in patients of this age range. Another reason for this age range is that many patients with head and neck malignancies undergo screening using TNE. Patients with head and neck malignancies undergoing total laryngectomy require screening for esophageal malignancy. Dysphagia is one of the swallowing problems found in older people. Dysphagia can be in the form of anatomical or physiological deficits in the mouth, pharynx, larynx and esophagus. The process of swallowing changes with age. Increasing age will cause a decrease in the quality and effectiveness of the swallowing process¹³. The results of this study are in accordance with the literature.

Transnasal esophagoscopy with indications for evaluation of head and neck malignancies was performed on 38 patients. The role of TNE in head and neck malignancies is growing, both routine screening, suspected esophageal tumors and post chemotherapy and radiation to head and neck malignancies¹⁴. TNE is the best alternative for the initial screening of esophageal malignancies in patients with head and neck malignancies, whereas rigid esophagoscopy is used for malignancies for which the primary is unknown and in large malignancies of the base of the tongue.¹⁵

The evaluation of esophageal foreign bodies was 13.13%. The use of TNE for the extraction of foreign objects at outpatient unit in our hospital had never been done because there was no extraction forceps available. One study reported the use of TNE for the extraction of foreign bodies. Most of the foreign body of the esophagus was successfully extracted but 32% had to undergo rigid esophagoscopy because of the large size of the foreign body, its sharp shape and the risk of esophageal perforation¹⁶. The use of TNE in the diagnosis of foreign bodies is superior to that of plain neck radiographs. The sensitivity and positive

predictive value of plain radiographs in diagnosing the presence of foreign bodies were only 59% and 52% because most of the foreign bodies were radiolucent¹⁶. Bennett, et al., States that TNE improved the diagnosis and management of the extraction of several types of foreign bodies compared to the rigid esophagoscopy method, but that certain foreign bodies cannot be extracted by this method because of their large size¹⁷.

Esophageal abnormalities in this study were found in 42% of the 99 patients examined by TNE. The most common abnormality was esophageal stenosis as much as 18.18%. Other esophageal abnormalities were esophageal mucosal lesions in 14.14% and tumors in 11.11%. The number of positive findings as much as 42% is according to reports from several researchers. Research by Chung et al (2014) reported positive findings in 38.7% of 137 patients⁹. Meanwhile, Abou-Nader et al (2014) reported positive findings in 44% of 257 patients¹⁰. Bellafsky (2001) reported positive findings on the TNE examination in 44% of 96 patients¹¹. Research by Shariff, et al., Reported that there was a strong association between Barrett's esophageal images found by TNE (98% sensitivity, 100% specificity) and conventional esophagoscopy¹⁸.

There were no complications in the 99 patients who were examined using TNE in this study. The study on 257 patients reported that none of the patients had complications¹⁰. A study by Postma, et al., Reported that out of 592 patients who underwent TNE examinations in America, 2 patients had complications (0.33%).¹⁹.

Conclusion

Transnasal esophagoscopy is a procedure that is experienced by many patients in the 51-60 years age range, with a male to female ratio of 2.3: 1. Dysphagia, reflux or globus are the most common indications for transnasal esophagoscopy. In this study, there were no complications.

Competing interests

All authors declare that no competing interests were disclosed.

Grant information

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Ethics and consents: Ethical clearance was approved by the Ethics Medical Research Committee of the Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (Ref. No.: 0442/LOF/301.4.2/IV/2021) on April 16, 2021.

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Comparison of the Effect of Glycemic Control on the Incidence of Fetal Macrosomia and Large for Gestational Age in Gestational Diabetes Mellitus Patients; A Systematic Review

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Abstract

Background: Gestational diabetes mellitus (GDM) can be managed with blood glucose control management, which includes a healthy lifestyle, insulin therapy, and oral anti-diabetic drug (OAD) medications when needed. Sub-optimally or poorly managed GD Mmay lead to arisk of complications, one of which is an abnormal growth in the fetus. This study aimed to compare the effect of blood glucose control management on the incidence of fetal macrosomia and large for gestational age (LGA) births in patients with GDM. **Methods:** This systematic review study obtained data from formerly published studies from the Science Direct database. The article search method used the characteristics of PICO (Population, Intervention, Comparison, Outcome) and compiled using the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) method. **Conclusion:** The use of medical nutrition therapy (MNT) and exercise in the therapeutic regimen, as well as routine monitoring of glycemic levels are very important to control the patient's glycemic level. The use of metformin can increase the success of therapy due to reduced levels of LGA and macrosomia in GDM patients.

Keywords: *diabetic pregnancy, glycemic control, macrosomia, large for gestational age, diabetes control, diabetic therapy*

Introduction

Diabetes is the most common metabolic disease that occurs during pregnancy.¹ Diabetes in pregnancy that is not managed optimally can cause morbidity in both mother and baby. A cohort systematic study showed that pregnant women with hyperglycemia had a greater risk of complications during delivery,

particularly macrosomia in the newborn and preeclampsia in the mother.² The incidence of macrosomia in GDM with poor glycemic control is 40%.³

According to 2013 WHO classification, hyperglycemia first detected during pregnancy must be categorized into 1) Pregestational diabetes, which can be determined by the same criteria as diabetes in nonpregnant persons; and 2) Gestational diabetes mellitus, which is diabetes diagnosed in the second or third trimester of pregnancy that did not exist before gestation.⁴ GDM therapy consists of two regimens:

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1) Non-pharmacological (medical nutrition therapy/ MNT/diet and exercise), and 2) Pharmacological (insulin).⁵ This study aims to find out and analyze the comparison of the effect of blood glucose control management on the incidence of macrosomia and large for gestational age in patients with gestational diabetes.

Materials and Methods

The material used in this study is the result of studies and analyses that have been carried out about the effect of blood glucose control management on the incidence of fetal macrosomia and large for gestational age in patients with gestational diabetes mellitus. Articles that have been collected are managed using the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) method. All articles were identified, screened, assessed for their eligibility, and included in the review.

A complete Science Direct search was done to obtain secondary data from articles published that included the search term containing gestational diabetes AND macrosomia AND (diet OR insulin OR metformin OR glyburide OR treatment) in their title and abstract. Boolean Operators including OR/AND were used. The keywords used are entered together into the electronic database search engine using advanced search, then selected using the PRISMA flow according to the inclusion and exclusion criteria that have been set. Articles are selected quickly by using the find word feature of the Google Chrome browser to find keywords. Types of studies that meet the inclusion criteria are quantitative studies that can be cross-sectional, cohorts, or clinical trials. This study

focused on published articles papers from 2016 to 2020. Seventeen articles were selected in total. The chosen papers were published from 2016 to 2020.

Results and Discussion

Seventeen quantitative research studies have been identified by search (Au *et al.*, 2016; Kanai *et al.*, 2016; Shi *et al.*, 2016; Silva *et al.*, 2016; Bogdanet *et al.*, 2017; Bianchi *et al.*, 2018; Eid *et al.*, 2018; Huhtala *et al.*, 2018; Khin *et al.*, 2018; Zamstein *et al.*, 2018; Zygula *et al.*, 2018; Lu *et al.*, 2019; Manoharan *et al.*, 2019; Meghelli *et al.*, 2019; Kapustin *et al.*, 2020; Pazzagli *et al.*, 2020; Penager *et al.*, 2020). Data extracted can be seen in **Table 1**. This study was conducted at one or more institutions from various countries, with the research subjects being patients with GDM who received MNT (medical nutrition therapy), exercise, insulin, and/or OAD (oral anti-diabetic drugs). All studies contain evidence of anti-hyperglycemic therapy and related outcomes of macrosomia or LGA outputs from GDM patients.

GDM Therapy Used in Research

GDM therapy primarily consisted of low glycemic index dietary modification.⁶ In several studies, patients received dietary recommendations from nutritionists.^{7,8} According to the recommendations of the American Diabetes Association (ADA), insulin therapy is then given when the glycemic target cannot be achieved only with dietary modifications, MNT, and lifestyle changes^{6,9}, namely if fasting blood glucose (FBG) > 92 mg/dl or 1 hour. Post-prandial (PP) blood glucose > 130 mg/dl in at least 20% of measurements in a week.⁸

Table 1. Journal Summary Matrix

No	Author, Year of Publication, Type of Study	Sample	Therapy Used	LGA Outcome	Macrosomia Outcome
1	Au et al. 2016, cross-sectional	67 single term babies born to GDM patients between September - October 2010 at the Royal Prince Alfred Hospital, Sydney, Australia	MNT and exercise (n = 38) MNT, exercise, insulin (n = 29)	MNT and exercise 5% (n = 2) MNT, exercise, and insulin 10% (n = 3)	N/A
2	Kanai et al. 2016, cohort	38 patients with mild GDM according to IADPSG criteria between 2009 - 2010 at Tsukuba University Hospital, Japan	Without therapy (n = 38)	Without therapy 26.3%	Without therapy 2.6%
3	Shi et al. 2016, retrospective cohort	488 GDM patients aged 21-44 years who were treated by the associate Department of Clinical Nutrition and Obstetrics of the China-Japan Friendship Hospital between 2008-2012	MNT (n = 307) [With insulin 20.25% (n = 63)] Without MNT (n = 181) [With insulin 42.54% (n = 77)]	N/A	MNT 9.77% (n = 30) Without MNT 27.62% (n = 50)
4	Silva et al. 2016, retrospective cohort	705 GDM patients in general maternity hospitals between July 2010 - August 2014	MNT + exercise 41.6% MNT + exercise + metformin 35.5% MNT + exercise + insulin 15% MNT + exercise + metformin + insulin 7.9%	MNT + exercise 26.31% (n = 30) MNT + exercise + metformin 28.07% (n = 32) MNT + exercise + insulin 26.31% (n = 30) MNT + exercise + metformin + insulin 19.29% (n = 22)	1.9% (n = 13)
5	Bogdan et al. 2017, retrospective cohort	1319 GDM patients according to IADPSG criteria between 2009 - 2014 from the ATLANTIC DIP database	MNT + exercise (n = 567) MNT + exercise + insulin (n = 752)	MNT + exercise 12.5% (n = 71/566) MNT + exercise + insulin 19.7% (n = 143/727)	MNT + exercise 12.7% (n = 72/565) MNT + exercise + insulin 22.2% (n = 165/744)
6	Bianchi et al. 2018, retrospective cohort	1198 pregnant women were referred to the diabetes clinic at The University Hospital of Pisa from January 2010 - March 2015 for GDM screening	MNT + exercise 67% MNT + exercise + insulin 33%	No differences were observed	No differences were observed
7	Eid et al. 2018, RCT	250 GDM patients at Alglaa Teaching Hospital antenatal clinic from March 2016 - June 2017	MNT + exercise + insulin (n = 113) MNT + exercise + metformin (n = 116)	MNT + exercise + insulin 15.5% (n = 18) MNT + exercise + metformin 11.5% (n = 13)	MNT + exercise + insulin 5.2% (n = 6) MNT + exercise + metformin 2.8% (n = 3)
8	Huhtala et al. 2018, RCT	319 GDM patients at Turku University Hospital, Turku, Finland, from June 2006 - December 2010, 216 of whom were randomized to receive insulin or metformin therapy	MNT (n = 103) MNT + insulin (n = 107) MNT + metformin (n = 109)	MNT 11.7% (n = 12) MNT + insulin 15.9% (n = 17) MNT + metformin 14.3% (n = 15)	MNT 4.9% (n = 5) MNT + insulin 0.9% (n = 1) MNT + metformin 4.6% (n = 5)
9	Khin et al. 2018, retrospective cohort	138 GDM patients on diet and metformin therapy in a UK district hospital, from January 2009 - December 2012	MNT + exercise + metformin (n = 61) MNT + exercise + metformin + insulin (n = 77)	MNT + exercise + metformin 6.5% MNT + exercise + metformin + insulin 13% (n = 10)	MNT + exercise + metformin 3.8% (n = 2) MNT + exercise + metformin + insulin 5.2%

Cont... Table 1. Journal Summary Matrix

10	Zamstein et al. 2018, population-based cohort	10184 GDM patients with singleton pregnancies who gave birth at a tertiary referral hospital from 1991-2014 59 GDM patients from January 2011 - January 2013	MNT + exercise (n = 9460) MNT + exercise + insulin/OAD (n = 724)	MNT + exercise 11% MNT + exercise + insulin/OAD 18%	MNT + exercise 10% MNT + exercise + insulin/OAD 13.3%
11	Zygula et al. 2018, cohort	59 GDM patients from January 2011 - January 2013	MNT (n = 44) Insulin (n = 15)	N/A	MNT 0% Insulin 13%
12	Lu et al. 2019, case-control retrospective	68 GDM patients who delivered at term at People's Hospital of North Jiangsu Province from January 2017 - June 2017	MNT (n = 56) Insulin (n = 12)	N/A	MNT 16.1% (n = 9) Insulin 16.7% (n = 2)
13	Manoharan et al. 2019, cross-sectional	40 primi gravida GDM patients on insulin therapy in the South Indian Tamil population	MNT + insulin (n = 40)	MNT + insulin 17.5% (n = 7)	N/A
14	Meghelli et al. 2019, retrospective cohort	121 singleton gestational diabetes mellitus patients with BMI before gestation > 40 kg/m ² from January 1996 - December 2014	MNT (n = 56) MNT + insulin 52.9% (n = 63)	MNT 35.2% (n = 19) MNT + insulin 29.5% (n = 18)	N/A
15	Kapustin et al. 2020, longitudinal prospective	40 GDM patients according to IADPSG criteria	MNT (n = 20) Insulin (n = 20)	N/A	MNT 20% (n = 4) Insulin 35% (n = 7)
16	Pazzagli et al. 2020, cohort	2467 GDM patients in Sweden from 2012-2016 who had recently received insulin or metformin therapy at 2nd or 3rd trimester	Insulin 88% (n = 2182) Metformin 7.6% (n = 187) Insulin + metformin 4.3% (n = 107)	N/A	Insulin 20% (n = 436/2182) Metformin 9.7% (n = 18/187) Insulin + metformin 21.7% (n = 23/107)
17	Penager et al. 2020, case-control retrospective	113 cases of birth in singleton gestational GDM patients with complicated macrosomia (only 81 data on therapy)	MNT 76%, MNT + insulin 24% (n = 26) [Insulin was more used in macrosomia group than in normal control group]	N/A	N/A

In several other studies, metformin is used as the first line when glycemic control cannot be achieved with MNT and physical activity alone.¹⁰ If the glycemic control target remains unattainable with the maximal dose of metformin (2,5 g), the patient is then advised to take insulin therapy. In some cases of severe GDM, insulin can be given immediately, without having to use metformin first. GDM is considered severe if the fetal abdominal circumference exceeds the 90th percentile, the patient's FBG > 100 mg/dl, and the 1 hour-PP blood glucose level > 140 mg/dl.¹¹

Shi *et al.* in 2016 used MNT guidelines published by the Chinese Diabetes Society and the China Medicine Doctors Association Nutrition Doctor Specialized Committee. MNT is regulated based on the

patient's body type before pregnancy, gestational age at diagnosis of GDM, weight gain during pregnancy, blood pressure, and lipid levels. MNT regulates food types and measures recommended intakes to ensure a balanced intake of the necessary nutrients, especially those with a low glycemic index. MNT also provides mealtime recommendations based on routine blood glucose monitoring data. MNT recommends small but frequent meals, to reduce the glycemic load at each meal, and suggests postprandial exercise.⁷

Suggestions for postprandial exercise include walking for 10 minutes a few days a week and gradually adding 5-10 minutes of exercise each day. For the majority of patients, the goal is to walk for 30 minutes most days of the week. Patients are also

advised to add more time to their daily physical activities. This exercise is recommended not to be done excessively, and only in a light form.¹²

Effect of GDM Therapy on Glycemic Control

Data on glycemic control are only available in a few articles. Au *et al.* in 2016 found that good glycemic control was achieved in most of the subjects, with 56 of 62 (90%) patients meeting the control targets. All patients in this study used MNT and exercise, while those who had not reached the target with both were given insulin in their regimen. Both groups showed the same results on the results of glycemic control.⁶

However, in another study, it was found that in patients on insulin therapy, patients with excess body weight, and/or BMI > 30 kg/m², glycemic control was only sub-optimal, and HbA1C values were higher. This was subsequently associated with higher levels of LGA and macrosomia in patients receiving insulin therapy, compared to those receiving MNT alone.⁹ This phenomenon was also found in a study by Penager *et al.* in 2020, who found that poor glycemic control was found three times more in the group with macrosomia, whereas in this group higher insulin use was also found compared to the control group without macrosomia.¹³ The use of a combination of metformin and insulin, was found to increase the average score of glycemic control when compared to insulin alone.¹⁰

In another study, the use of metformin in MNT and exercise regimens also showed good results in GDM control and was shown to reduce unwanted neonatal outcomes.¹¹ This result is quite different from the study by Huhtala *et al.* in 2018, which found that patients who were given a combination of MNT and metformin with MNT and insulin did not show different results on glycemic control.¹⁴

Comparison of Macrosomia Incidence in Various Therapies

Of the seventeen studies analyzed, only twelve had data regarding the number of incidences of macrosomia. Of these, only eleven studies contained more than one group of therapeutic modalities so that they could be compared. Ten studies suggested an association between insulin use and a higher incidence of macrosomia.^{9,10,12,13,15,16,17,18} Three studies found this phenomenon in patients treated with insulin alone^{16,17,18} and two studies found this phenomenon in patients receiving MNT, exercise, and insulin.^{9,12}

When viewed by percentage, the average macrosomia level was found to be highest in the group without MNT, which was 27.62%⁷ and was followed by the metformin and insulin combination group with 21.7%.¹⁹ Other moderately high results were found in the insulin-only group, with a mean of 21% (range 13-35%).^{16,17,18} The results obtained indicate a relationship between insulin use and high levels of macrosomia. This can be explained by the existence of a therapy protocol that insulin will only be given or added to the therapeutic regimen if a patient is not able to achieve good glycemic control with lifestyle modification alone. The results also demonstrate the importance of using lifestyle modifications that accompany insulin therapy, compared to the use of insulin alone.

Different results were obtained in a study by Huhtala *et al.* in 2018, who found that the incidence of macrosomia was found to be higher in patients with MNT alone, although the incidence of LGA was more common in the use of MNT and insulin. The different results obtained could be explained by the excellent glycemic control achieved in both groups. There were no differences in maternal weight before and after therapy, as well as other baseline characteristics. The variation in glucose levels in this study was very small, leading to the outcome differences between the two groups were not significant.

Data regarding therapeutic modalities associated with the lowest levels of macrosomia were only obtained in ten studies. Four studies found the lowest levels of macrosomia in the group with MNT alone^{7,16,17,18}, two studies found this phenomenon in groups with MNT and exercise.^{9,15}, and two other studies found this phenomenon in the group with MNT, exercise, and metformin.^{12,10} The rest of the studies found this phenomenon in the group treated with metformin alone¹⁹ and MNT and insulin.¹⁴ Most of the studies that contain data regarding the lowest levels of macrosomia (6 out of 10) found that the use of lifestyle modification as the first line in the GDM therapy protocol was able to reduce the level of macrosomia. The absence of additional therapy also implies that the patient's glycemic balance is achieved, resulting in a lower macrosomia outcome. This phenomenon can prove that the GDM therapy protocol that has been applied has good and appropriate outcomes as expected.

In terms of percentage, the average macrosomia level was found to be the lowest in the MNT and insulin therapy group, which was 0,9%. This study showed excellent glycemic control in all groups of therapeutic modalities so that even though insulin was used, the macrosomia level was still very low.¹⁴ The percentage of the smallest macrosomia level was then followed by the group without any therapy with 2,6%. However, this may be influenced by the severity of GDM in the study sample which was still mild.²⁰ The next four treatment regimens had additional metformin as a treatment modality. MNT, exercise, and metformin occupy the third-lowest percentage level, with an average of 3,3%% (range 2,8-3,8%)^{10,12}; MNT and metformin group with a percentage of only 4,6%¹⁴; followed by the MNT, exercise, metformin, and insulin group with 5,2%¹⁰; and metformin therapy alone with a macrosomia incidence of 9,7%.⁹ This phenomenon can support the idea of using metformin in the treatment regimen

before insulin is given.

Comparison of LGA Incidence in Various Therapies

LGA is defined as neonate weight > P90 according to gestational age and sex.⁶ Of the seventeen studies, only nine have data related to the number of LGA incidents. Among these, there were only eight studies that contained more than one therapeutic modality group that can be compared. Six studies found a link between insulin use and a higher incidence of LGA. Three of them found this phenomenon in patients with MNT, exercise, and insulin.^{6,9,12} Different results were obtained in the study by Silva *et al.* in 2016, who found that the incidence of LGA was higher in patients with MNT, exercise, and metformin therapy, compared to patients treated with lifestyle modification alone, lifestyle modification and insulin, or lifestyle modification with a combination of insulin and metformin.¹¹ The incidence of LGA is also seen to have a fairly large percentage in the group with MNT therapy alone in Meghelli *et al.* 2019, which is 35.2%. However, this may occur due to the sample of this study which is GDM patients with obesity. Obesity is an independent risk factor for macrosomia, so its effect is synergistic with GDM in aggravating LGA in infant patients. This study implies that optimal therapy is important to minimize the risk of macrosomia.²¹

All studies containing outcomes in the form of LGA and macrosomia have synergistic results, except in the study by Huhtala *et al* in 2018, which found differences in the treatment group which gave more incidences of outcomes. This study found a higher incidence of LGA in the MNT and insulin groups, which was 15,9% of the total sample, while the incidence of macrosomia was higher in the group of patients treated with insulin alone, which was 4,9% of the total sample.¹⁴

Manoharan *et al.* in 2019 found that the LGA outcome in patients receiving MNT and insulin was 17,5%.²² Another study also found that mild GDM patients without therapy found the incidence of LGA of 26,3%.²⁰

Data on therapeutic modalities associated with the lowest LGA levels were obtained in eight studies. Three of them found this phenomenon in the group with MNT and exercise.^{6,9,15} Two studies found this phenomenon in groups with MNT, exercise, and metformin.^{10,12} The rest of the studies found this phenomenon in the MNT, exercise, metformin, and insulin therapy group¹¹; MNT alone¹⁴ and MNT and insulin.²¹ When viewed from the percentage of LGA incidence, therapeutic modalities with the best outcomes are the MNT and exercise group, with a range of 5-13%, and an average of only 10%.^{6,9,15}

The next three treatment groups with the best outcomes used metformin in their treatment regimen. MNT and metformin are the therapeutic modalities with the second smallest percentage, which is only 14,3%¹⁴; followed by the group receiving MNT, exercise, and metformin with an average of 15,36% (range 6,5-28,07%)^{10,11,12}; and the MNT, exercise, metformin, and insulin groups with an average of 16,15% (range 13-19,29%).^{10,11} Further research is needed on the safety of using metformin in GDM patients.

Conclusion and Acknowledgement

In conclusion, the use of MNT and exercise in the therapeutic regimen, as well as routine monitoring of glycemic levels are very important to control the patient's glycemic level. The use of metformin can increase the success of therapy due to reduced levels of LGA and macrosomia in GDM patients. Therefore, glycemic control with MNT, exercise, and administration of metformin or insulin optimally as indicated, is very important to reduce undesirable

GDM outcomes, especially macrosomia and LGA.

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Abbreviation

- 1) ADA — American Diabetes Association
- 2) FBG – Fasting Blood Glucose
- 3) GDM – Gestational Diabetes Mellitus
- 4) IADPSG – *International Association of the Diabetes and Pregnancy Study Groups*
- 5) LGA – Large for Gestational Age
- 6) MNT – Medical Nutrition Therapy
- 7) N/A – Not Available
- 8) OAD – Oral Anti-Diabetic Drug
- 9) PICO – Population, Intervention, Comparison, Outcome
- 10) PP – Post-Prandial
- 11) PRISMA – Preferred Reporting Items for Systematic Review and Meta-Analysis
- 12) RCT – Randomized Controlled Trial
- 13) WHO – World Health Organization

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Antibacterial Activity of Antimicrobial Peptide Indolicidin against Multidrug-Resistant *Klebsiella pneumoniae* Isolated from Patients with Burns

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Abstract

The emergence of multidrug resistant *Klebsiella pneumoniae* has become a significant problem worldwide and also being a major threat to patients with burns infections. The therapeutic action of antimicrobial peptides derived from humans or animals or synthetic peptides attracted attention as alternatives for antibiotics in order to treat the resistant strains especially with strains isolated from burn patients. The current study investigated the role of antimicrobial peptide Indolicidin as an antibacterial agent with multidrug *K. pneumoniae* isolates from burns. The collection of study samples has taken place at the period between November 2020 and completed at end of March 2021, it has included 250 clinical specimens as burn swabs from inpatients with burn infections admitted in four hospitals in Baghdad. The results of selective media, biochemical tests, and Vitek2 system identified 40 isolates (16%) as *K. pneumoniae* from all collected bacterial cultures. The results of the antimicrobial susceptibility test by using the disc diffusion method for the isolates under study showed that *K. pneumoniae* clinical isolates were moderate resistant to the majority of the antibiotics tested. The majority of *K. pneumoniae* isolates were high resistant to Erythromycin (100%) and Ceftazidime (85%), also, it was obvious resistance to Ceftriaxone, Cefepime and Cefotaxime, while the lowest percentage of resistance was for Imipenem (25%) and Meropenem (38%). The results of minimum inhibitory concentrations (MICs) of indolicidin against (10) *K. pneumoniae* isolates which multidrug resistant and formed the strong biofilm, revealed that range of concentrations of indolicidin was (0.7-100 µg/ml) and it was obvious that there is a significant effect of indolicidin on the growth of *K. pneumoniae* at very low concentrations. In this study, we believe that the development of these antimicrobial peptides may become a new generation of urgently needed antimicrobials that can overcome bacterial resistance mechanisms.

Keywords: Antibacterial, Burns, Indolicidin, *Klebsiella pneumoniae*

Introduction

The high prevalence of multidrug resistant bacteria in burn units is likely a consequence of several factors,

including high antibiotic pressures, high colonization pressures, need for intensive medical, surgical therapy, and a vulnerable immunocompromised patient population^[1]. *Klebsiella pneumoniae* accounts for about one-third of all Gram-negative infections such as urinary tract infections, cystitis, pneumonia, surgical wound infections, burns, and septicemia

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[2]. Treating pathogens is becoming challenging because of multidrug resistance and availability of limited alternative therapies which has further confounded this problem [3]. Antimicrobial peptides (AMPs) are promising candidates as antibacterial agent against resistant bacteria, which reduce the likelihood of resistance evolving compared to the use of antibiotics. Furthermore, combinations of AMPs and traditional antibiotics with different mechanisms of action could facilitate the revival of ineffective drugs based on the enhanced or synergistic activity of the combination against human pathogens [4]. Due to the importance and wide spread of these bacteria and spread of antibiotics resistance and to achieve important of antimicrobial peptide to treatment, this study was aimed to evaluation the role of the short antimicrobial peptides indolicidin as therapeutic agents against the multidrug resistant *Klebsiella pneumoniae* isolated from burn patients.

Materials and Methods

Isolation and identification of *K. pneumoniae*

This study was performed at Hospitals in Baghdad, Iraq, between November 2020 and March 2021. Out of 250 burn swabs, a total of 40 isolates of *K. pneumoniae* were collected from patients with burns Infections. CHROMagar Orientation, Blood agar and McConkey agar were used for isolation *K. pneumoniae*. These isolates were identified using traditional bacteriological methods and biochemical testing, with VITEK 2 system (bioMerieux, France), according to the manufacturer's recommendations.

Antibiotic Susceptibility Test

Antimicrobial susceptibility test was conducted by using disc diffusion method. Briefly, *K. pneumoniae* overnight growth were prepared on McConkey agar and then resuspended in Mueller-Hinton broth (Oxoid). The turbidity of the suspension is adjusted to an equivalent 0.5 McFarland and this suspension

was used to inoculate on Mueller-Hinton agar (Oxoid) plates. The antibiotics discs used in this study as the following: Kanamycin (K), Gentamicin (GM), Imipenem (IMI), Meropenem (MEM), Ceftazidime (CAZ), Cefotaxime (CTX), Ciprofloxacin (CIP), Tetracycline (T), Ampicillin sulbactam (SAM), Erythromycin (E), Cefepime (CPM) and Cefoxitin (FOX), (MAST, UK) were placed on the medium. The agar plates were incubated at 35 °C for 24 h. and then the inhibition zone was measured and interpreted by the percent of susceptible, intermediate, or resistant isolates as defined by CLSI breakpoint interpretative Criteria (CLSI, 2020).

Minimum inhibitory concentrations (MIC).

MIC was determined using the microdilution method (Microtiter Plate Assay with Resazurin Dye) as described by the Clinical and Laboratory Standards Institute (CLSI, 2020). Briefly, 1:2 serial dilutions of Indolicidin peptides in Mueller Hinton Broth (MHB) were placed in a 96-well round-bottom plate at concentrations ranging from 100 to 0.7 µg/ml. The bacterial inoculum was prepared from a subculture of *K. pneumoniae* in LBB incubated for 18–24 hours at 35 ± 2°C before to the test. The bacteria suspension was diluted to 1x10⁸ colony forming units (CFU)/mL, to obtain a turbidity equivalent to 0.5 on the McFarland scale, confirmed by spectrophotometry upon reaching an absorbance between 0.08–0.1 at a wavelength of 625 nm; then a 1:200 dilution in MHB was performed to obtain a final concentration of 5x10⁵ CFU/mL. The diluted bacterial suspension was added to the 96-well plate containing the serially diluted peptides. The final volume of 200 µL per well consisted of 100 µL of the compound and 100 µL of diluted bacteria suspension. Negative and positive growth controls were performed by adding only MHB or *K. pneumoniae* with MHB to the wells, respectively. After incubation for 24 h at 37 °C, resazurin (0.015 %) was added to all wells (30 µl per well), and further incubated for 2–4 h for

the observation of colour change. On completion of the incubation, columns with no colour change (blue resazurin colour remained unchanged) were scored as above the MIC value. At the end of the incubation time, MIC was determined as the lowest compound concentration at which no bacterial growth was observed.

Results and Discussion

Isolation and characterization of *K. pneumoniae*

The two most important distinguishing characteristics of *Klebsiella* spp. are positive lactose fermentation on MacConkey agar medium and the viscosity of the colonies. *Klebsiella* spp. isolates (such as *K. pneumoniae*, and *K. oxytoca*) showed positive result on the MacConkey agar after 24-48 hours of incubation at 37°C, as shown in figure (1).

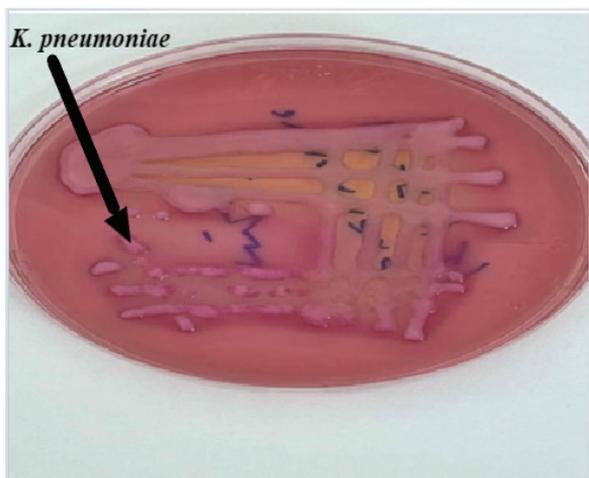


Figure (1): *Klebsiella* spp. on MacConkey agar plate.

All suspected *Klebsiella* colonies were detected by culturing on blood agar medium (supplemented with 5% human blood) showing large shiny, mucoid, whitish-grey and round colonies with no hemolysis^[5]. Moreover, as described in Figure (1), mucous colonies of *K. pneumoniae* were touched with a standard inoculating loop and the loop was lifted vertically from the surface of the agar plate, mucoid isolates adhered to the loop and stretched more than 5 mm in length as it was lifted from the plate^[6].



Figure (2): String test for *Klebsiella pneumoniae* mucoid colonies.

CHROMagar Orientation medium was used for specific isolation of urinary tract pathogens. On CHROMagar, isolates of *Klebsiella* appeared as metallic blue colonies at 37°C for 24 hours as shown in Figure (3) this medium also has selectivity for other Urinary tract pathogens with specific color for each bacterial genus, where the colonies of *Escherichia coli* appeared as pink red colonies



Figure (3): Metallic blue colonies of *K. pneumoniae* on chrome agar surface after 24 h at 37°C of incubation (Selective test).

Chromogenic agars are reliable media for the detection aerobic Gram negative bacteria by easier

recognition of different colonies on these media. CHROMagar Orientation medium is preferred medium because of the high accuracy and the rapid identification with very low false positive rates [7]. The use of CHROMagar Orientation medium reduces the need for further reagents and extra confirmatory tests suggesting that CO medium is a cost-effective replacement for conventional urine culture methods and its significantly reduced workload in the microbiology laboratory compared to that for Blood agar and MacConkey agar, and should be considered

as an alternative to conventional culture methods for detecting and reporting uropathogens [8]. *Klebsiella* isolates found to be non-motile which differentiate them from other motile *Enerobacteriaceae* genus. All isolates gave negative results for oxidase test and positive results for catalase and urease tests. Glucose was fermented with the production of acid and gas, H₂S was not produced. Most of clinical *K. pneumoniae* isolates obtained in presented study exhibited hypermucoviscosity by forming a string ≥ 5 mm in length [9]. The results of biochemical test were summarized in table 1.

Table (1): The results of some biochemical test of *Klebsiella pneumoniae* and others bacteria.

ID	Biochemical tests		K. pneumoniae
1	Gram stain		Negative
2	Motility		Negative
3	indole		(-)ve
4	Simmon citrate test		(+)ve
5	Urease test		(+)ve
6	Triple sugar iron	H ₂ S	(-)ve
7		CO ₂	(+)ve
8		Acid	A/A
9	Methyl Red test		(-)ve
10	Voges Proskauer(VP)		(+)ve
11	Catalase Test		(+)ve
12	oxidase		(-)ve
13	String		Positive (≥ 5 mm)

The VITEK 2 system was used to confirm a final diagnosis of *K. pneumoniae*. This system was detected bacteria faster, efficient and away from the contamination that may prevent detection of the pathogen. Results of the tests used in this system confirmed the results obtained from morphological, biochemical, so all isolates (40) that previously identified as *Klebsiella* spp. are proved to be *Klebsiella*

pneumoniae.

Antibiotic Susceptibility of *Klebsiella pneumoniae*

The antibiotics resistance and sensitivity of *K. pneumoniae* isolates using disc diffusion method was evaluated for all 40 isolates with 12 antibiotic discs as shown in Figure (3).

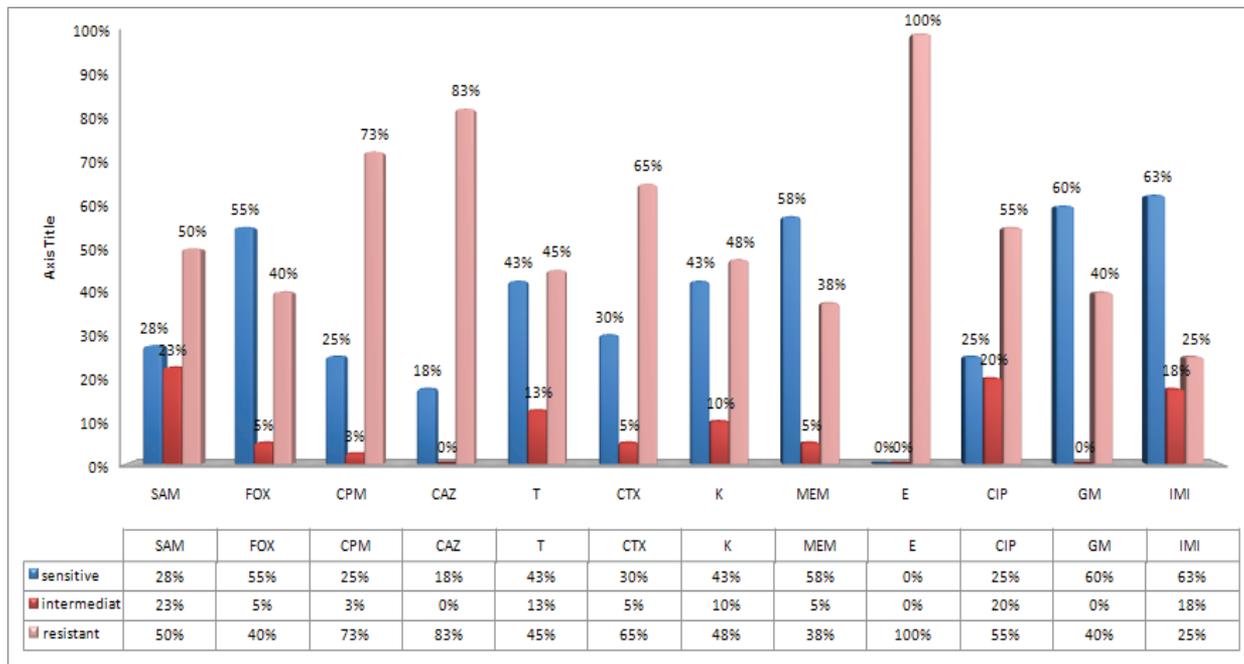


Figure (4): Antibiotic susceptibility results for 40 *K. pneumoniae* isolates with 12 antibiotics. (Kanamycin (K), Gentamicin (GM), Imipenem (IMI), Meropenem (MEM), Ceftazidime (CAZ), Cefotaxime (CTX), Ciprofloxacin (CIP), Tetracycline (T), Ampicillin sulbactam (SAM), Erythromycin (E), Cefepime(CPM) and Cefoxitin (FOX)).

The results of this study showed that the highest percentage of sensitivity of antibiotic against *K.pneumoniae* was for impenem (63%), Gentamicin (60%) and Meropenem (58%), while the lowest percentage was for Erythromycin (0.00%) and Ceftazidime (18%). In case of intermediate, the highest percentage of intermediate activity of antibiotic against *K.pneumoniae* was for ampicillin-sulbactam Ampicillin sulbactam (23%), Ciprofloxacin(20%), impenem (18%) and Tetracycline (13%), while the lowest percentage

was for Erythromycin, Gentamicin, Ceftazidime (0.00%), Cefepime(3%), Cefoxitin, Cefotaxime (5%) and Kanamycin (10%). Finally, the highest percentage of antibiotic resistance by *K.pneumoniae* was for Erythromycin (100%) Ceftazidime(85%), Cefepime (73%)and Cefotaxime (65%) while the lowest percentage was for impenem (25%) and Meropenem (38%).

The antibiogram results showed that a significant resistance to the most of antibiotics used in this study. In a local study on *K. pneumoniae* isolates

from inpatients with and burns infections in Al-Kufa hospital in Al-Najaf province, Iraq. A total of 43 *K. pneumoniae* strains were isolated. The highest resistance rate was observed for amoxicillin, and amoxicillin+clavulanic acid (97.67%) while the lowest resistance rate was observed for imipenem (9.30%) [10].

One of the studies about the antimicrobial susceptibility patterns of *Klebsiella isolates* from burn patients. Out of 883 isolates from 1294 patients 195 were found to be *Klebsiella* spp. Based on the biochemical properties 153 isolates were *Klebsiella pneumoniae*. In this study it was found that 54% of the *Klebsiella isolates* were multidrug resistant as they were resistant to at least one antibiotic of three or more different groups of antibiotics[11]. Two hundred and

seventy-two wound swabs from burnt patients were collected from Burn Intensive Care Unit of Eastern India, out of which 62.8% (n = 185) were revealed as positive for the presence of bacteria. *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *E. coli* were discovered to be the most common organisms in patients. Isolated bacteria were least resistant to tigecycline and colistin. All the *K. pneumoniae* isolates were resistant to ampicillin, cefuroxime, ceftriaxone and cefepime [12].

Minimum Inhibitory Concentrations (MICs) of Indolicidin against *K. pneumoniae* isolates

By using Microtiter Plate Assay with Resazurin Dye, Minimum Inhibitory Concentrations (MICs) of Indolicidin against *K. pneumoniae* isolates were detected as shown in Figure (6) and Table (3).

Table (2): The Minimum Inhibitory Concentrations (MICs) of AMP (indolicidin) against *Klebsiella pneumoniae* Isolates.

The Isolate cod	Minimum Inhibitory Concentration (MIC) (µg/ ml)							
	100	50	25	12.5	6.25	3.125	1.5	0.7
K 3	+	+	+	+	+	+	+	+
K 5	+	+	+	+	+	+	+	+
K 6	+	+	+	+	+	+	+	+
K 19	+	+	+	-	-	-	-	-
K 20	+	+	+	+	+	-	-	-
K 21	+	+	+	+	+	-	-	-
K 24	+	+	+	+	+	-	-	-
K 25	+	+	+	+	+	-	-	-
K 30	+	+	+	+	-	-	-	-
K 33	+	+	+	+	+	+	+	+

- = Growth; + = Inhibition

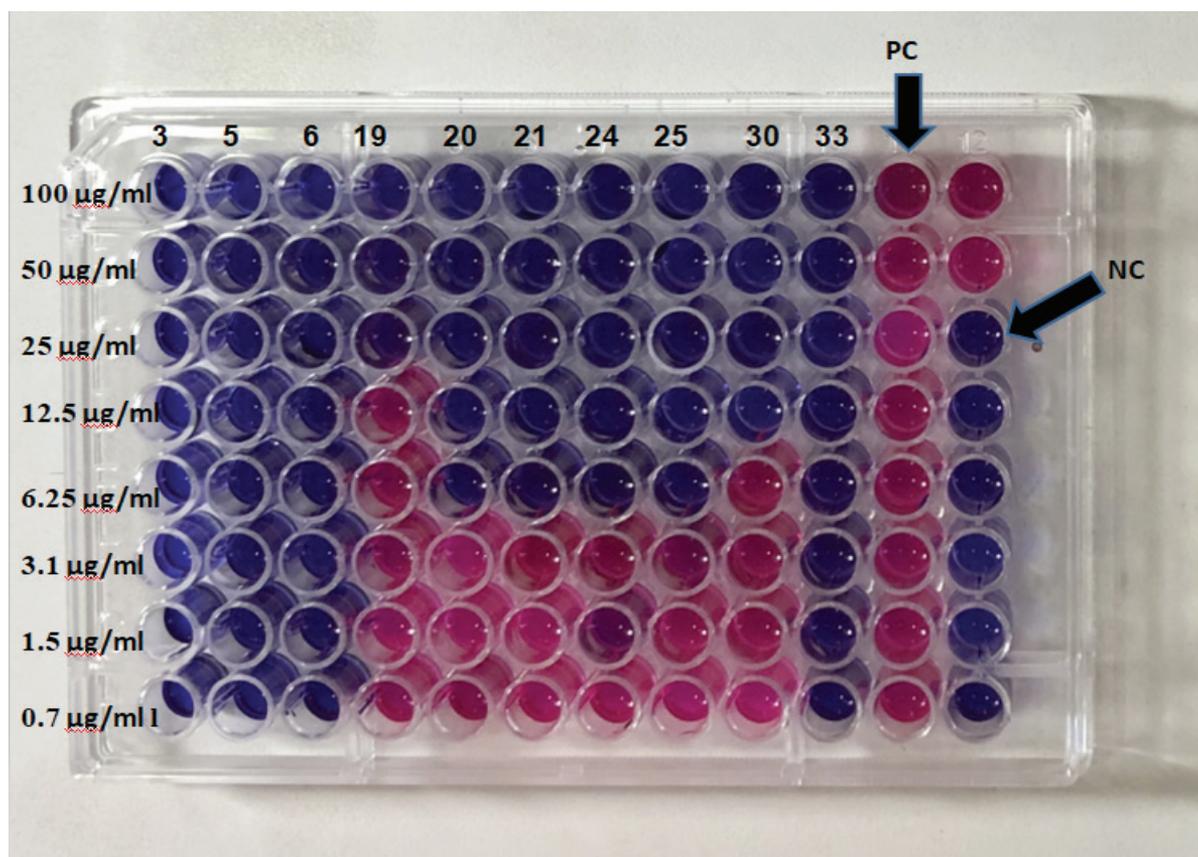


Figure (4): The minimum Inhibitory Concentrations (MICs) of AMP (indolicidin) at the Concentrations (0.7-100 µg/ml) Against *klebsiella pneumoniae* Isolates by Microtiter Plate Assay with Resazurin Dye.

The present study demonstrated that MIC was different from strain to another, where it was found that the isolates K3, K5 and K6 were very resist to the Indolicidin concentrations used in this study (0.7-100).

The minimum inhibitory concentration of the isolates K20, K21, K24 and K25 was 6.25.125, while for the isolates K19 and K33 was 25, and 12.2 was recorded for the isolate K3. It was reported that the production of a hybrid molecule composed of AgNPs and indolicidin had antibacterial activity, where the AgNP antibacterial activity was evaluated versus oral Gram-positive and Gram-negative bacteria. This study found that the coated nanoparticles' antibacterial activity strongly inhibited the growth of microorganisms, with very low minimum inhibitory concentration (MIC) values in the range of 5–12.5

µg/mL, and this effect depended on the specific characteristics of the metal surface coated with indolicidin [13]. The cytoplasmic membrane was the site of action of indolicidin as assayed in *E. coli* by the unmasking of cytoplasmic beta-galactosidase due to membrane permeabilization. The mechanism for this activity was shown to be the ability of the peptide to cause an increase in the transmembrane current of planar lipid bilayers. The small size and unique composition of indolicidin, it was capable of killing Gram-negative bacteria by crossing the outer membrane and causing disruption of the cytoplasmic membrane by channel formation [14].

In a previous study, Among these 15 antimicrobial peptides (AMPs), melittin, indolicidin and mastoparan showed good activity against both colistin-susceptible

and colistin-resistant *A. baumannii*, where Indolicidin showed MICs of 8 and 16 mg/L for colistin-susceptible *A. baumannii* and colistin-resistant *A. baumannii*, respectively [15]. Indolicidin was used as a positive control since it is known to be active against *S. aureus*. The MIC of indolicidin was determined to be 16 µg/ml (MSSA) and 32 µg/ml (MRSA), and Indolicidin possesses a broad antimicrobial activity against a range of Gram-positive and Gram-negative bacterial strains due to its high affinity for lipopolysaccharides and membrane proteins [16].

Conclusion

The potent activity of antimicrobial peptide Indolicidin against antibiotic-resistant strains of *K. pneumoniae*, suggests this peptide could be a critical advancement in the development of new treatments for *K. pneumoniae* infection, especially in burn and wounds.

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Funding: self

Ethical Clearance: Not required

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The Relationship between Stress Level and Nutritional Status of Students at SMP Negeri 56 Surabaya

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Abstract

Background: Stress prevalence increases by 5% in 15-year-olds and over in Surabaya. Stress causing negative effects is called distress. It may affect teenagers' nutritional status without considering their diet, leading to growth inhibition if it occurs continuously. It follows Riskesdas's (2013) data stating that 13-15-year-olds have a nutritional status of 3.3% very thin, 7.8% thin, 8.3% overnutrition, and 2.5% obese. Therefore, this study aimed to discover the relationship between stress level and nutritional status at SMP Negeri 56 Surabaya due to its dense academic and non-academic activities. Based on JPNN.com, several students hurt themselves using a blade due to psychological problems. **Methods:** This study used an observational analytical study design through a cross-sectional approach. Data collection was performed using the DASS-21 questionnaire and weight and height measurements, then analyzed using the Pearson Correlation or Rank Spearman test. **Conclusion:** the stress level was not related to the nutritional status of eighth-grade students of SMP Negeri 56 Surabaya, observed from the current nutritional status (BB/U), past nutritional status (TB/U), and body proportion (IMT/U).

Keywords: DASS-21, Nutritional status, Stress level, Teenagers

Introduction

Stress prevalence in 2011-2012 was 428,000 cases (40%) of a 1,073,000 total cases. Based on the Basic Health Research data, stress prevalence in teenagers annually increases by 6%, where Indonesian populations of 15-year-olds and over experience mental disorders of stress, anxiety, and depression.¹ In 15-year-olds and older in Surabaya, the mental disorder prevalence increases by 5%.² Stress occurs due to an imbalance between pressure and individual ability to respond to such pressures. Stress with negative effects is called distress. Initial symptoms of

distress include anxiety and depression.

Most people experiencing stress are teenagers since adolescence is the transition from childhood to adulthood. Various stressors may affect the emotional increase in teenagers, i.e., personal, family, school, and social factors.³

If teenagers continuously experience stress, it leads them not to consider their diet and affects their nutritional status, resulting in growth inhibition. Riskesdas data in 2013 revealed that 13-15-year-olds were 3.3% very thin and 7.8% thin. Overnutrition prevalence in 13-15-year-olds was 8.3%, and 2.5% was obese.¹

Based on research Bitty et al. (2018) obtained a relationship between stress and nutritional status with the strength of strong correlation relationships and

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positive direction.⁴ Meanwhile, Kusuma et al. research (2010) had no relationship between stress levels and nutritional status.⁵ Based on this background, the researchers were captivated to examine the relationship between stress level and nutritional status of eighth-grade students of SMP Negeri 56 Surabaya since this middle school has dense academic and non-academic activities and various achievements from the students, teachers, and school. Moreover, based on JPNN.com, 56 students of SMPN 56 Surabaya hurt themselves using a blade due to psychological problems.⁶

Materials and Methods

The study was performed using an observational analytical study design with a cross-sectional approach, having a total of 53 samples of eighth-grade students at SMP Negeri 56 Surabaya meeting the inclusion criteria: aged 12-16 years, having a complete family

is a biological child, living with parents, and without congenital disorders. The exclusion criterion did not have a chronic illness. The independent variable was stress level, and the dependent variable was nutritional status. Data collection was carried out using a DASS-21 questionnaire distributed to students and weight and height measurements, which were then analyzed using the Pearson Correlation test if the data were distributed normally or the Rank Spearman test if the data were distributed abnormally.

Results and Discussion

The sample size that met the inclusion and exclusion criteria amounted to 53 students comprising 27 male students and 26 female students. The minimum age was 13 years, while the maximum age was 16 years, with a mean age of 14.3 years on a 0.61 standard deviation. The highest age distribution was 14 years, where male students dominated the females (tabel 1.)

Tabel 1. Frequency Distribution of Student Gender in Percentage Based on Age

Gender	Age								Total	
	13		14		15		16			
	n	%	n	%	n	%	n	%	n	%
Male	0	0.0	19	55.6	7	40.7	1	3.7	27	100
Female	2	3.8	19	46.2	4	46.2	1	3.8	26	100

Distribution of Student Stress Level on Gender

In the table 2., most students did not experience stress by 88.67% from 53 students, where males dominated females. Also, female students were more susceptible to stress than male students. The most commonly experienced Stress level was moderate.

Based on the study result, most students did not experience stress by 88.67%, while students experiencing mild stress were 9.43%, and moderate stress was 1.90%. Female students were more susceptible to stress than males, where 60% of female students had a mild stress category, and

100% had a moderate stress category. It follows a study by Matud (2004), reporting the same result that stress level in females was higher than males since females have a coping mechanism focusing on emotions or feelings, leading them to more somatic symptoms and psychological pressures than males.⁷ It is also reinforced by McDonough and Walter in 2001, asserting that the distress score of females was higher than males due to different stress responses between them are related to hypothalamic-pituitary-adrenal (HPA) activities related to cortisol hormone adjustment and sympathetic nervous system.⁸

It contrasts a study by Kaistha et al. (2013) in India that male students had a higher stress percentage than males by 53.3%.⁹ Theoretically, this difference is due to increased hormones in females from the premenstruation cycle that increases cortisol, leading

to stress,¹⁰ and when exposed to stressors, the HPA (Hypothalamic Pituitary Adrenal) axis will secrete ACTH (Adrenocorticotropic Hormone). Females are more sensitive than males; therefore, cortisol is easy to build and causing stress.¹¹

Tabel 2. Frequency Distribution of Student Stress Level in Percentage Based on Gender

Stress Level	Gender				Total	
	Male		Female			
	n	%	n	%	n	%
Normal	25	53.2	22	46.8	47	100
Mild	2	40.0	3	60.0	5	100
Moderate	0	0.0	1	100.0	1	100

Distribution of Student Nutritional Status on Gender

In the table 3., based on the nutritional status during the study, male students had a better nutritional status than female students. It contrasts the past nutritional status of female students, in which they had a better nutritional status than males. During the study, female students’ body was more proportional than male students. The thin body was observed more in male students, while female students dominated obese body.

Based on the frequency distribution table of nutritional status in percentage based on gender, male students had better nutritional status than female students. It is in line with a study in New Delhi, demonstrating female children with worse malnutrition conditions than males. It is due to the high poverty rate that reduces nutritional intake and gender discrimination where male children have a better social value.¹² However, it does not follow a study by Dian in 2016, revealing that the female child frequency with good nutritional status was higher than males due to the faster growth rate in females.¹³

Conversely, the past nutritional status of female students was better than males. Buhendwa in 2017

stated that female children had a better nutritional prevalence than males, with a short prevalence of 9.8% in males and 3.4% in females.¹⁴ However, it is different from Kunwar’s study, where the prevalence of short female children was higher than in male children.¹² During the study, male students’ body was more proportional than female students. Obese and scrawny body figures were discovered more in female students. It follows a study by Buhendwa in 2017, stating that female children had a more disproportional body than male children by 8.6%.¹⁴ Besides, a study by Lazzeri in 2008 revealed that male children had a taller body proportion than female children, by 63.9%.¹⁵

In theory, Eberhardie stated differences between the nutritional needs of males and females. Male physical activities are more significant than females; hence, their nutritional need is higher. Furthermore, females consider their body image more than males; thus, many females postpone or reduce their diet to have a perfect body image. Determining the amount of nutritional need for teenagers is vital since teenagers’ growth and development are different between males and females.¹⁶

Tabel 3. Frequency Distribution of Student Nutritional Status in Percentage Based on Gender

Nutritional Status Based on the Anthropometric Index and Z-Value		Gender				Total	
		Male		Female			
		n	%	n	%	n	%
BB/U	< -3	1	100.0	0	0.0	1	100
	-3 to <-2	3	75.0	1	25.0	4	100
	-2 to 2	8	53.3	7	46.7	15	100
	> 2	0	0.0	0	0.0	0	0.0
TB/U	< -3	0	0.0	0	0.0	0	0.0
	-3 to <-2	1	100.0	0	0.0	1	100
	-2 to 2	0	0.0	2	100.0	2	100
	> 2	0	0.0	0	0.0	0	0.0
IMT/U	-3 to <-2	1	100.0	0	0.0	1	100
	-2 to 1	11	47.8	12	52.2	23	100
	> 1 to 2	1	20.0	4	80.0	5	100
	> 2	1	100.0	0	0.0	1	100

The Relationship between Stress Levels and Nutritional Status

The table 4. illustrates that non-stressed students had a good nutritional status during the study. Conversely, the past nutritional status was malnutrition for stressed students. During the study, students with a proportional body mostly did not experience stress.

Based on the normality test of nutritional status, the p-value in BB/U was 0.279, 0.697 for TB/U, and 0.762 for IMT/U with a significance level of $p > 0.05$, indicating that the nutritional status based on BB/U, TB/U, and IMT/U indices was normally

distributed. The normality test of stress level obtained a significance value (p) of 0.363 with a significance level of $p > 0.05$, indicating that stress level was normally distributed.

Based on the relationship analysis between stress level and nutritional level of BB/U index using the Pearson Correlation test and a significance level of $\alpha = 5\%$, the correlation coefficient score was 0.078, meaning that the relationship strength between stress level and nutritional status according to BB/U was 0.078, i.e., no correlation with a negative relationship an a significance value (p) of 0.743. It is higher than

the significance level of $\alpha = 5\%$, indicating that stress level had an insignificant relationship with nutritional status based on BB/U.

In TB/U, using the Pearson Correlation test and a significance level of $\alpha = 5\%$, the correlation coefficient score was 0.199, meaning that the relationship strength between stress level and nutritional status according to TB/U was 0.199, i.e., no correlation with a negative relationship an a significance value (p) of 0.801. It is higher than the significance level of $\alpha = 5\%$, indicating that stress level had an insignificant relationship with nutritional status based on TB/U.

Based on the relationship analysis between stress level and nutritional level of IMT/U index using the Pearson Correlation test and a significance level of $\alpha = 5\%$, the correlation coefficient score was 0.013, meaning that the relationship strength between stress level and nutritional status according to IMT/U was 0.013, i.e., no correlation with a negative relationship an a significance value (p) of 0.944. It is higher than the significance level of $\alpha = 5\%$, indicating that stress level had an insignificant relationship with nutritional status based on IMT/U.

Based on the frequency distribution table of the stress level based on nutritional status, stressed students had good nutritional status during the study. Conversely, the past nutritional status revealed that most stressed students were those with nutritional issues. During the study, students with a proportional body mostly did not experience stress.

The Pearson Correlation analysis test results obtained an insignificant relationship between stress

level and nutritional status based on BB/U, TB/U, and IMT/U indices with a significance value (p) of 0.743, 0.801, and 0.944, respectively. It is in line with a study by Saat in Kuala Lumpur, demonstrating an insignificant relationship between stress and nutritional status. This condition may be caused by eating time allocation, environmental support, and individual factors.¹⁷ However, it contrasts the study results from Masdar, showing a statistically significant relationship between stress and respondents' nutritional status with $p=0,003$. Stress will affect one's diet to lead them to consume high-calorie or high-fat food.¹⁸

In theory, Nasrani stated that the primary hormone responses in stress are activating CRH and ACTH systems. The process incorporates hypothalamus stimulus, causing corticotrophin-releasing hormone (CRH) secretion. It then stimulates the hypophysis anterior to secrete ACTH. CRH and CTH secretion increase cause cortex adrenal to excessively release cortisol. During stress, the body will release the cortisol hormone. A high cortisol hormone level will trigger the body to release insulin, leptin, and the neuropeptide Y (NPY) system that causes hunger, resulting in the desire to eat. It causes visceral fat accumulation and increases IMT.¹⁹ Besides, Sominsky's theory states that in an acute stress condition, released CRH can inhibit neuropeptide Y (NPY)/agouti-related peptides (AGRP) in the arcuate hypothalamus (ARC) nucleus. Neuropeptide Y and AGRP can stimulate eating behavior and suppress energy release. Therefore, acute stress will suppress appetite. Urocortin, a CRH family, can also hinder appetite. Urocortin is reported to inhibit ghrelin secretion, a hormone stimulating appetite.²⁰

Tabel 4. Frequency Distribution of Stress Level in Percentage Based on Nutritional Status

Stress Level	Nutritional Status												Total Students Based on Stress Level	
	BB/U				TB/U				IMT/U					
	Normal		Abnormal		Normal		Abnormal		Normal		Abnormal			
	n	%	n	%	n	%	n	%	n	%	n	%		
Normal	15	68.4	4	31.6	1	50.0	1	50.0	20	76.9	6	23.1	47	100.0
Stress	0	0.0	1	100.0	1	100.0	0	0.0	3	75.0	1	25.0	6	100.0

Conclusion and Acknowledgement

The study results conclude that stress level did not relate to the nutritional status of eighth-grade students of SMP Negeri 56 Surabaya, both the current status (BB/U and IMT/U indices) and the past status (TB/U). It is due to different assessments among individuals against stressors and the difference in stressor intensity perceived by individuals. Therefore, a prolonged study period with the same stressor intensity is required to illustrate the relationship between stress level and nutritional status. Furthermore, samples have unequal distribution and a small sample size. Thus, it is recommended for future researchers to have a bigger sample size.

Conflict of Interest: No conflict of interest.

Ethical Clearance: Health Research Ethics Committee Universitas Airlangga School of Medicine Surabaya, Indonesia: No. 223/EC/KEPK/FKUA/2020

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Abbreviation

- 1) BPS -Badan Pusat Statistika
- 2) DASS - *Depression, Anxiety Stress Scale*
- 3) IMT/U - BMI for Age
- 4) TB/U - Height for Age
- 5) BB/U - Weight for Age
- 6) UNICEF - *United Nations Children's Fund*
- 7) WHO - *World Health Organization*
- 8) SMP - Sekolah Menengah Pertama/ Junior High School

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Meta Analysis: Relationship of Husband's Knowledge and Support During Pregnancy with Antenatal Care (ANC) Visits by Pregnant Mothers

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Abstract

Antenatal Care (ANC) is a health service by trained health workers for mothers during their pregnancy, which must be carried out routinely, according to standards and in an integrated manner. ANC services are indispensable and recommended for pregnant women in order to maintain the health of the mother and fetus during pregnancy, detect and treat abnormalities early and prepare the mother for safe delivery. In fact, there are still many pregnant women who do not access ANC services according to standards, so this situation can contribute to morbidity and mortality rate (MMR). The research was conducted in November 2020 – March 2021 by searching for secondary data from two electronic databases, namely Google Scholar and Pubmed (2015 to 2020). Studies were selected through the PRISMA protocol and critically assessed using JBI's Critical Appraisal Tool. Test for heterogeneity, summary effect and publication bias using the JASP application version 0.14.1. Odd ratio with 95% confidence level (CI) was used to calculate the effect size. From the selected data, the husband's knowledge and support factors have quite a lot of differences in research results, so it is necessary to re-analyze the relationship of husband's knowledge and support during pregnancy with ANC visits by pregnant women to produce more accurate conclusions. Based on the inclusion criteria, there were 17 studies that could be included in the meta-analysis, with 3.956 respondents from Indonesia, Ethiopia and Vietnam. Homogeneous data with the combined OR value of knowledge of pregnant women with ANC visits is 2,48 (95% CI; 2,01-3,03) and the relationship of husband's support during pregnancy with ANC visits is 2,03 (95% CI; 1,65-2,51), and there are publications biased. This meta-analysis confirms that there is a significant positive relationship between husband's knowledge and support during pregnancy and ANC visits.

Keywords: *Antenatal Care Visits, Knowledge, Husband's Support, Meta Analysis.*

Introduction

Antenatal Care (ANC) is one of the government's efforts in accelerating the reduction of the Maternal

Mortality Rate (MMR), but unfortunately not all pregnant women can make ANC visits so that in 2017 Indonesia was ranked third with the highest MMR at 177/100,000 KH.^[1] Globally in 2018 pregnant women who accessed ANC services K1 were 86% and K4 were 65%^[2]. National data in 2016 shows that K1 coverage is 100% and K4 coverage has met the target

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of the Indonesian Ministry of Health's Strategic Plan (74%) which is 85.35%, but there are still 9 provinces that have not reached the target [3]. These data indicate that not all pregnant women continue their prenatal care according to standards and have a risk of pregnancy. The behavior of pregnant women who do not perform antenatal care will reduce the coverage of ANC, especially K4, and can increase the risk of maternal mortality. Mothers who never or less than 4 times have their pregnancy checked, have a 3.5 times risk of maternal death than mothers who have more than 4 pregnancy checks [4].

The high maternal mortality rate reflects the low utilization of maternal health services [5,6]. According to the 2016 Ethiopian Demographic and Health Survey, the utilization of antenatal care services was 62% where only 20% of pregnant women underwent the first antenatal care (K1) and only 32% had repeat visits (K4)[7]. Dahiru and Oche (2015) reported that around 54% of pregnant women experienced delays in antenatal care[8]. Some areas in Indonesia also show that more than 50% of pregnant women do not regularly/obediently do antenatal care visits[9,10,11,12], the majority of ANC visits by pregnant women (52.6%) were not up to standard [13] and Tufa et al (2020) stated that in general timely initiation of ANC for pregnant women was not ideal[14].

Research by Boamah et al (2016) and Prasetyaningsih (2020) states that the use of antenatal care is influenced by individual factors and interpersonal factors[15,16]. Safitri (2020) and Wahyutri et al (2015) state that the knowledge factor is the most dominant variable that affects ANC visits and pregnant women who have good knowledge have a 13.7 times higher chance of making ANC visits according to standards [13,17]. In addition, according to Wolde et al (2019) the delay of pregnant women to start antenatal care (K1) is due to poor knowledge

about the importance of early ANC initiation [17,18,19]. However, this is contrary to the results of research by Puspitasari et al. (2019) and Wahyutri et al. (2015) which state that there is no significant relationship between knowledge and ANC visits [16,20].

According to Laksono et al (2020) the ability of pregnant women to use ANC services cannot be separated from the influence of their families, especially their husbands. Husband has an important role in maternal health [21,22,23]. However, gender inequality positions women as those who have to give in and follow their husband's decisions for themselves. The husband as the decision maker in the family causes pregnant women to not get the opportunity to make decisions even though it is related to their health [24]. This patriarchal culture still exists, especially in rural areas [25].

The husband's involvement in maintaining maternal health will have an impact on the good health status of the mother. These roles include providing material and emotional support in obtaining optimal health services[26]. In addition, husband's support can be shown by providing nutritious food, healthy housing, and transportation to health services[27,28]. Pregnant women who get a lot of support from their families will be more regular in making ANC visits[29]. A husband who accompanies his wife during ANC can increase his husband's knowledge about pregnancy and childbirth and his readiness when complications occur [30]. Husbands who have knowledge/knowledge or understanding of ANC are able to provide explanations and support for their wives to carry out healthy lifestyle behaviors during pregnancy[31]. Husband's support is a determining factor because it will provide support to partners, reinforcers in motivation to conduct ANC visits and influence decisions made[32,33]. When complications occur, quick decisions will save the mother from

death^[34], so husband’s support has a very important role in determining the success of ANC^[9,11] and women are more likely to use ANC services when their husbands accompany them for ANC visits^[35].

Several other research results also state that there is no significant relationship between husband’s support and antenatal care visits^[36,37,38,39]. As stated by Bello et al (2019) that women who are more empowered are less likely to have husbands accompanying them during ANC visits^[40], this is related to the difficulty of pregnant women in getting permission from their husbands ^[41]. Based on this description, a review is needed to get answers to the gaps in the results of previous studies by systematically

and quantitatively summarizing and re-analyzing the relationship between knowledge and husband’s support during pregnancy with Antenatal care visits so that new data are quantitative.

Method

The secondary data search was systematically carried out using the PubMed and Google Scholar databases published in the last 5 years (2015-2020) and stages based on the PRISMA protocol. The results of this analysis are presented based on the PRISMA diagram ^[42]. The identified articles were assessed for quality using the Critical Appraisal Tool for cross-sectional studies^[43] and inclusion criteria.

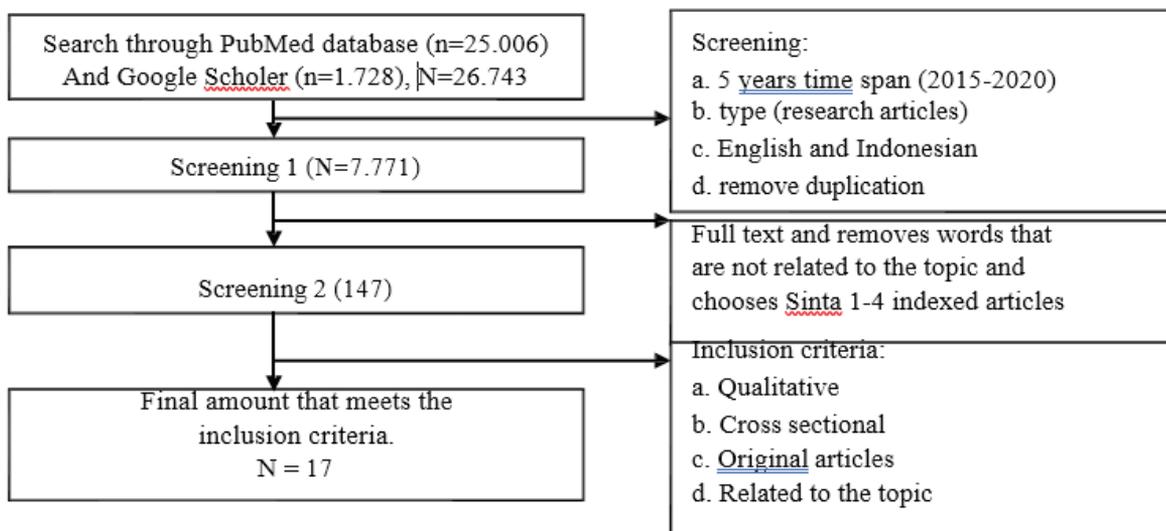


Figure1. PRISMA Diagram(45)

Data analysis using Microsoft Excel and JASP version 0.14.1. The value of Odds Ratio (OR), Prevalence Ratio (PR), Crude Odds Ratio (COR) and 95% confidence interval were used to calculate the effect size. Statistical meta-analysis of the random effects model was used to estimate heterogeneity with values Q, I^2 dan T^2 . The Q value is compared with the p value < 0.05 , the level of heterogeneity of the study is seen from the value I^2 dan $T^2 > 25\%$. The forest

plot is used as a graphical representation of the effect size of each study and the summary effect. Funnel plot, rank test correlation and Egger’s test used to identify publication bias. Trim and Fill tests are used to estimate missing studies and which when combined in meta-analyses can overcome publication bias ^[44].

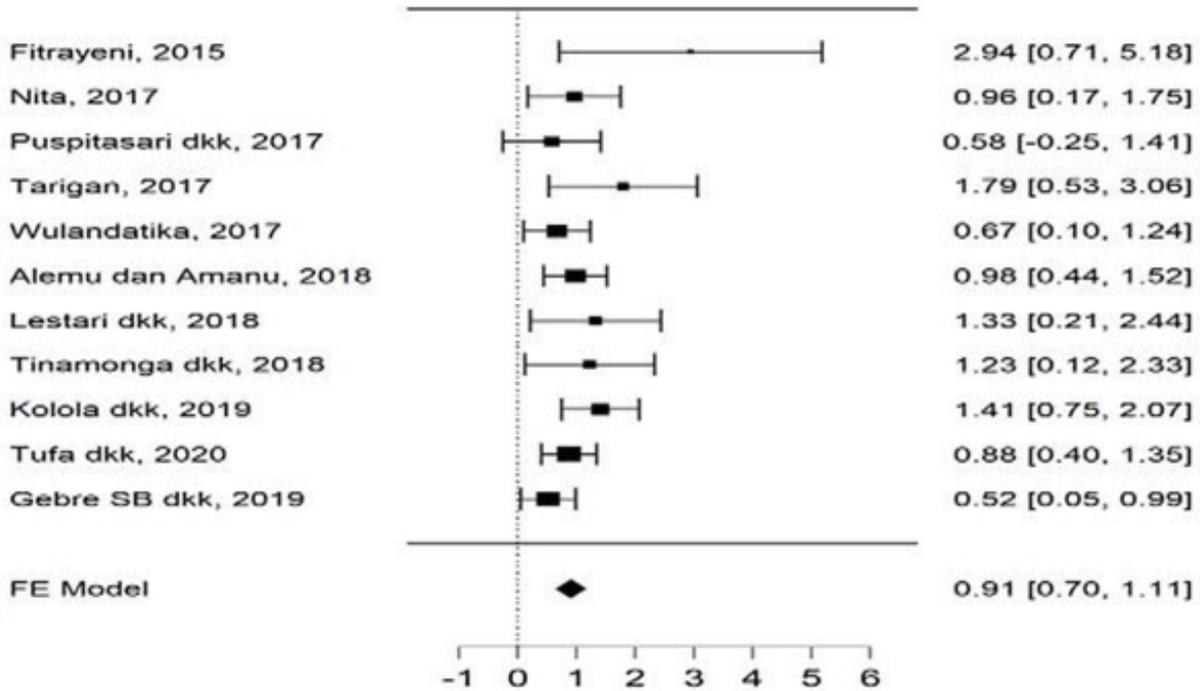
Result

A total of 26,743 articles were identified from 2

databases PubMed and Google Scholar. In accordance with the quality assessment and discussion, there are 17 articles that can be used as data in the meta-analysis (figure 1), the characteristics of the study are described in table 1). Among the included studies, 10 studies were conducted in Indonesia [10,12,21,30,32,37,45,46,47,48], 6 in Ethiopia [14,20,49,50,51,52] and 1 from Vietnam [22]. The sample is 3,956 respondents, the average age is between reproductive age (20-35 years), the majority of junior high and high school education, have been married and have a husband and have been pregnant before. Respondents are pregnant women and mothers who have given birth <12 months ago.

Knowledge of ANC

Of the 11 studies analyzed showed that the results of the heterogeneity test showed the value of Q (12.139) with p value (0.276), I² value (7.9%), T² value (0.011) which statistically showed insignificant heterogeneity (homogeneous). So for the combined effect test using a fixed effect model with the acquisition of the OR nilai value 2.48; CI 95%; 2.01-3.03, which means knowledge is significantly related to ANC visits. Pregnant women with good knowledge have 2.48 times more chances of making ANC visits than pregnant women with less knowledge about ANC.



Note:

OR=EXP (Log OR=estimate), OR=EXP (0.91), OR=2.48, lower limit EXP (0.70), lower limit =2.01
 upper limit=EXP (1.11), upper limit =3.03

Figure 2. Forest Plot Knowledge with ANC visit

Husband's support during pregnancy

Of the 12 studies analyzed showed that the results of the heterogeneity test showed the value of Q (13,457) with p value(0.264),I² value (22.369%),T² value (0.045)which statistically showed insignificant heterogeneity (homogeneous). So for the combined effect test using a fixed effect model with the acquisition of the OR value 2.03; CI 95%;1.65-2.51, which means that pregnant women who have husband's support during pregnancy have 2.03 times more chance of making ANC

visits compared to pregnant women who do not have husband's support.

Note:

OR=EXP (Log OR=estimate),OR=EXP (0.71),OR=2.03,Lower limit=EXP (0.50),Lower limit =1.65

Upper limit=EXP (top value),upper limit=EXP (0.92), Upper limit =2.51

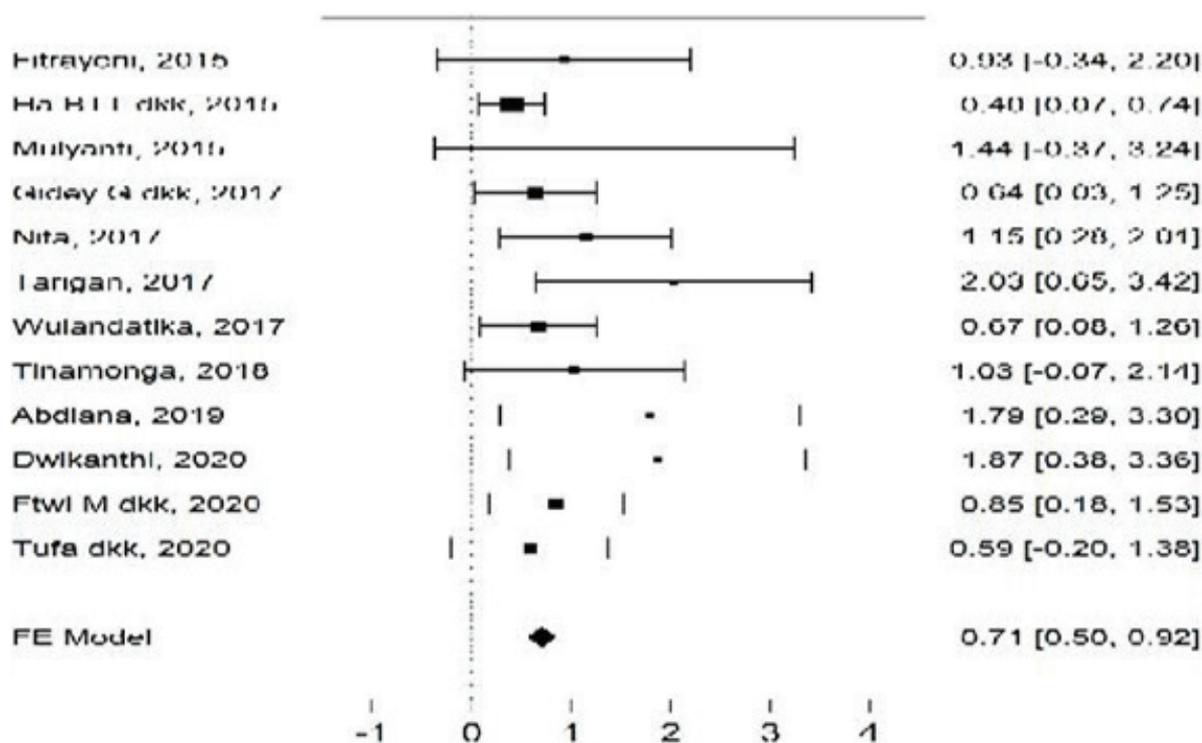


Figure 3. Forest Plot of Husband's Support with ANC Visit

Publication Bias on Knowledge Variable

The results of the publication bias statistical test on the knowledge independent variable show that the funnel plot shows that the black dot above forms an asymmetry pattern where there are no black dots in the middle and bottom left or indicates that research

with a small sample size is not statistically significant, missing or not being published. The rank correlation statistic test and Egger's Test showed p values = 0.010 and 0.012 so that the p value <= 0.05, this means funnel plot asymmetry or publication bias has been identified (Fig. 4).

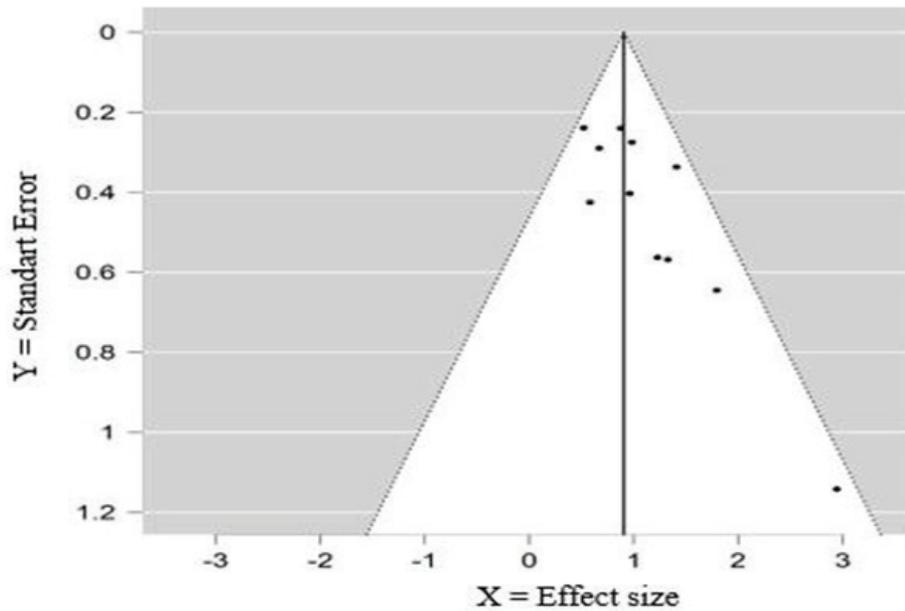


Figure 4. Funnel Plot on the relationship between knowledge and ANC visits

Publication bias on husband’s support variable

The results of the statistical test of publication bias on the independent variable of husband’s support during pregnancy showed that the funnel plot appeared black dots forming an asymmetry pattern where there

were no black dots in the middle and lower left or indicated that studies with small sample sizes were not statistically significant missing or not published. The rank correlation statistic test and Egger’s Test showed p values = 0.014 and 0.001 so that the p value < = 0.05, this means funnel plot asymmetry or publication bias has been identified (Fig. 5)

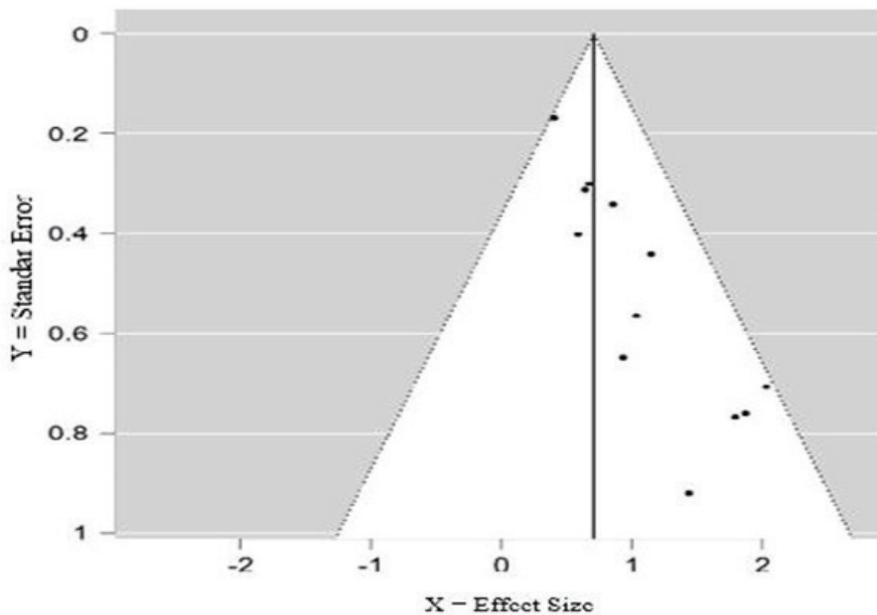


Figure 5. Funnel Plot on the relationship between husband support and ANC visits

Discussion

From this meta-analysis, it was found that husband's knowledge and support during pregnancy had a significant positive relationship with ANC visits by pregnant women. Good knowledge about ANC is 2.48 times more likely to make ANC visits compared to pregnant women with less knowledge. Likewise with husband's support, pregnant women who have husband's support during pregnancy are 2.03 times more likely to make ANC visits compared to those who do not have husband's support. Knowledge is a very important domain in shaping one's behavior^[53] and positive behavior can be formed if someone understands the meaning and benefits of something^[54], so that from the knowledge possessed, it will raise awareness of the importance of regular pregnancy check-ups^[12]. From the results of the analysis, it was found that good knowledge about ANC is related to the completeness of ANC^[45], more frequency of ANC visits^[30,47], compliance in ANC visits^[10,12] and knowledge also has a relationship with the appropriate timing of the ANC examination^[20,49,50].

Of the 11 studies on the relationship between knowledge and ANC visits analyzed, most of the respondents have good knowledge (average above 59.3%), but initiation of ANC is still relatively low and starting ANC late is in the second trimester^[14]. In addition, there are still many pregnant women who are at risk factors for pregnancy, namely age < 20 years and > 35 years, so it is necessary to increase knowledge by adolescents about reproductive health to prevent unwanted pregnancies and the importance of delaying pregnancy at an early age as well as increasing knowledge in accessing family planning services. and avoiding risky pregnancies in the age group > 35 years. Another finding from this analysis is that there is a similarity in decision making in the household, which is with the husband^[20,22,48], so

it is important to increase husband's knowledge about ANC and gender equality so that pregnant women have husband's support during their pregnancy.

From this study, it can also be seen that the knowledge possessed by pregnant women comes from positive experiences from previous pregnancies. So it is expected that service providers, especially midwives, can provide quality ANC services by increasing effective communication functions. In addition, knowledge must also be possessed by husbands to support the achievement of quality ANC visits. The knowledge possessed by the husband will be able to produce various decisions that are more appropriate, such as the selection of places and birth attendants, so that the decisions made can or are able to improve the health status of the mother and baby.

Husband's support is also significantly associated with ANC visits. Pregnant women who have husband support will have complete ANC visits^[22,45], on time^[51,52], and more obedient^[10]. This is in accordance with the theory of Fisbean and IcekAjen (1980) which states that a person's intentions are influenced by social factors where the person who is considered the closest can influence it^[53]. Family environment is a factor that affects a person's health status^[55] and support from husbands is needed by pregnant women during pregnancy^[32]. The support needed by pregnant women is physical and psychological support.

Husband's support can be started from the knowledge by the husband about the changes experienced by pregnant women, to raise an understanding of the needs of the mother during her pregnancy and can make the right decisions. Husband's support will not only affect the mother's pregnancy and ANC visits but will also have an impact on the utilization of other health services such as delivery in health facilities and birth attendants by professional health workers, the successful implementation of

exclusive breastfeeding and prevention of stunting and completeness of immunization for babies. This is because at the ANC visit this knowledge will be given during counseling so that the husband can know and provide support.

In this meta-analysis study identified publication bias, both on the knowledge variable and husband's support. This is because the sample used in the study only uses journal articles that have been published online. Trim and Fill analysis was used to estimate how many studies should be included in order to avoid publication bias. The results of this test indicate that in the study of the relationship between knowledge and ANC visits, 4 studies are needed and in the study of the relationship between husband's support during pregnancy and ANC visits, 6 studies are required to be included in the meta-analysis. If research that is relevant to the criteria that the researcher has determined is not included in the meta-analysis, it will result in less information, wider confidence intervals, and less powerful tests, although they do not have a systematic and too large impact on the effect size.

Conclusion and Acknowledgment

The results of the meta-analysis confirmed that husband's knowledge and support during pregnancy was associated with ANC visits by pregnant women. Early knowledge about reproductive health and pregnancy is important to be given early and at the latest to the group of married couples so that the pregnancy that occurs is a desired and well-planned pregnancy. In addition, the husband's support (physical and psychological) must be obtained by pregnant women during their pregnancy, so that pregnancy that occurs becomes a positive experience for pregnant women. We would to say thank you for Faculty of Medicine, Lambung Mangkurat University supported this research.

Declaration of Conflicting Interest

The authors declared no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

Ethical Clearance: There is no ethical test because it uses the journal analysis method

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Use of ICT (Information and Communication Technologies) in Health Facilities During the COVID-19 Pandemic: Case of Morocco

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Abstract

Background: The Covid-19 pandemic represents an unprecedented global crisis. It has placed many strains on the health systems of countries around the world. They had to, among other things, fight simultaneously against this pandemic, treat the affected people, preserve the rest of the population, while continuing to take care of the other patients. Faced with this situation, the development of the use of ICT in health establishments is a necessity today more than ever in order to limit any kind of contamination in hospitals, especially that caused by the spread of covid-19 on the one hand, and to digitize the relationship between caregiver and patient on the other hand. All these challenges require the availability of digital tools in health institutions and require health professionals well informed and trained in this sense. In this respect, the use of digital tools and telehealth (E-health, telemedicine, telecare....) is a priority step to consolidate health systems, as these new technologies allow patients to receive accessible, safe and adapted virtual care. **Objective:** The aim of our study is to evaluate the level of adaptation of the Moroccan health system to the covid-19 pandemic through the use of information and communication technologies (ICT) in health facilities (hospitals and primary health care facilities), in order to combat coronavirus contamination in the latter (stop contamination: professional to professional, professional to patient and patient to patient). **Methods:** The online questionnaire technique allowed us to collect 500 responses from health professionals spread over all Moroccan regions. **Results:** (86%) of the interviewees declared the existence of a covid-19 service within their institutions, with a majority of (91%) never received training in the use of ICT. (51%) of the health, professionals confirmed the absence of digital tools in the covid-19 services. (87%) announced that the covid-19 services do not have applications for the registration of patients suspected or affected by SARS covid-19. In addition, (79%) of the respondents expressed their dissatisfaction with the use of ICT. In the same context, (72%) of the participants stated that the use of ICT can limit the spread of SARS covid-19 in health facilities.

Conclusion: The health crisis has confirmed the importance of digitalization in the health sector. To do so, the state must place digital tools at the center of its interests as a major reform project for the Moroccan health system. The adoption of new technologies in the health sector can help health care practitioners to efficiently provide quality care to patients in order to limit any kind of contamination.

Keywords: ICT, covid-19, health facility, digitalization, telehealth.

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Introduction

Digital health, or “e-health”, refers to all areas where information and communication technologies (ICT) are used for health purposes ⁽¹⁾. According to

the World Health Organization (WHO), “e-health” is defined as the use of information and communication technologies (ICT) to support health care, and these technologies form the backbone of services to prevent, diagnose and treat disease ⁽²⁾.

Since December 2019, the world is facing a new pandemic known as SARS-CoV-2 (Covid-19), which has spared virtually no country. Its expansion and exponential contagiousness have imposed the confinement of most, if not all, countries in the world, and have left hospitals out of breath and decimated a significant portion of health care personnel ⁽³⁾. Telehealth has been the means of both containing the health crisis and ensuring the continuity of care ⁽¹⁾.

In France, as a derogation, to deal with the Covid-19 pandemic, healthcare professionals can use digital tools and “general public” communication applications when they take care of patients.⁽¹⁾ ICT enable remote collaboration between healthcare professionals and a relationship between healthcare professionals and the patient at a distance, rather than in contact.⁽⁴⁾

In addition, the use of telehealth offers, firstly, the possibility of ensuring the remote management of patients with Covid-19 and, secondly, an effective response to the risk of spreading the virus. It also provides an effective response to the risk of spreading the virus, as it protects healthcare professionals from infection, as well as the patients they care for.⁽¹⁾

Similarly, the practical value of telemedicine was brought to light by the health crisis related to the Covid-19 pandemic, which facilitated its social acceptance by health professionals and patients.

Indeed, as soon as the first contaminated cases of Covid-19 appeared and after the state of health emergency declared by the Kingdom of Morocco, the Moroccan authorities took the necessary precautions to curb its spread.⁽⁵⁾

From this point of view, the hospital centers in Morocco are facing a new state, in which the fight against the spread of the virus, the isolation and the healing of people affected by the coronavirus remains the major concern of all the officials of the Kingdom. Numerous measures have been decided to escape the inexorable advance of the new coronavirus, qualified as a pandemic by the WHO.⁽⁶⁾

Faced with this situation of confinement of the population, the use of ICT is a necessity today more than ever in order to improve access to health care, increase the efficiency of interventions, reduce barriers to participation, improve adherence to treatment...⁽⁷⁾

In this perspective, this study aims at testing the following hypothesis: “Increase of the use of the ICT in the hospitals equals decrease of the cases contaminated by the coronavirus”. In order to assess the following points:

- v Have health professionals been prepared and trained in ICT to deal with this unpredictable situation?
- v The availability and use of ICT during covid-19;
- v The importance of the Electronic Patient Record (EPR) in covid-19;
- v The role of ICT in obtaining a computerized patient database;
- v The need for a new generation of digital hospitals in Morocco;

Materials and Methods

Setting and type of study.

Our study is both qualitative and quantitative; it is focused essentially. On the use of the digital in the health establishments under the Kingdom of Morocco. This study was conducted over a period of six months,

from January 1 to June 30, 2021.

Sampling technique.

The survey was carried out via an online self-questionnaire of the "Google form" type, which lasted for a period of two months. We collected 500 responses from health professionals with different categories.

Indeed, the advantages of the online questionnaire are multiple, it is less expensive, faster, allows to question the most people, its answers are more reliable and more easily exploitable... Etc. But during this pandemic, the use of online questionnaires is a necessity in order to avoid the spread of the coronavirus.

Similarly, the present work is based on a literature review aimed primarily at scientific journals on topics related to the use of information and communication technologies in the health field. The Google Scholar scientific search engine was also used to identify more recent studies citing the identified journals or articles.

Finally, observation reflects what the researcher notices, "observes" by living with people, by sharing their activities; moreover, we experienced in a provincial hospital in the city of Sidi Kacem Morocco all the events related to the pandemic, namely the realization of covid-19 tests, the isolation of suspected cases, and the hospitalization of infected cases. The recovery and discharge of patients ...etc.

Results and Discussion

1. General:

Our study showed the participation of different categories of health professionals according to a workforce of 46% of nurses and health technicians, 28% of doctors, 14% of administrators and 12% gather other categories constitute teachers and technicians. All the respondents who answered our survey, 69% of whom are women and 31% men, cover all the regions of the Moroccan territory with 38% located in the Rabat-Salé-Kenitra region and distributed in the health establishments (H.E) as follows

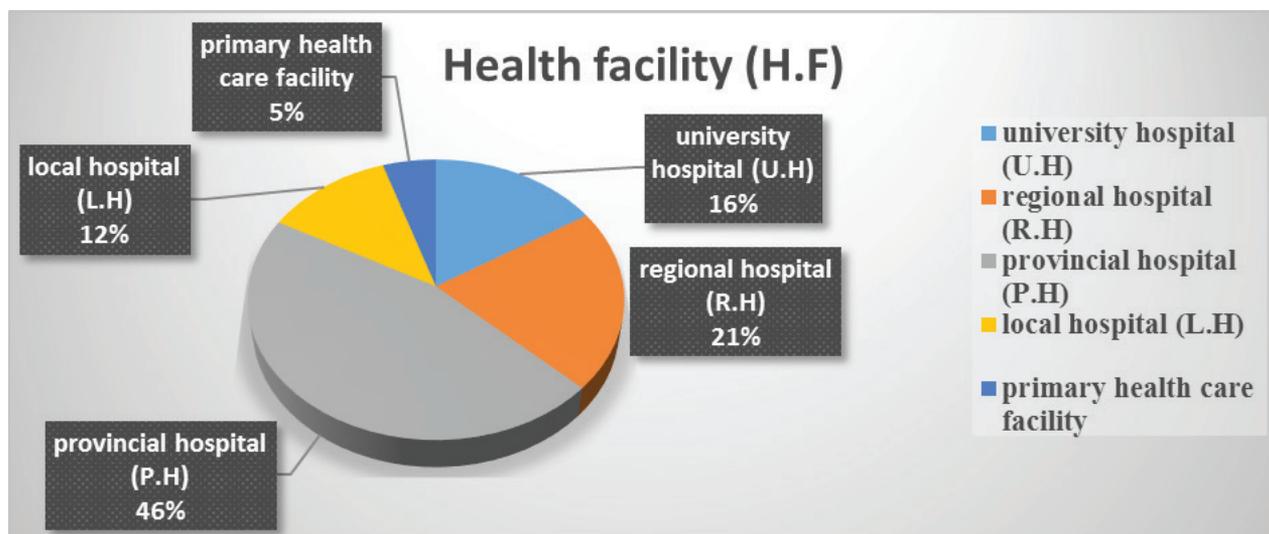


Figure 1: Distribution of health professionals by health facility.

A percentage of 86.3% of the interviewees declared the existence of a covid-19 service within their facilities (about 432 people in our sample) while 13.7% confirmed the non-existence of this service in these health facilities. Of these professionals, 79% had the opportunity to work in the covid-19 service at different times. (26.7% having worked for a period of one month, 36.7% having worked between one month and six months and 36.6% having worked from the beginning of the pandemic).

2. Knowledge and availability of ICT.

According to the results of our survey, we found that only 28.4% of the health professionals in our sample have knowledge about ICT, while 71.6% do not. A majority of 91% have never received training in the use of these technologies, while 9% have. Furthermore, 89% of the participants are unaware of

the terms related to digital health (e-health, telehealth, telecare).

We can explain this ignorance of ICT by the lack of programs related to digital health in basic and continuing education during the educational and professional pathway, a Moroccan study has highlighted the lack of integration of ICT in initial or continuing medical training. On the other hand, 42.5% of the students surveyed declared that they had difficulties using ICT for their training.⁽⁸⁾

About 90% of these interviews stated that their health institutions had never organized a training session on the importance of using ICT during the covid-19 pandemic. From these results, we can deduce that the use of ICT in the basic training programs of health professionals is necessary for future professionals qualified in digital health.

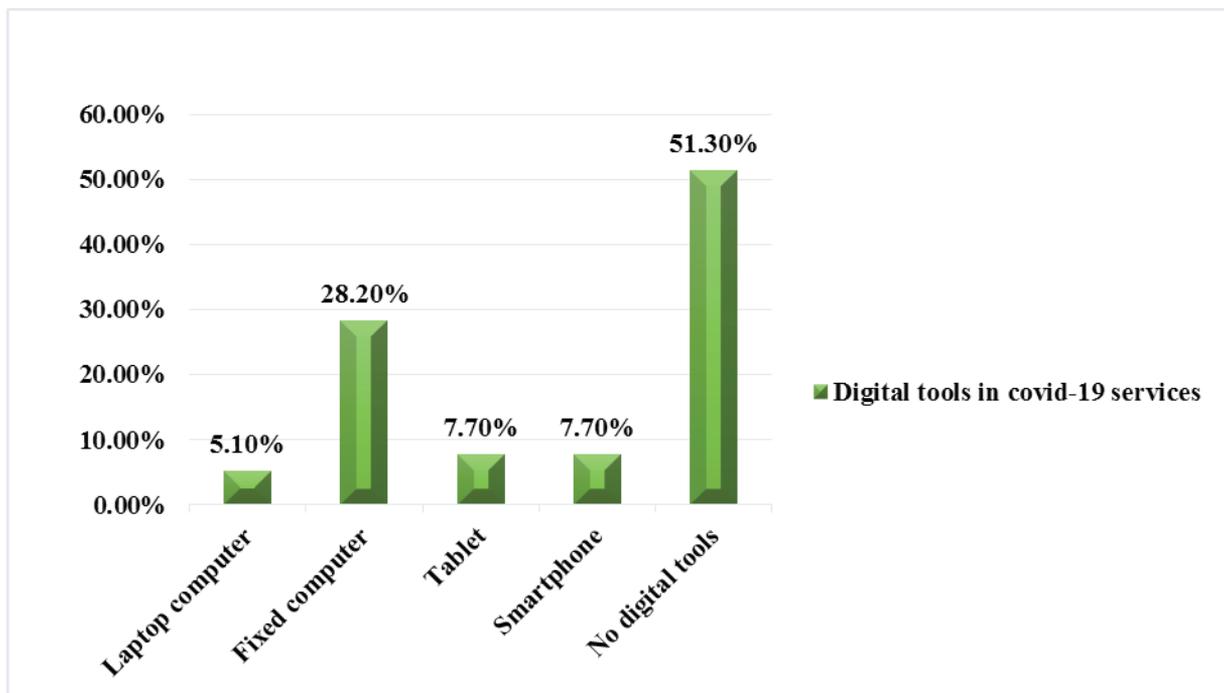


Figure 2: availability of digital tools in the covid-19 service.

The results show that, 51.30% of the health professionals confirm the absence of digital tools in the covid-19 services, of which 28.20% estimate the availability of fixed computers, while 15.40% announce the existence of tablets and smartphones, while only 5.10% chose the answer in accordance with the existence of laptops. On the other hand, 42% claim that Internet access is unavailable in their establishments, while 58% of the professionals announce that Internet access is available. The latter are less satisfied with this accessibility and unanimously agree that access is available only for professionals and not for patients.

As local and regional health authorities around the world work tirelessly to respond effectively to the

covid-19 crisis, it is extremely important to recognize that, in times of crisis or beyond, digital tools must act as a catalyst to achieve the goals of the relevant authorities. They must safeguard the health of citizens, promote social cohesion, and protect human rights, including at the digital level.⁽⁶⁾

3. ICT usage: professional to professional:

According to the results, the coronavirus disease affected about 59.5% of the health professionals in our sample, of which 55.6% of them announced that the coronavirus contamination was in the hospital environment, 33.3% during the exercise at the covid-19 service and 11.1% outside the hospital.

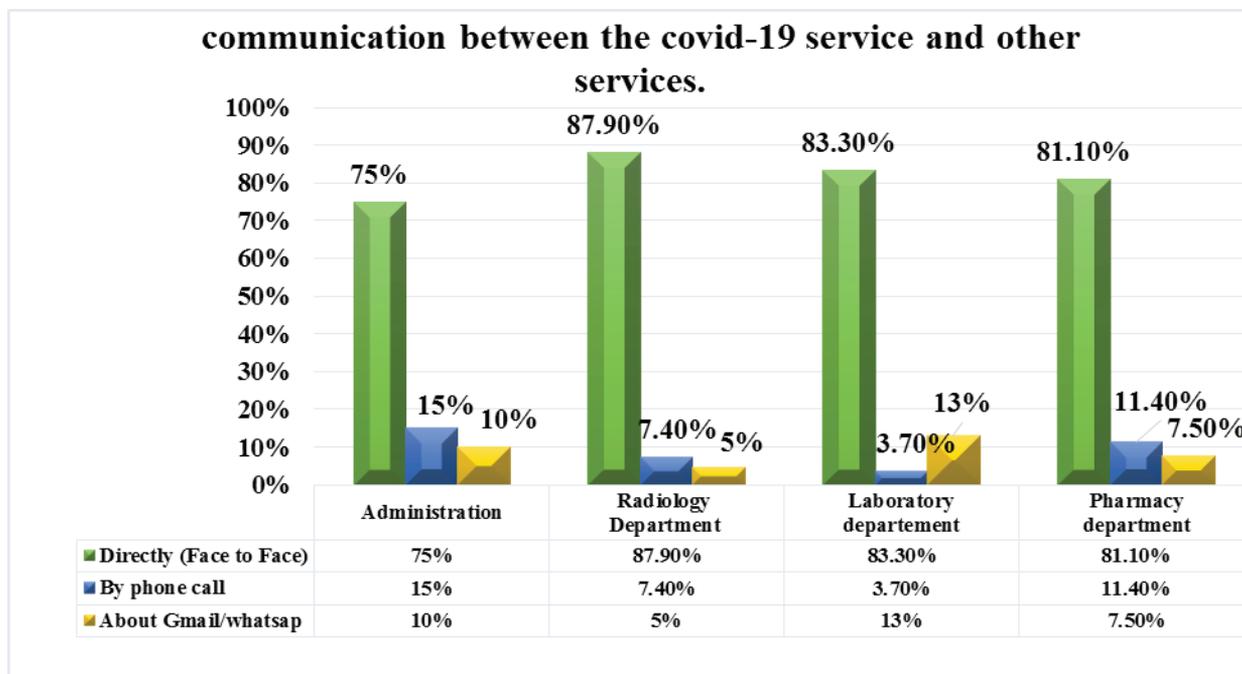


Figure 3: Communication between the covid-19 service and other services.

It should be noted that communication between the covid-19 service and the various hospital services (Administration, Radiology, Laboratory, Pharmacy) is most often carried out by direct contact (face to face) as shown in the statistics in (Figure 3) a study has shown that the transition to "paperless" has enabled us

to significantly reduce the risk of contamination of the covid-19 when exchanging documents from hand to hand.⁽⁷⁾

On the other hand, the majority (82%) of these interviews never participated in a remote meeting

organized by their managers during this pandemic, while 18% rarely participated in such meetings.

In addition, the purpose of medical teleassistance is to allow a medical professional to remotely assist another health professional during the performance of an act. The participants confirm that the technique of medical teleassistance is adopted by 37.8% of them against 62.2%. Despite the importance of this technique; health professionals timidly adopt it.⁽⁹⁾

The answers to the question "Have any texts been adopted to ease the conditions for the use of ICT during this pandemic?" show that 82.4% of the respondents have never received an organizational text, from either the government or the Ministry of Health.

In addition, several countries have relaxed telehealth laws during this pandemic, for example, In the United States the U.S. Department of Health and Human Services (HHS) relaxed the requirements of the Health Insurance Portability and Accountability Act (HIPAA) early in the pandemic, so that health care professionals could quickly implement new telemedicine and video interactions.⁽¹⁰⁾

4. ICT usage: professional to patient:

Medical teleconsultation allows a health professional to carry out a remote consultation with a patient, through the use of an information transmission medium⁽¹¹⁾, despite the importance of this technique, especially in this period of covid-19. 63.9% have never used it, 19.4% rarely used it and 16.70% are often adopted this technique.

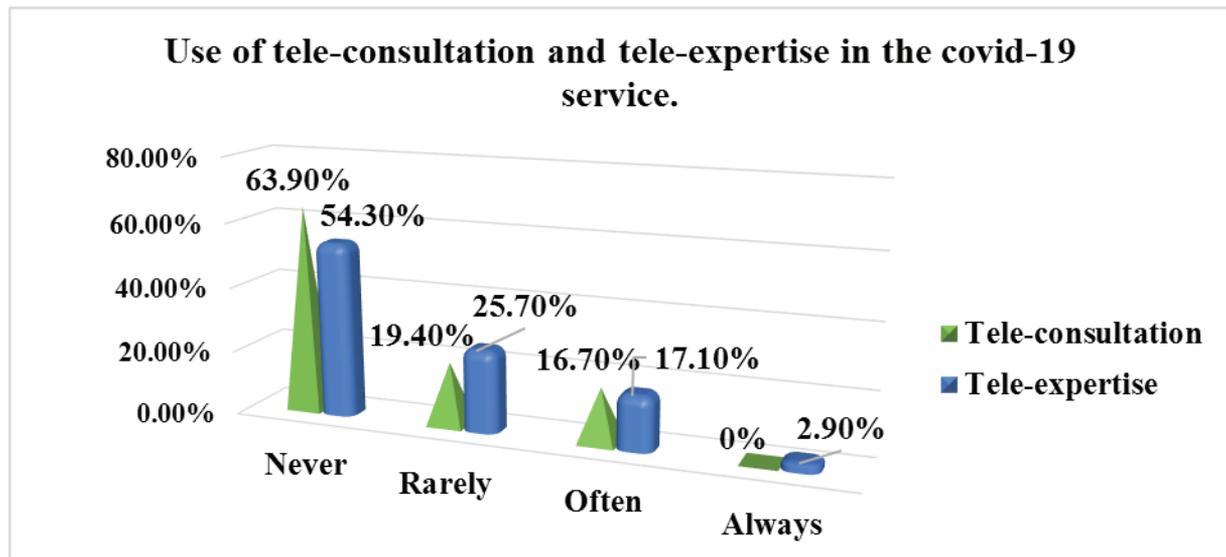


Figure 4: Use of teleconsultation and tele-expertise in the covid-19 service.

A French study has shown that, "The contribution of teleconsultation has been major because it has made it possible to absorb a volume of patients that we would not otherwise have been able to handle.⁽¹¹⁾ Easy to implement, teleconsultation is characterized by its absence of risk of contamination, and allows us to isolate suspected patients directly and, if necessary,

to refer them to the appropriate care structure".⁽¹²⁾

Similarly, the purpose of tele-expertise is to allow a medical professional (known as the "requesting" medical professional) to seek the opinion of one or more medical professionals (known as the "requested" medical professional) remotely, on the basis of their

training or particular skills, on the basis of medical information related to the management of a patient.⁽¹³⁾

However, 54.3% have never used this technique. Furthermore, a French study shows that the tele-expertise platform allows the GP's initial requests to be redirected, thus allowing for better care to be provided. It is undoubtedly through the speed and precision of the advice given on a difficult diagnosis, with reasoned and precise requests for additional examinations, or the scheduling of a closer consultation, that the platform allows for a reduction in hospitalizations in a given territory.⁽¹⁴⁾ For all these reasons, the benefits of this technique are so important during this pandemic.

It should be noted that the majority, namely 87.1%, say that covid-19 services do not have applications for the registration of patients suspected or affected by SARS-covid-19. At the same time, and in the interest of digitizing the relationship between caregiver and patient, because in the era of the coronavirus, digital tools are major assets in the prevention of the pandemic and the detection of infected patients. Despite the importance of all the above, we found that all participants are unanimous on the non-existence of

the computerized patient record (CPR) in the isolation units. Indeed, studies show that paper-based patient records have been clearly identified as vectors favoring rapid transmission of SARS-CoV-19 between patient/staff and staff/staff⁽⁶⁾.

In addition, in the framework of limiting the contamination and reassuring the family of the patient with sars covid-19. Most of the participants, about 88%, consider that the absence of listening cells can favor the spread of the virus. In France, in the context of monitoring patients with covid-19 and in order to avoid overcrowding of the health care system and to relieve practitioners as well as to avoid hospital contaminations, the remote monitoring device COVIDOM was set up urgently at the scale of Ile de France . This shows that the creation of e-health applications with the aim of communicating with patients and their families is an alienable asset in order to absorb the volume of visitors and limit their travel⁽¹²⁾.

In addition, 73% said that the lack of remote patient monitoring was due to the unavailability of cameras in the covid-19 wards.



Figure 5: Healthcare professionals' level of satisfaction with ICT use.

The results concerning the use of ICT in health establishments still reflect the poor exploitation of these technologies, since more than 79% of those interviewed consider themselves dissatisfied with the use of ICT, about 12% are not very satisfied, while only 9%, i.e. 45 people of those interviewed, express their satisfaction (Figure 5).

In the same context, 72% of participants said that the use of ICT can limit the spread of covid-19 disease in health facilities, while 28% saw things differently. In a Swiss study, reports indicate that covid-19 mortality rates in Chinese populations with low access to digital health resources have exceeded those in areas of China with higher access⁽¹³⁾.

This shows that the massive and diversified adoption of new technologies to protect against the virus and limit its spread in the hospital environment.

We have observed through this study that the use of ICT can limit the contamination by the coronavirus, and subsequently lower the rate of contamination in the ranks of health care personnel. Indeed, the unavailability of digital tools, the lack of training in these technologies and the absence of a clear will of the government regarding telehealth are the main causes of the poor exploitation of digital resources during this pandemic.

Situations experienced by respondents of our survey sample confirm that a rate of patients, asymptomatic carriers of Covid-19, come to the emergency department for consultation, they go through a circuit that begins with the contact of other patients, security guards, housekeepers, nurses and doctors.... etc.. Which generates a heavy spread of the virus. According to you, this spread is avoidable thanks to tele-consultation and the use of ICT.

Conclusion

The crisis of Covid-19 has accelerated the

development of telehealth worldwide. Indeed, despite the efforts made by the Moroccan state in order to succeed the construction and the revolution of the kingdom, the investment in ICT in the health sector is still low especially in this period of Covid-19. The director of the National Health Insurance Service (NHIS) recalled that the fact that a fully digitized health system and an e-government system are already in place was instrumental in managing the pandemic.⁽¹⁵⁾

Telehealth allows for better coordination of healthcare professionals in the monitoring of patients, in particular through the shared medical record (DMP). This digital health record stores and secures all of the patient's health information (treatments, test results, allergies, etc.), which can be shared with the health professionals of their choice.⁽¹⁶⁾

At the same time, ICT are today an essential lever for the modernization of the public sector. Faced with a constantly changing environment and an increasingly demanding and diversified demand, the Administration, with all its components, is called upon to adapt and simplify its processes in order to succeed in the project of transformation through ICT and to make an electronic administration emerge. The introduction of ICT has positively changed the relationship between the Administration and its users.

In conclusion, the coronavirus crisis is a test for countries. It must be a starting point for research and investment in e-health, because the world will be exposed to other pandemics that may be more vicious than the covid-19.

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Source of Funding: The source of the research cost from self.

Ethical Clearance : Nil

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The Role of IL-5, IL-33 and Total IgE in a Sample of Workers Suffering from Paints and Mills Asthmatic Patients

Ruaa Adnan Ali

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Abstract

A case-control retrospective study was conducted during November 2020 on 88 asthmatic patients (38 male mill and 50 male paints workers) with age ranged between (15-25), and 44 control with age ranged between (15-25) years to assess the role of total IgE, interleukin 5 (IL-5), and interleukin 33 (IL-33) serum levels in pathophysiology of disease using ELISA and multiplex immunoblot kits. Some hematological changes such as (Eosinophil and Basophil%) by Beckman coulter. The patients' samples were obtained from Nature Dyes Company Ltd in the industrial region of Aweerij Industrial, as well as Al-Dora flour mill in Baghdad, which is part of the general company for grain processing. The tests were carried out at the Specialized Center of Allergy in Baghdad/Al-Rusafa from October 2020 to April 2021. Specialized respiratory disorders and Asthma diagnose these cases, which are clinically diagnosed according to international guide lines. The present study confirms the highly significant role of total IgE and IL-5, IL-33 levels compared with control in pathophysiology of bronchial asthma and its correlation with disease severity and allergen type in adults. Results showed that there was a highly significant increase in Eosinophil and basophil % in cases of asthma of paints and mills patients with control group. In addition, there was a positive correlation between Eosinophil and Basophil in asthma of paints, and in asthma of mills. There was a positive correlation between total IgE with IL-5 and IL-33 in asthmatic group.

Keywords: *Asthma, Eosinophil, Basophil, Total IgE, IL-5, IL-33*

Introduction

Hypersensitivity Reactions (allergy) are immune responses that are amplified or inappropriate in response to an antigen or allergen. Asthma is a heterogeneous condition characterized by persistent airway inflammation and marked by repeated symptoms of wheezing, shortness of breath, chest tightness, and coughing that differ in duration and

severity and are associated with variable expiratory flow limitation⁽¹⁾. Work-related asthma is the term used to define asthma worsened by the workplace and encompasses both Occupational Asthma (OA) and work-exacerbated asthma^(2,3). OA is de novo asthma induced by either sensitization to a specific substance or a chemical at work, which is termed Sensitizer-Induced Occupational Asthma (SI-OA) or by exposure to high concentrations of an inhaled irritant found in the workplace, which is termed Irritant-Induced Occupational Asthma (II-OA)⁽⁴⁾. The diagnosis of OA is difficult, requiring confirmation for the diagnosis of asthma, plus evidence that the asthma was caused

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by workplace conditions. An accurate diagnosis of OA is a very important viewing of the significant health consequences for affected workers, but also the substantial socio-economic impact⁽⁵⁾. The level of exposure to a sensitizing agent is the most recognized environmental risk factor for OA but evidences suggest that occupational exposure to vapors, dust, gas and fumes increases prevalence of asthma⁽⁶⁾. Several host factors have been associated with OA. Atopy is a strong risk factor for OA due to High Molecular Weight HMW agents (e.g., bakers/pastry makers, laboratory animals workers)⁽³⁾. Abundant eosinophilia and elevated levels of immunoglobulin E (IgE) are inflammation⁽⁷⁾. It has addressed that IgE is a key immunoglobulin in triggering the inflammatory response in asthmatic patients, as well as asthma evolution and chronicity⁽⁸⁾. IgE plays a role in immediate hypersensitivity. cytokine with pro-inflammatory properties, and development of inflammation⁽⁹⁾.

Interleukin 5 is a 13-amino acid protein that forms a 52-kDa homodimer related to both Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) and IL-3⁽¹⁰⁾. The early response is characterized by marked synthesis of IL-3, IL-4, IL-5 and IL-13, as well as the chemokine ligand-5 and (GM/CSF). These mediators recruit neutrophils, eosinophils, basophils, macrophages and T lymphocytes to the site of inflammation in the late allergic response⁽¹¹⁾. IL-5 is synthesized and secreted by eosinophils, TH2 cells, mast cells, CD34+ progenitor cells, Natural Killer (NK) T cells, and type 2 innate lymphoid cells as⁽¹²⁾.

Interleukin 33 is produced as a 30 kDa pro-peptide, like IL-1b and IL-18, and is cleaved by caspase-1 to generate mature 18 k D IL-33. The IL33 is a regulatory cytokine from IL-1 cytokine family and it consider as an alarmin that alerts the immune system, its produce by many types of cells like epithelial cells of (skin, lungs, and gastrointestinal tract that exposure

to the environmental allergens), endothelial cells, osteoblast, fibroblasts, adipocytes, smooth muscle cells, macrophages and dendritic cells⁽¹³⁾.

Materials and Methods

A case-control retrospective study was conducted during November 2020 on 88 asthmatic patients (38 male mill and 50 male paints workers) with age ranged between (15-25) year, and 44 control with age ranged between (15-25) years to assess role of total IgE, interleukine5(IL-5), and interleukin33(IL-33) serum levels in pathophysiology of disease using ELISA and multiplex immunoblot kits. Some hematological changes such as (Eosinophil and Basophil) by Beckman coulter. The patients' samples were obtained from Nature Dyes Company Ltd in the industrial region of Aweerij Industrial, as well as Al-Dora flour mill in Baghdad, which is part of the general company for grain processing. The tests were carried out at the Specialized Center of Allergy in Baghdad/Al-Rusafa from October 2020 to April 2021.

Eight ml of blood was drawn from each subject using 10 ml disposable syringes in sterile conditions. (2ml) of blood was collected in a sterile EDTA tube for hematological assay (Basophil and Eosinophil%) by Beckman analyzer counter.

In the gel tube, 6 ml of blood was obtained and allowed to clot at room temperature. After blood clotting, the serum was centrifuged at 3000 rpm for 5 minutes before being separated into equal parts and stored at (-20°C) for immunological testing of total-IgE, IL-5, and IL-33 levels⁽¹⁴⁾.

Data Analysis

The data of this study were analyzed for normality, homogeneity and normal distribution firstly to determine the suitable statistical tests for calculating the mean, standard error, standard deviation, and the probability by using student T-test, ANOVA test,

Pearson’s chi- square test and Pearson’s correlation via using the Graphpad Prism version 4.0⁽¹⁵⁾. The probability considered significant when it (P<0.01), (P<0.05).

and control(2.164±0.9421).There were highly significantly in asthmatic groups(p=<0.0001) (P<0.01).

Results

Asthma patients were further characterized according to severity of disease, family history and allergen type. The (mean±SD) of eosinophil, basophile, levels of T IgE, IL-5, and IL-33

Eosinophil Cell Count (%)

The results in studied groups were compared to the Eosinophil Cell (%); asthma of paints was(4.806±1.289),asthma of mills was(5.083±1.519),

Basophil Cell Count (%)

The results in studied groups were compared to the Basophil Cells (%), asthma of paints was (0.4068±0.1944), and asthma of mills was (0.3816±0.2137). While control was (0.2734±0.1479). There were highly significantly in asthma of paints with control (P=0.0021) (P<0.01), and there were significantly in asthma of mill with control (P=0.0264) (p<0.05). But there were no significant in asthmatic groups (0.8046) (P>0.05). As shown in table(1) .

Table (1)The percentage of Eosinophils and Basophils in asthmatic groups

Hematology assay	A.Paints(n=50)	A.Mills(n=38)	Controls(n=44)	P-Value
	Mean ±S.D	Mean ±S.D	Mean ±S.D	ANOVA
Eosinophil count (%)	4.806±1.289	5.083±1.519	2.164±0.9421	<0.0001****HS (p<0.01)
Basophil count (%)	0.4068±0.1944	0.3816±0.2137	0.2734±0.1479	0.0020**HS (P<0.01)

The Serum level of Total Immunoglobulin IgE(T-IgE) in studied groups.

The results total IgE in patients with asthma of paints, asthma of mills and control were (Mean±SD) (373.9±252.2), (219.6±158.4), (46.02±25.32). There were highly significantly in asthmatic groups(p=<0.0001) (P<0.01) with control, as shown in Table (2).

Table (2) Distribution of total IgE level in studied groups

Immunological assay		No.	Mean	Std. Deviation	Std. Error	P- value
Total IgE (IU/ml)	A .paints	50	373.9	252.2	35.66	<0.0001** HS
	A .Mills	38	219.6	158.4	25.70	
	Controls	44	46.02	25.32	3.817	
	Total	132				

(**HS) Highly significant , (P<0.01) .

Interleukin -5 levels

The results of the level of IL-5 increased in the serum of asthmatic group. The level of IL-5 (mean±SD) in asthma of paints were (129.9±119.2) pg/ml, and asthma of mills were (47.19±26.89), While in control were (54.05±20.27) pg/ml. There were highly significantly in asthmatic groups with control, but there were no significantly between asthmatic groups.

Interleukin-33 level

The results of the level of IL-33 increased in the serum of asthma group patients. The level of IL-33 (mean±SD) in asthma of paints were (24.91±22.01) pg/ml, and asthma of mills were (20.19±14.86) pg/ml. While (13.57±6.039) pg/ml in the control. Statistical analysis showed that there were highly significantly in asthmatic groups with control group (P=0.0038) (P<0.01).

Table (3) comparison the serum levels of interleukins(IL-5,IL-33)between asthmatic groups.

Immunology assay	A.paints(n=50)	A.mills(n=38)	Controls(n=44)	P-value
	Mean ±S.D	Mean ±S.D	Mean ±S.D	ANOVA
IL-5 (Pg/ml)	129.9±119.2	47.19± 26.89	54.05± 20.27	< 0.0001** HS(P<0.01)
IL-33(Pg/ml)	24.91± 22.01	20.19± 14.86	13.57± 6.039	0.0038**HS (P<0.01)

The correlation between the total IgE and interleukins (IL5-IL33) in asthmatic group with age group

The present study that there was a relationship between the total IgE with IL-5 and IL-33, where it was found a positive correlation with significantly in age group in asthma of paints (r=-0.3141)(p=0.0263*) (p<0.05), and no significantly in asthma of mills(r=-0.1492) (p=0.3712)(p>0.05).

Table (6) The correlation between total IgE and IL-5, IL-33 in asthmatic group with age groups

Total IgE		Age	IL-5	IL-33
Asthma Paints	r	-0.3141	-0.06075	0.05557
	p	0.0263*	0.6751	0.7015
Asthma mills	r	-0.1492	-0.1234	-0.2693
	p	0.3712	0.4605	0.1020

Discussion

The results showed increased Eosinophils. There were highly significantly in asthmatic groups with control. Eosinophilic gastrointestinal disorders have increased levels of eosinophils in portions of the gastrointestinal tract (esophagus, stomach, small intestine, large intestine, or multiple segments)⁽¹⁶⁾. Eosinophils release cytokines, growth factors, and leukotrienes, which induce additional inflammation and generate the disorder's characteristic and frequent symptoms, such as hyperactivity in response to various stimulating events⁽¹⁷⁾. Eosinophils are linked with the development of allergic asthma in the respiratory tract and are recruited to the lungs by cytokines produced by activated Th2 cells as part of the inflammatory response. The authors of this new research into the general population want to identify the factors linked to high blood eosinophil levels. Interpret the blood eosinophil count with a more nuanced approach, suggesting that age, sex, comorbidities, and lifestyle should all be considered⁽¹⁸⁾.

Also, the results showed increased of Basophils. There were highly significantly in asthmatic group with control. Basophils, which make up less than 1% of all leukocytes in the blood, have been found to play an important role in the immune system and to produce high amounts of Th2 cytokines like IL-4⁽¹⁹⁾. This study agrees with certain Iraqi studies, such as⁽²⁰⁾. Basophils have several characteristics of tissue-resident mast cells, such as the presence of basophilic granules in the cytoplasm, the display of the IgE receptor (FcεRI) on the cell surface, and the release of chemical mediators in response to various stimuli⁽²¹⁾.

The result appears increased total IgE there were highly significantly in asthma group with control. Both innate and adaptive immune responses play a role in the severity and pathogenicity of allergic asthma. Immunoglobulin E is a well-known component of allergic responses, as it is generated

during the sensitization process, which begins with the first exposure to the allergen^(22,23). High IgE levels and a high eosinophil count were seen in 33% of their English patients with severe allergic asthma. Similarly,⁽²⁴⁾ they found that allergic asthmatic patients produced more total and specific IgE than non-allergic and healthy subjects, and they also confirmed that total and specific IgE were produced locally in both allergic and non-allergic asthmatic patients. They found that individuals with allergic asthma had higher IgE levels than healthy people, suggesting that increased IgE was a key risk factor for chronic childhood asthma⁽²⁵⁾.

The results appear increasing of IL-5. There were highly significant differences between asthma groups with control. T-helper 2 cells generate and release IL-5 after a complex activation process involving allergens inhaled and dendritic cells⁽²⁶⁾. Interleukin 5. The reason for the reduction in IL-5 levels is that it can control the Th1/Th2 balance and improve the airway inflammation produced by acute Bronchial Asthma (BA), as well as the clinical symptoms and lung function of individuals with acute BA⁽²⁷⁾. The results are consistent with a previous study⁽²⁸⁾, which found a strong association between blood eosinophilia and IL-5. Furthermore, they found that IL-5 is the main cytokine responsible for eosinophil maturation, activation, proliferation, and survival⁽²⁹⁾. T cells are the primary generator of IL-5 in the lungs, and IL-5 is required for eosinophil recruitment, proliferation, survival, and activation. Furthermore, in severe asthma, neutrophils play a role in eosinophil stimulation to cause airway inflammation by forming extracellular traps⁽³⁰⁾.

The results of this study disclosed that the level of IL-33 increased in the serum of asthma group patients. There were highly significant difference between asthma of patients compared with control, whereas no significant differences between asthma of mills compared with control⁽³⁰⁾ in vivo studies

discovered that IL-33 plays critical roles in allergic inflammation, type-2 immunity, and eosinophil homeostasis, and added that IL33 is released after cell damage and necrosis and activates allergic inflammation by increasing the synthesis of inflammatory and chemotactic proteins that increase allergic inflammation, such as IL-4, IL-5, and IL-13⁽²³⁾.

Conclusions

There are highly significantly in total IgE and IL-5, IL-33 between the asthma group and control, and there were significant positive correlation between total IgE and IL-5, IL-33 in patients' group with age groups ($P > 0.01$). There were highly significantly in eosinophils and Basophiles asthma groups with control.

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The Relation between the Plasma Level of Testosterone Hormone and the Severity of COVID-19 in Iraqi Patients

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Abstract

Coronavirus disease 2019 discovered in December 2019, Wuhan, China. It was transmitted globally producing the present COVID-19 pandemic. Concerns have been raised about the potential impact of COVID-19 on male reproductive organs and male fertility as the number of infections in the male community has increased. The objectives of current study are studying the relationship between the plasma levels of testosterone and the markers of immune reaction with the severity and mortality in a sample of COVID-19 patients. A cross section study included NO= 103 male patients affected by SARS- CoV-2 pneumonia, diagnosed by PCR and chest CT scan, (≥ 18 years old), and recovered in the respiratory intensive care unit (RICU). Several biochemical risk factors were determined Free Testosterone, sex hormone binding globulin (SHBG) were measured by Enzyme-Linked Immunosorbent Assay(ELISA), D-dimer, Ferritin, CRP, Urea, Creatinine were measured by automated method by using Abbott Architect c4000 and Complete Blood Count(CBC). The results show that the serum free testosterone and SHBG levels a significant lower in non-survivor patients than survivor patients with COVID-19. While the other biomarkers (D-dimer, Ferritin, Urea, Creatinine) were significant higher in non-survivor patients than survivor patients. The CRP, WBC and lymphocyte showed that no significant between the both group of patients. In conclusion the study showed that lower free testosterone and SHBG levels enable significant role in increasing risk of COVID-19 mortality amongst adult male patients.

Keywords: COVID-19 ,Testosterone level, Severity.

Introduction

In the second half of 2019, a pneumonia with an unclear etiology was discovered in Wuhan, Hubei Province, China. The pathogen was quickly isolated and named 2019 Novel Coronavirus (2019-nCoV) on 12 January, 2020 ⁽¹⁾. The virus has spread

to Italy and other European countries, as well as the United States, after being contaminated in China and causing thousands of deaths, and the number of new confirmed cases is rising every day. Owing to the wide spread infectivity and high transmission rate, the World Health Organization(WHO) called the disease coronavirus disease 2019 (COVID-19) and declared it a pandemic. Human coronaviruses typically cause respiratory and enteric infections ⁽²⁾. To date, there have been over 140 Million confirmed cases of COVID-19 and over 3 Million deaths across

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the globe. The COVID-19 begins when COVID-19 is transmitted from one person to another through inhalation or oral ingestion of virus-containing droplets. COVID-19 spike protein binding to the angiotensin-converting enzyme 2 (ACE2) receptor may allow the virus to penetrate epithelial cells in the nasal or oral cavities⁽³⁾. The COVID-19 is more common in men than in women, according to epidemiological data from China and Europe. A meta-analysis of 39 observational studies found that males (57.4%) were more likely than women to require hospitalization as a result of COVID-19 infection⁽⁴⁾. However, there are gender disparities in the prevalence of COVID-19, and it is an unexplained phenomenon why men appear to be more susceptible to COVID-19 infection than women, with a greater fatality rate. The COVID-19 pathogenesis is aided by the angiotensin-converting enzymes 2 (ACE2) receptor, which aids direct host cell destruction. Binding of COVID-19 virus with the ACE2 receptors enables its cellular entry and replication. As a result, cells with increased ACE2 expression may appear to be more sensitive to COVID-19 infection. Having considered these reports, it is a breakthrough revelation in male fertility research that testes have the highest ACE2 mRNA and protein expressions levels of all body tissues. The seminiferous duct cells, spermatogonia, Leydig cells, and Sertoli cells are the four main testicular cell types that express ACE2⁽⁵⁾.

Materials and Methods

This study was conducted at the Department of Biochemistry, College of Medicine, University of Baghdad, at Khalis General Hospital and Baquba Teaching Hospital in Diyala during the period from November 2020 to January 2021. It involved 103 COVID-19 male patients who were diagnosed by PCR and chest CT scan. Their age \geq 18 years old. Patients

who taking testosterone and tamoxifan drugs, taking sports stimulants, suffer from excessive obesity and the females were excluded from the study.

Blood Samples:

About 6 ml of blood samples were obtained from veins for COVID-19 patients. Each blood samples divided into three parts:

1. The first part 1ml of whole blood for measuring CBC.
2. The second part 2ml put in anticoagulant tube separated by centrifugation at 3000 rpm for 10 min for obtained plasma to measuring D-Dimer.
3. The third 3ml in plane tube separated by centrifugation at 3000 rpm for 10 min, the resulting serum divided into aliquot for:

Aliquot 1: Biochemistry measurement for ferritin, CRP, urea and creatinine.

Aliquot 2: The rest were stored at (-20 °C) until assayed for serum free testosterone and sex hormone binding globulin(SHBG).

Results

The current study is cross sectional study that involve 103 COVID-19 male patient, with mean \pm SE of age 45.7 ± 1.7 years. Enrolled patient have been tested for several biomarker level to evaluate their clinical condition and the severity of disease that include CBC, S. ferritin, plasma D-Dimer, CRP, S. urea and creatinine. In order to evaluate association of disease severity and gender difference free testosterone and SHBG were measured.

all those details are presented in table 1.

Table 1: Study laboratory characteristics of participants, frequency and percentage.

	Reference range		Frequency	Percent
Age group		below 35	19	18.4%
		age range 35-55	43	41.7%
		above 55	41	39.8%
CRP level (mg/dl)	0.5 \geq	normal level	2	1.9%
		high CRP level	101	98.1%
lymphocyte level (10 ³ /uL)	1.00 – 3.70	Low lymphocyte count	55	53.4%
		Normal lymphocyte count	48	46.6%
WBC level (10 ³ /uL)	3.00 – 15.00	normal WBC count	21	20.4%
		Leukocytosis	82	79.6%
D-dimer level(ng/ml)	198	normal D-dimer level	2	1.9 %
		high D-dimer level	101	98.1%
Urea (mmol/l)	< 50 yrs(3.2- 7.4) >50 yrs(3.0 -9.2)	High Urea level	51	49.5%
		Normal Urea level	51	49.5%
		Low Urea level	1	0.97%
Creatinine(umol/l)	63.6 -110.5	Normal creatinine level	49	47.5%
		High creatinine level	34	33%
		Low creatinine level	20	19.4%
Ferritin level (ng/ml)	30 – 300	normal ferritin level	11	10.7%
		high ferritin level	92	89.3%
Testosterone level (pg/ml)	19-55 yrs(1.00-28.28) >55yrs (0.70-21.45)	normal or low testosterone level	73	70.9%
		high testosterone level	30	29.1%
SHBG level (nmol/l)	16.8 - 113.2	low or normal SHBG	98	95.1%
		high SHBG	5	4.9%
Fate				
		Alive	63	61.2%
		Dead	40	38.8%
		Total	103	100.0%

In table 2, the patient is divided into two groups according to their fate, the mean \pm SE of biomarker for reach group was measured

Table 2: Mean \pm SE of the studied biomarkers.

	Alive(n= 63)	Dead(n= 40)	p-value
	Mean \pm SE	Mean \pm SE	
age yrs.	52.77 \pm 2.04	57.80 \pm 2.93	0.15
CRP (mg/dl)	182.91 \pm 27.83	194.29 \pm 23.005	0.77
Ferritin(ng/ml)	1182.7 \pm 142.45	1777.8 \pm 126.02	0.005*
WBC (103/uL)	14.27 \pm 0.70	16.18 \pm 0.93	0.10
LYMPH(103/uL)	0.98 \pm 0.09	0.82 \pm 0.07	0.24
D-dimer(ng/ml)	1857.50 \pm 291.21	3985.42 \pm 1224.95	0.04*
Urea(mmol/l)	9.92 \pm 1.07	15.10 \pm 2.07	0.01*
Creatinine(umol/l)	96.17 \pm 8.54	174.61 \pm 29.92	0.003*
Free Testosterone (pg/ml)	18.81 \pm 0.92	11.77 \pm 1.35	0.001*
SHBG (nmol/l)	62.49 \pm 3.87	44.10 \pm 4.88	0.001*

*Significant p-value \leq 0.05

To evaluate the correlation between free testosterone and SHBG with other biomarker in both dead and alive group, Pearson correlation coefficient r was measured and as showed that in table 3 and table 4.

Table 3: The correlation between free testosterone with other markers.

	Alive (n= 63)		Dead (n= 40)	
	r	p-value	r	p-value
WBC(103/uL)	0.25	0.04*	0.01	0.918
Lymphocyte(103/uL)	0.04	0.75	-0.01	0.94
CRP (mg/l)	0.06	0.63	-0.08	0.62
Ferritin (ng/ml)	-0.03	0.8	-0.008	0.96
D-Dimer (ng/ml)	-0.17	0.18	-0.03	0.83
Urea (mmol/l)	-0.06	0.62	-0.12	0.43
Creatinine (μ mol/l)	-0.19	0.23	-0.11	0.38
SHBG (nmol/ml)	0.47	0.001*	0.21	0.19

Table 4: The correlation between SHBG with other marker in both groups(dead and alive) .

	Alive (n= 63)		Dead (n= 40)	
	r	p-value	r	p-value
WBC (103/ μ l)	0.16	0.19	0.15	0.3
Lymphocyte(103/ μ l)	0.07	0.56	-0.12	0.43
CRP (mg/l)	0.23	0.06	0.01	0.95
Ferritin (ng/ml)	0.03	0.79	-0.008	0.96
D-Dimer (ng/ml)	-0.14	0.25	0.45	0.003*
Urea (mmol/l)	-0.08	0.51	-0.07	0.65
Creatinine (μ mol/l)	-0.08	0.52	-0.11	0.49
Free testosterone (pg/ml)	0.47	0.00*	0.21	0.91

Discussion

The current findings showed that there was significant increased (98.1%) in CRP levels and (79.6%) in leukocyte count in COVID-19 patients, these results in agreement with ⁽⁶⁾. These increment of levels might be linked to the overproduction of inflammatory cytokines in patients with COVID-19 levels ⁽⁷⁾. And the current results showed that no significant different in the non-survivors and survivors patients in CRP levels and WBC count , these results was disagreement with ⁽⁸⁾. The results of present study showed that the levels of lymphocyte count in blood of COVID-19 patients were decreased in (53.4%) of patients. These results in agreement with ^(6,9). Lower lymphocyte count might be associated with severe COVID-19, need for Intensive Care Unit and increased mortality⁽⁸⁾. These results disagree with the present study that showed the non-survivors with confirmed COVID-19 had lower lymphocyte count when compared with survivors but no significant difference between the survivors and non-survivors

patient group. The current findings showed that (89.3%) of the enrolled patients have high levels of ferritin ,these results were in agreement with ⁽⁶⁾. Patients with severe COVID-19 have been reported to have elevated ferritin levels as well as a cytokine storm. Because hyperferritinemia has been linked to inflammatory states in COVID-19 infection, ferritin could be a valuable marker for predicting disease severity and the scope of the cytokine storm ⁽¹⁰⁾. The present results found that a significant differences between non-survivors group which is higher than the survivors group of patient, which in turn is a higher than the normal level this results was in agreement with ⁽¹¹⁾.

D-dimer which is the fragments produced when plasmin cleaves fibrin to break down clots was high in about (98.1%) of patients. This increment in D-dimer levels is linked to venous thromboembolism (VTE) and acute pulmonary embolism (APE), both of which are associated with a high fatality rate ⁽¹²⁾. The current study found that D-dimer a significant

differences between dead group which is higher than the alive group of patients which was higher than reference range this results was in agreement with (12). Higher D-dimer levels in COVID-19 patients ,hinting coagulation abnormalities (10). Testosterone level was taken as an additional marker for disease severity markers in the present study and showed that (70.9%) of the participants have normal or low testosterone level , which comes in agreement with (6), this study found that COVID-19 has the potential to lower testosterone levels. Lower testosterone levels at baseline were associated with a significantly increased risk in term ICU admission and mortality. The current findings showed that free testosterone levels was lower significant in COVID-19 non-survivor patients compared with survivor patients , which was also lower than normal range , this results was in agreement with (6). Low Testosterone levels appear to be associated with increased susceptibility of respiratory diseases, as well as a predictor for adverse outcomes and mortality from COVID-19 (13). Çayan et al (13) showed that COVID-19 could decrease circulating testosterone levels, and lower testosterone levels at baseline were linked to a higher risk of severe symptoms requiring ventilator support because of the ACE2 receptor upregulation in respiratory cells ,elevated risk of lung damage and mortality. Testosterone is linked to the immune system of respiratory organs, and low testosterone levels might increase the risk of respiratory infections. The ACE2 is a constitutive product of adult type Leydig and Sertoli cells. In addition, it plays a critical function in lung protection (14). Coronavirus disease 2019 can penetrate epithelial respiratory cells because ACE2 is activated and down-regulated by the virus's spike protein (15). As a result, viral binding to the ACE2 receptor may reduce its expression causing degradation in a lung protective pathway, and might affect testosterone production in male COVID-19 patients , resulting in an increase in pro-inflammatory

cytokines in COVID-19 infected patients (16). The current findings also showed that (95.1%) have low or normal sex hormone binding globulin (SHBG). Sex hormone binding globulin levels in the current study showed that low significant in non- survivor patients compared with survivor patients , which was also lower than normal range. This decrease in the levels of SHBG might be due to it's direct relation to testosterone. Although COVID-19 primarily affects the respiratory system, it has also been found to damage multiple organs, such as the kidneys . Because the kidneys have the highest level of ACE2 expression (17). The present study showed that (49.5%) of patients having high level of urea. While (33%) of patients having high level of creatinine. And showed that both urea and creatinine are significant differences between non-survivors group which were higher significant than survivors group. These results were in agreement with (18) who found that (35%) of patients with COVID-9 had increase in urea and creatinine levels. These data suggest that COVID-19 mediated impaired kidney function may be one of the main causes final death in COVID-19 patients.

The current findings showed that no significant correlation between the free testosterone with other biomarkers in both groups. But found that a significant positive correlation between SHBG and free testosterone in the survivors group might be normal due to it's direct relation to testosterone. And found that positive significant correlation between WBC count and free testosterone in the survivors group, further more studies are needed to understand the correlation between WBC and free testosterone in survivors patients. The findings in the present study showed that no significant correlation between SHBG with other biomarkers in both groups. But found that a significant positive correlation between SHBG and free testosterone in the survivors group, and a significant positive correlation between SHBG and D-dimer in the non-survivors group.

Conclusions

Free testosterone and SHBG levels decrease in all patients with COVID-19 and it was more clear in non-survivors patients compared with survivor patients might be good taking a marker of severity.

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The Effects of Water Provision and Education on Students' Hydration Status, Cognitive Abilities, and Fine Motor Function in A Full-Day Primary School

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Abstracts

Background/aim: Dehydration due to inadequate water intake in children may disrupt brain functions. This study aimed to investigate the effects of hydration education with water provision on hydration status, cognitive functions and motor performance.

Materials and Methods: Forty-eight children in primary full-day school (age 8 – 11 years) were recruited in this study. The intervention of hydration education was performed for two days, both for students and teachers. The water was provision for 12 days. Letter cancellation task (LCT), symbol digit modalities test (SDMT), and direct image different test (DIDT) were used to assess cognitive functions. Motor performance was evaluated using a finger tapping test (FTT). Hydration status was determined with urine specific gravity (USG) and urine color (UC). Before and after interventions, all parameters were evaluated.

Results: Most students found dehydrated (>65%). The students had lower USG and UC after the intervention and it was correlated with each other (before; $r_s=0.45$, $P = 0.001$ and after; $r_s=0.82$, $P = 0.001$). All cognitive and motor functions were significantly higher after an intervention ($P < 0.05$).

Conclusion: Hydration education to students and teachers combined with water provision at primary full-day school increases hydration status and enhances cognitive and fine motor skills.

Keywords: *Urine color, Attention, Motor function, Dehydration*

Background

Growing evidence shows an increasingly widespread lack of total fluid intake, mostly water,

among children worldwide⁽¹⁻⁴⁾. Children and adolescents (aged 4–17) drank less than 500 mL of water daily^(3,4). Besides, the fact that water is the most abundant part of the body of children places them at a high risk of dehydration^(5,6).

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Increased awareness of the inadequate water intake induces dehydration of school-aged children, and access to water in schools has been raised⁽⁷⁾. Stookey and Konig found significant variations in fluid availability for school-aged children in Mexico,

Brazil, Argentina, China, and Indonesia. In some states, more than half of students do not have free access to drinking water while in school⁽⁸⁾, particularly in hot and dry areas⁽⁷⁾. According to Kausik et al., promoting adequate school water intake can help students' biological functioning⁽⁹⁾.

Several investigations discovered that dehydration could impair cognitive function^(10,11). In addition, education intervention⁽¹²⁾ and water supplementation were sufficient to prevent dehydration^(11,13). Acute water supplementation increases the student's visual focus and motor abilities⁽¹³⁾. Therefore, the effect of combining intervention (education and water provision) on cognitive and fine motor functions have not been explicitly studied in primary full-day school.

The current study examined the effects of hydration education and water provision for 12 days on urine hydration markers (USG and UC), cognitive functions (LCT, SDMT, and DIDT), and fine motor performance (FTT). We used all parameters as a baseline before the intervention. The degree of change in each parameter was measured after the intervention.

Methods

Participants

This study was carried out in September 2019 in Masohi, Central Maluku, Indonesia. Before collecting data, primary school principals were asked for permission. Selection criteria included grade (4-6) and health. Chronic disease or medication use were excluded. Their teachers informed their parents about the research. Before data collection, participants and their guardians signed consent forms. Only 48 of the 58 participants with parental consent completed the study. In this study, the Helsinki Declaration's ethical principles were followed (1964). All PoltekkesKemenkes Maluku procedures were approved by the Ethical Committee (LB.02.02/6.2/2910/2019).

Study design and education intervention

The study was quasi-experimental. All participants received hydration education and water provision. The research had three stages. Pre-intervention assessments included hydration, cognition, and fine motor. This lasted only one day. After that, teachers and participants learned about daily fluid requirements, dehydration symptoms, and rehydration options. Participants' water intake was monitored for two weeks by trained teachers who also drank water at school. Participants were observed at school from 7:00 am to 4:00 pm. Each class got refillable water. A 500 mL water bottle from home was also required. All school activities resume as scheduled. On the last day, we did post-intervention. Pre-intervention parameters were assessed.

Hydration status assessment

Pre- and post-intervention hydration status was assessed between 3:30 and 4:00 p.m. using USG and UC. Each participant received one sterile urine container. USG was measured twice with a portable refractometer (Cole-Parmer RSA-BR82T). Between samples, the refractometer was calibrated with distilled water. The sample was placed in a transparent container against a white background to calculate UC using a validated eight-color scale.

Cognitive and motor assessments

Letter cancellation task

The LTC requires participants to locate and cross a target letter within a grid of distractor letters. This test measured visual attention. This study used the one-letter version. Subjects had to cancel 'U' between O and V letters. Participants had 60 seconds to hit all targets. Scores were calculated by subtracting errors from the number of correct targets.

Symbol digit modalities test

The SDMT's cognitive demand is on visual information processing speed and attention. The top of the paper sheet has nine stimulus symbols and nine Arabic numbers from 1 to 9. Above the key were 120 blank boxes with a single pattern. Initial practice (5 min) for each participant, with orders. Participants have 90 seconds to fill in the blank boxes with the key. The number of empty squares that could be filled with the correct symbol determined the score.

Direct image difference test

This test was for visual attention. Two similar images were shown side by side. Participants had 60 seconds to spot the differences between the two images. In this case, more correct differences found meant better results (maximum =12).

Finger tapping test

Fine motor assessment was done by tapping digitally (CNS-Finger Tapping Test, Tushar Kalra). Participants had 60 seconds to practice with the instrument before the test. The digital app records several taps in a 3-trial by a preferred hand (10 s for each). On the digital screen until the trial is finished. The fine motor score was the average of three taps.

Statistical Analysis

The statistical analyses were performed using GraphPad Prism 9.0.0 and SPSS version 21 software programs. The data were presented in means \pm standard deviation (SD) or median \pm interquartile range (IQR) based on the normality distribution. Significance levels were set at $p < 0.05$.

The participants' characteristics were analyzed between males and females using unpaired t-test or Mann-Whitney test, and Kolmogorov-Smirnov test. The mean difference of participants' scores on FTT was analyzed with paired t-test. The mean rank differences of UC, USG, and cognitive assessments test pre and post-intervention using Wilcoxon test.

The differences of cognitive and motor assessments between hydration status were analyzed using one-way ANOVA or Kruskal Wallis test. Spearman correlation test was used to assess the correlation between US and USG levels.

Results

Participant characteristics

Table 1 shows participant characteristics for boys and girls. The Kolmogorov-Smirnov test revealed that both boys and girls had similar school grades ($p > 0.05$). Age, weight, BMI, and hydration status were not different between genders ($p > 0.05$). Only 22% of boys and 33% of girls had well-hydrated urine color samples. According to USG, one-third of boys and one-quarter of girls were severely dehydrated (≥ 1.030).

Hydration status based on urinary markers

Figure 1 presents data on the effect of hydration education and water provision at class on participants' urine hydration markers. There was a significant difference in the UC ($p < 0.0001$) and USG ($p < 0.0001$) between pre (Fig. 2a) and post interventions (Fig. 2b). Both urine hydration marker values were lower after intervention than that before the intervention.

Spearman correlation test showed a significantly positive correlation between the US and USG levels on pre and post-intervention ($p = 0.001$). The correlation coefficient between the US and USG levels before treatment with hydration education and water provision was moderate ($r_s = 0.45$), while after treatment was intense ($r_s = 0.82$).

Cognitive and motor performances

The results of LCT, SDMT, DIDT, and FTT before and after treatment are presented in Figure 2. There were significant differences in the mean rank of LCT, SDMT, and DIDT between pre and post-

intervention ($p < 0.0001$). All cognitive performances improved after treatment. The paired t-test also found significant differences in the mean of FTT between the time of assessments ($p < 0.0001$).

The cognitive and motor performances pre and post-intervention were also analyzed based on hydration status, specifically USG value (Table 2). The LCT values pre and post-treatment with hydration education and water provision were significantly differences (one-way ANOVA, $F_{3, 44} = 3.84$; $p = 0.016$ and $F_{3, 44} = 2.86$; $p = 0.048$, respectively). Post-hoc analyses of these data revealed that the significant dehydration group showed a significant decrease in LCT than well-hydrated ($p = 0.003$ and $p = 0.005$, pre and post) and minimal dehydration ($p = 0.008$

and $p = 0.040$, pre, and post) groups. Interestingly, there was no significant decrease in LCT scores on the serious dehydration group than the well-hydrated group, both pre and post-intervention.

The SDMT mean rank did not change significantly between pre- and post-treatment ($p > 0.005$). Dehydration also reduces DIDT score post-intervention ($p = 0.041$). The FTT data showed a significant difference between groups post-intervention (one-way ANOVA, $F_{3, 44} = 6.99$; $p = 0.001$) but not pre-intervention. The serious dehydration group had significantly fewer FTT scores than the well-hydrated ($p < 0.0001$), minimal dehydrated ($p < 0.0001$), and significant dehydrated ($p = 0.001$) groups.

Table 1 Characteristics of participants between male and female

Characteristics	Boy (n = 27)	Girl (n=21)	P value
Age (years) ^a	10 ± 3	9 ± 1	0.135*
8 – 9	12 (44%)	12 (57%)	
10 – 11	15 (56%)	9 (43%)	
Grades at school			
4th grade	11 (41%)	13 (62%)	0.665#
5th grade	9 (33%)	7 (33%)	
6th grade	8 (26%)	1 (5%)	
Body weight (Kg) ^b	28.3 ± 14	27.5 ± 10.5	0.442*
BMI-b	-0.28 ± 1.7	0.54 ± 0.9	0.435 [†]
Underweight	3 (11%)	0	
Normalweight	16 (59%)	21 (100%)	
Overweight	4 (15%)	0	
Obese	4 (15%)	0	

^aData presented as mean ± SD. ^bData presented as median ± IQR.

*Mann-Whitney test. #Kolmogorov-Smirnov test. [†]Unpaired t-test. n=number of participant.

Table 2. Cognitive and fine motor performances of participants in pre and post intervention based on USG

Cognitive and motor assessments		Hydration status based on USG				p value
		WH	MD	SiD	SeD	
	n Pre/post	15/36	13/16	6/4	14/2	
LCT	Pre	29.8 ± 4.4	28.9 ± 6.2	20.5 ± 9.3*#	25.7 ± 6.4	0.016a
	Post	33.1 ± 4.6	32.3 ± 3.8	25.6 ± 7.9*#	33.0 ± 2.8	0.046a
SDMT	Pre	21 ± 25	13 ± 24	17 ± 12.3	20 ± 11.3	0.426b
	Post	25 ± 9.8	30.5 ± 19.8	25.3 ± 6.3	19	0.207b
DIDT	Pre	7 ± 3	5 ± 2	6 ± 2	5 ± 3	0.041b
	Post	7 ± 3	8.5 ± 1	8.5 ± 6	5	0.161b
FTT	Pre	51.3 ± 6.9	48.8 ± 8.9	48.3 ± 6.2	48.6 ± 4.9	0.685a
	Post	53.4 ± 5.5	52.2 ± 3.1	52 ± 4.5	41.5 ± 3.5*#§	0.026a

^aThe data were analyzed using One-way Anova. ^bThe data were analyzed using Kruskalwallis test. *<0.05 vs. WH ;#<0.05 vs. MD ; §<0.05 vs. SiD. WH, Well-hydrated; MD, Mild dehydration; SiD, Significant dehydration; SeD, Serious dehydration; LCT, Letter cancelation task; SDMT, Symbol digit modalities test (SDMT); DIDT, Direct image difference test; FTT, Finger tapping test.

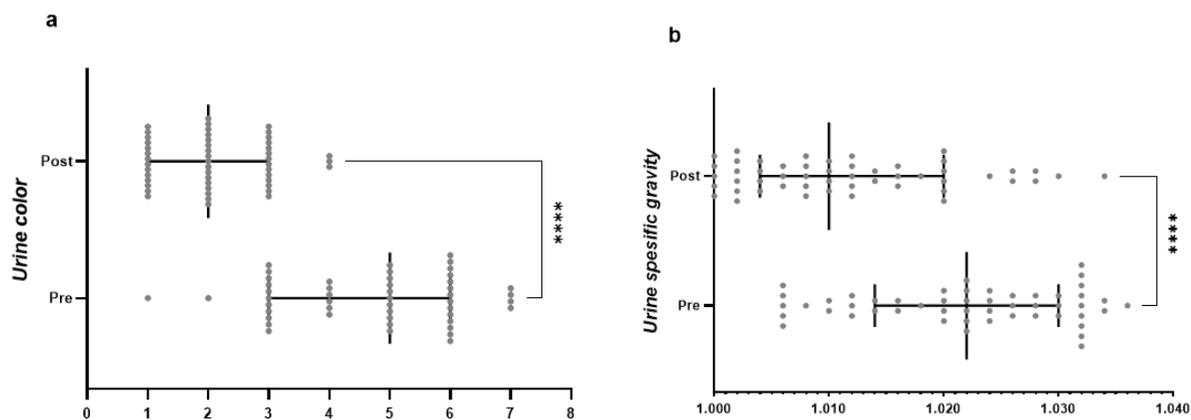


Figure 1

The effect of hydration education and water provision of drinking water at class on participants’ urine hydration markers. a. Urine color. b. Urine specific gravity. The data are presented as median±IQR (n=51).

****p<0.0001 (Wilcoxon test).rs, Spearman rank correlation coefficient. p, p-value

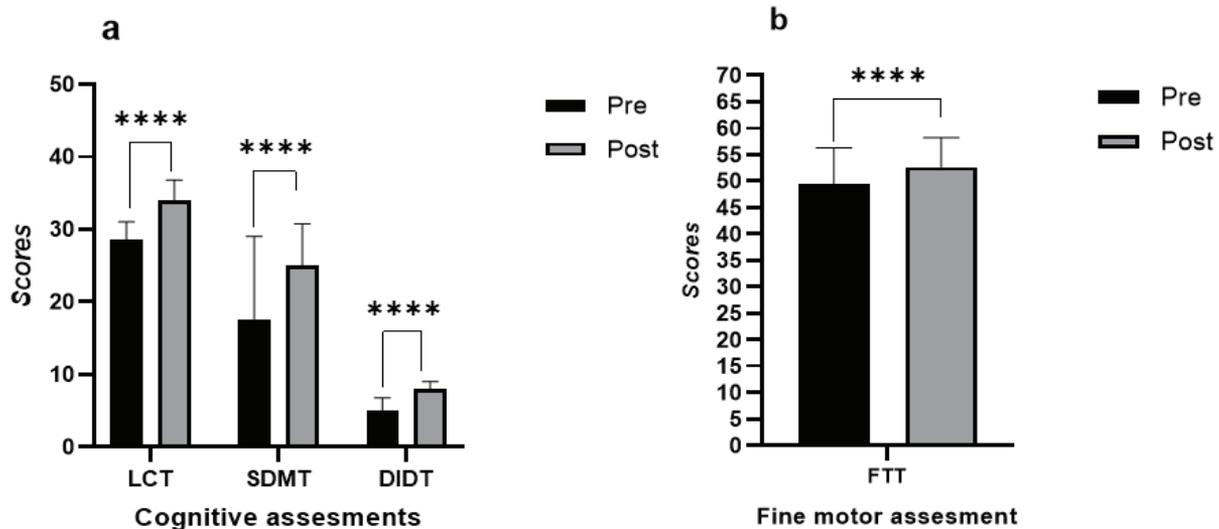


Figure 2

The effect of hydration education on cognitive and motor performances of participants. **a. Cognitive assessments.** The data are presented as median±IQR (n=48). ****p<0.0001 (Wilcoxon test). **b. Fine motor assessment.** The data are presented as mean±SD (n=48). ****p<0.0001 (Paired t-test).

LCT, Letter cancelation task; SDMT, Symbol digit modalities test (SDMT); DIDT, Direct image difference test; FTT, Finger tapping test.

The effect of hydration education and water provision of drinking water at class on participants' urine hydration markers. **a. Urine color.** **b. Urine specific gravity.** The data are presented as median±IQR (n=51). ****p<0.0001 (Wilcoxon test). r_s , Spearman rank correlation coefficient. *p*, *p*-value

Discussion

The current study found that two weeks of hydration education and water provision improved students' hydration and cognitive abilities in primary full-day school. Improved hydration status (lower USG and UC values) influenced cognitive and fine motor performance based on dehydration levels.

Our study had shown that the incidence of dehydration before the intervention was high (68% - 73%). This research finding was consistent with previous studies^(10,14,15). These findings show that once children have reached school, they do not drink enough water⁽¹³⁾. As a result, the risk of dehydration increases with extended schooling time.

The hydration status of boys and girls did not differ statistically (Table 1). Some experiments had varying results. They found that boys' urine osmolality and USG were higher than girls⁽¹⁶⁻¹⁹⁾. The reason for our result remains uncertain at present since we did not perform a drinking diary or food recall, but it may be linked to variations in the race or ethnicity of participants⁽²⁰⁾ in this study compared to those previous reports⁽¹⁶⁻¹⁹⁾.

The current study found that after two weeks of intervention, USG and UC decreased significantly (p<0.05), indicating an increase in school-based water intake. It has been proposed that increasing water consumption is more important than other drinks in reducing dehydration⁽²⁰⁾. It is well-known that providing water at school increases student water intake and hydration^(11,13,15,21). Water access and verbal hydration education reduced urine osmolality, USG, and UC in children attending sports camps⁽¹²⁾. However, teachers' role as role models and monitoring

student hydration seems to have helped improve students' hydration status. According to Schätzer et al., healthy school hydration initiatives should help empower teachers⁽²²⁾. A lower urine osmolality or adequate hydration were found in schools that provided water, drinking education, and structured drinking agreements⁽²³⁾. This study's approach to reducing child dehydration includes both hydration education and teacher empowerment.

It has been conveyed that a loss of 2% of body weight due to inadequate hydration could detract cognitive and psychomotor performances⁽²⁴⁾. Kozioł-Kozakowska and colleagues reported that dehydration was demonstrated as a risk factor (Odds Ratio= 2.85) for impairment of students' attention⁽²⁵⁾. These dehydration effects on brain function have been restored during water intake⁽²⁶⁾. Along with improved hydration, the study found improved cognitive function on the LCT, SDMT, and DIDT scores (Fig. 2a) and fine motor abilities FTT (Fig. 2b). The LCT, SDMT, and DIDT are recognized for determining visual attention⁽¹⁴⁾. Our results matched those of previous studies^(15,21,27,28).

As shown in Table 2, there were statistically significant ($p < 0.05$) differences in mean LCT scores between groups of hydration status. Also, the SDMT scores were identical in both studies. Pre-intervention DIDT test scores showed a significant difference between hydration status groups, whereas post-intervention FTT scores showed no difference. This result suggests that hydration status specifically affected cognitive performances. Previous research found that body water adequacy was domain-specific rather than generalized^(21,29).

The group with the worst attention scores on LCT pre- and post-intervention was significantly dehydrated ($p < 0.05$), according to post hoc analysis (Table 2). Surprisingly, the LCT score was similar in both groups ($p > 0.05$). The severe dehydration

group also had shallow fine motor performances on FTT post-intervention. Extreme dehydration affected children's fine motor skills ($p > 0.05$), indicating that dehydration affects children's fine motor skills. Previously, studies evaluating dehydration-related mental performance showed a dose-response relationship. The more body fluids are lost because of dehydration, the worse the performance will be^(30,31). Our FTT results support these viewpoints, but not for LCT scores. In children with extreme dehydration, the attention is well-preserved on LCT after the intervention is possibly attributable to physiological mechanisms of adaptation. They were experienced extreme and severe dehydration since the pre-intervention period. Physiological systems respond acutely to hypohydration to reduce homeostatic disruption and probably adjust to chronic water deficit to increase tolerance⁽³²⁾.

Conclusion

Hydration education to student and teacher combined with water provision for 12 days at primary full-day school may return student USG and UC to well-hydrated levels. Improving the hydration status of students has been shown to enhance cognitive and motor skills. Our study's limitation does not consider the calculation of the daily amount of intake of water or other beverages and provided a control group. Further investigations on the effects of hydration education with water supplementation on different aspects or parameters of children's brain function are also warranted.

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Conflict of Interest Nil

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Effect of Lifestyle Modification Guidelines on Mild Preeclampsia

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Abstract

Background: Preeclampsia has an adverse effect on both mother and fetus, and may be life-threatening condition. Lifestyle modification can improve the clinical presentation of preeclampsia. **Methods:** Aim of the current study was to examine the effect of lifestyle modification guidelines on improving mild preeclampsia. A Quasi-experimental design was adopted on a purposive sample of 80 pregnant women with mild preeclampsia at the antenatal outpatient unit in Port Said Maternity Hospital, they were assigned into two groups; the study group received lifestyle modification guidelines alongside the routine care and pharmacological management, while the control group received the routine care and pharmacological management. Two tools were used for data collection, Interviewing Questionnaire Sheet and Follow Up Assessment Sheet. **Results:** The findings showed that lifestyle modifications guidelines lowering the mean of systolic and diastolic blood pressure, gestational weight gain, and proteinuria. Also, reducing risk of delivery before 34 weeks of gestation by 10.0%, delivery from 34 to 36 weeks of gestation by 30.0%, altered liver function by 22.5%, altered kidney function by 15.0%, and cardiac symptoms by 17.5%. **Conclusion:** Lifestyle modification guidelines alongside with routine care and pharmacological treatment are effective method in modifying mild preeclampsia and its complications.

Key Words: Lifestyle Modification, Lifestyle Guidelines, Maternal Outcome, Mild Preeclampsia, Severe Preeclampsia.

Introduction

Preeclampsia (PE) is an induced pregnancy hypertensin disorder, which may be resolves within six weeks postpartum. In addition, PE are

considered a multi system, progressive disorder, which characterized by elevated blood pressure (BP) (systolic blood pressure (SBP) is equal or more than 140 mmHg, and/or diastolic blood pressure (DBP) is equal or more than 90 mmHg), in previously normotensive women, in the absence of proteinuria or proteinuria +1 with dipstick reading, and edema at 20-24 weeks of gestation ^(1,2).

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Preeclampsia is accompanied with various of complications for mother^(3,4). Lifestyle can be described as the way that individuals, families, and communities can live, in terms of personal behaviors including nutrition, physical activity, and stress management. A healthy lifestyle can improve health and happiness⁽⁵⁾, and reduce the rate of morbidity and mortality⁽⁶⁾. During pregnancy, lifestyle modification may be an appropriate strategy for controlling PE in combination with pharmacological therapies⁽⁷⁾.

Preeclampsia is one of the most common life-threatening complications during pregnancy⁽⁸⁾. Nursing guidelines and counseling sessions for women with high BP during pregnancy have an important role in early control of its subsequent problems. Lifestyle modification could control hypertension (HTN) and reduce complications related to HTN to mother. Hence, the present study through the light on the effect of lifestyle modification guidelines on modifying mild PE.

Material and Method

Aim of the study:

Aim of the current study was to examine the effect of lifestyle modification guidelines on modifying preeclampsia.

Research hypothesis:

Pregnant women with mild preeclampsia who will follow lifestyle modification guidelines will show a low risk for developing maternal complications related to preeclampsia than those who don't.

Research Design:

A quasi-experimental (control and study groups) design was adopted in the current study.

Study Setting:

This study was conducted at the antenatal

outpatient unit affiliated with Port Said Maternity Hospital, Egypt.

Study Population and Sample:

The study sample included a purposive sample of 80 pregnant women at 20 – 24 weeks of gestation with mild PE assigned equally into two groups.

Tools of Data Collection:

Tool (1): Structured Interviewing Sheet.

It contains personal data, which include age, occupation, educational level, and marital status, obstetrical history, which contain gravidity, parity, history of abortion and preterm deliveries, and inter-pregnancy intervals.

Tool (2): Maternal Follow Up Assessment Sheet.

It deals with the follow-up data during the pregnancy period, such as BP, weight, proteinuria, severe PE, and maternal complications related to PE.

Pilot Study:

A pilot study was conducted on eight pregnant women with mild PE (10.0% of the total sample) to test the feasibility and applicability of the tool, and examine the clarity of the questions. The women who recruited in the pilot study were excluded from the study sample.

Validity and Reliability of Tool:

The tool was reviewed by jury composed of five experts in the field of Maternity & Newborn Nursing and Community Nursing to test the clarity and applicability of the tools. Reliability was tested by Cronbach's Alpha test to test the reliability of tools through their internal consistency, by Cronbach's Alpha test was equal (0.0843).

Field of Work:

Guidelines of lifestyle modification for pregnant women with mild PE was prepared to enrich them with knowledge about the appropriate lifestyle for mild PE as regards to nutrition, physical exercise, rest and sleep, stress management, smoking, follow up, measures to relieve signs and symptoms of mild PE, and dangerous signs of PE. Participants were allocated into two groups as the first 40 women were recruited in the control group, while the second 40 women were recruited in the study group. Participants was individually interviewed to collect the personal and clinical data. Then, participants in the control group were following the routine care and pharmacological treatment as the hospital protocol for the management of mild PE. While, the participants in the study group were following the hospital protocol for the management of mild PE beside lifestyle modification guidelines, it was explained using various methods of health teaching; Power Point show and interventional guideline handout. The pregnant women in both groups were assessed every two weeks until 36th weeks of gestation, then weekly until delivery for; BP, weight, proteinuria, and maternal complications related to PE.

Ethical Consideration:

An approval from the Research Ethics Committee, Faculty of Nursing, Port-Said University was obtained. Written approval was obtained from Port Said Maternity Hospital administrators and head of the concerned department. The aim and procedure of the current study was explained to each woman and an oral approval consent was obtained. They were assured about confidentiality and privacy. They have the right to withdraw from the study at any time without any reason.

Result

Table (1): shows the personal data of the studied

sample. The mean age of the control and study groups was (28.73±6.56 & 29.85±6.99) respectively. In addition, 60.0% & 75.0% of the control and study groups were housewives respectively. Also, 45.0% & 57.5% of the control and study groups had secondary education respectively. It was estimated that, all of them were married.

Table (2): mentions the obstetrical history among the studied sample. It was observed that, 82.5% & 92.5% of the control and study groups were multigravida respectively, and 72.5% & 57.5% of the control and study groups didn't have history of abortion. Also, the majority of the control and study groups didn't have a history of preterm delivery (97.5% & 87.5%) respectively. In addition, 52.5% & 57.5% of the control and study groups had less than 2 years inter-pregnancy intervals respectively.

Table (3): clarifies the maternal outcome among the studied sample. The study group had lower mean of SBP and DBP readings than the control group (145.98±8.32&151.75±6.84) & (89.91±3.98&93.43±3.42) with highly statistically significance (p= 0.001 & 0.000) respectively. Also, the study group had lower mean of GWG than the control group (13.74±4.48&16.10±5.99) with statistically significance (p= 0.049). In addition, the study group had lower mean of proteinuria than the control group (1.67±0.31 & 1.45±0.38) with statistically significance (p= 0.006). As shown in table (3), the study group had lower rate of developing severe PE (62.5%& 92.5%), altered liver function (15.0% & 37.5%), tachycardia and palpitation (7.5% & 25.0%) than the control group with statistically significance (p= 0.001, 0.022, & 0.034) respectively. Only one case of the control group developed eclampsia, while 30.0% & 15.0% of the control and study groups had altered kidney function without statistically significance (p=0.152&0.108) respectively.

Table (1): personal data among the studied sample (n= 80).

Variables	Control Group (n= 40)		Study Group (n= 40)		Significance
	No	%	No	%	
Age (year)					
20-	15	37.5	14	35.0	X ² = 1.892 P= 0.60
26-	9	22.5	6	15.0	
31-	8	20.0	7	17.5	
More than 35	8	20.0	13	32.5	
Mean (SD)	28.73±6.56		29.85±6.99		t= 0.742 P= 0.46
Occupation					
Occupied	16	40.0	10	25.0	X ² = 2.051 P= 0.23
Housewife	24	60.0	30	75.0	
Educational Level					
Illiterate	2	5.0	4	10.0	X ² = 6.443 P= 0.09
Primary	3	7.5	6	15.0	
Secondary	18	45.0	23	57.5	
University	17	42.5	7	17.5	
Marital Status					
Married	40	100.0	40	100.0	

Table (2): obstetrical history among the studied sample (n= 80).

Variables	Control Group (n= 40)		Study Group (n= 40)		Significance
	No	%	No	%	
Gravidity					
Primigravida	7	17.5	3	7.5	X ² = 1.829 P= 0.31
Multigravida	33	82.5	37	92.5	
Parity					
Nullipara	7	17.5	3	7.5	X ² = 1.835 P= 0.40
Primipara	11	27.5	12	30.0	
Multipara	22	55.0	25	62.5	
History of abortion					
None	29	72.5	23	57.5	X ² = 2.121 P= 0.35
Once	5	12.5	9	22.5	
Twice	6	15.0	8	20.0	
History of Preterm Labor					
Yes	1	2.5	5	12.5	X ² = 2.883 P= 0.20
No	39	97.5	35	87.5	
Pregnancy Interval (year)					
Nulliparous	7	17.5	3	7.5	X ² = 2.691 P= 0.44
Less than 2	21	52.5	23	57.5	
2 – 5	11	27.5	11	27.5	
More than 5	1	2.5	3	7.5	

Table (3): maternal outcome among the studied sample (n= 80).

Variables	Control Group (n= 40)		Study Group (n= 40)		Significance
	No	%	No	%	
SBP Mean (SD)	151.75±6.84		145.98±8.32		t= 3.388 P= 0.001*
DBP Mean (SD)	93.43±3.42		89.91±3.98		t= 4.251 P= 0.000**
GWG Mean (SD)	16.10±5.99		13.74±4.48		t= 1.998 P= 0.049*
Proteinuria Mean (SD)	1.67±0.31		1.45±0.38		t= 2.813 P= 0.006*
Severe PE Yes No	37 3	92.5 7.5	25 15	62.5 37.5	X ² = 10.323 P= 0.001*
Timing of delivery Less than 34 weeks 34 – 36 weeks 37 – more than 37	5 18 17	12.5 45.0 42.5	1 6 33	2.5 15.0 82.5	X ² = 13.787 P= 0.001**
Eclampsia Yes No	1 39	2.5 95.0	0 40	0.0 100.0	X ² = 2.051 P= 0.152
Altered liver functions Yes No	15 25	37.5 62.5	6 34	15.0 85.0	X ² = 5.230 P= 0.022*
Altered kidney function Yes No	12 28	30.0 70.0	6 34	15.0 85.0	X ² = 2.581 P= 0.108
Cardiac symptoms Yes No	10 30	25.0 75.0	3 37	7.5 92.5	X ² = 4.501 P= 0.034*

Discussion

A healthy lifestyle can control PE in combination with pharmacological therapies⁽⁷⁾. Use of booklets facilitate for the continuation of effective education as the guidelines become easier to be comprehended and adapted from the booklet and used in everyday life⁽⁹⁾. As mentioned by the findings of the present study, lifestyle modification guidelines lower the mean of SBP and DBP, proteinuria, gestational weight gain (GWG), eliminate the risk of developing severe PE, reduce the alteration of liver functions, and cardiac complications with statistically significance. While, it lowers the rate of eclampsia, and altered kidney functions without statistically significance differences.

These findings are emphasized by Alexander et al⁽¹⁰⁾, who stated that lifestyle modification is sufficient for the management of pregnant women with stage one HTN. In addition, Vamvakis et al⁽¹¹⁾ found that lifestyle modifications lower SBP and DBS. Also, ElSayed and Desoky⁽¹²⁾ concluded that the counselling sessions of lifestyle had a positive correlation on the status of pregnant women with mild PE and lower the readings of SBP and DBS with a high statistically significance.

These findings are going in the same line with Ahmed⁽¹³⁾, who found that lifestyle counselling lowers the BMI of pregnant women. Also, Horn et al⁽¹⁴⁾ recommended that antenatal diet and physical activities prevent excess GWG and benefit mother and child. Moreover, Ferrara et al⁽¹⁵⁾ found that diet, physical activity, and stress management had reduced weekly rate of GWG. On the other side, Asci and Rathfisch⁽¹⁶⁾ mentioned that lifestyle interventions show no statistically difference in GWG between the intervention group and control group.

These findings are supported by Attini et al⁽¹⁷⁾ suggested that a low protein-restricted, vegan diet,

plant-based diet might contribute to controlling proteinuria in pregnant CKD women. On the other hand, a study adopted by Reshma et al⁽¹⁸⁾ mentioned that relaxation therapy included deep breathing, progressive muscle relaxation, and guided imagery shown no significant difference in proteinuria. From the point of view, this conflict may be because the current study, including instructions regarding nutrition and exercise besides stress management.

The findings of the current study emphasized that lifestyle modification guidelines lower the risk of developing severe PE by 30.0%. These findings are supported by Mekie et al⁽¹⁹⁾ who found that nutritional counselling during ANC follow-up lower the risk of facing PE by 48.0%. Furthermore, Davenport et al⁽²⁰⁾ mentioned that, women who exercise had 30.0% less risk for developing gestational hypertensive disorders. In addition, Lewandowska and Wieckowska⁽²¹⁾ concluded that smoking in the first trimester increase the risk of PE.

The current study findings are in disagreement with Fulay et al⁽²²⁾ who concluded that adherence to the DASH diet during early pregnancy does not appear to be protective against hypertensive disorders in pregnancy. Also, Mol et al⁽²³⁾ reported that simple preventive measures, such as low-dose aspirin, calcium, and diet and lifestyle interventions, show small benefit. In addition, Vollebregt et al⁽²⁴⁾ reported that physical activity in pregnancy does not reduce the incidence of PE or gestational hypertension in nulliparous women. These differences due to different designs and methodologies of research, including the general characteristics of the studied sample, could have influenced the discrepancy.

Conclusion

Lifestyle modification guidelines alongside pharmacological treatment protocol are effective and safe management plan for pregnant women with mild

preeclampsia to improve maternal outcome; it can be used to reduce the adverse effect of preeclampsia on women. Health educational sessions about lifestyle modification should be implemented to pregnant women with mild PE.

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Dhikr and Prayer Guidance on Peace of Mind and Blood Pressure Control

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Abstract

Background: The Effectiveness of Dhikr and Prayer Guidance on Peace of Mind and Blood Pressure Control. WHO data (2015) indicate the 1.13 billion people worldwide with hypertension. The purpose of this study is to know the effectiveness of dhikr and prayer guidance on peace of mind and blood pressure control in the elderly with hypertension. **Methods:** The research design was a quasi-experiment with a pretest-posttest approach. The samples are 24 respondents. **Results:** The results showed that the $p_{\text{value}} 0.036 < 0.05$ means no significant effect on the peace of mind of the elderly with hypertension in the intervention group who given dhikr and prayer guidance. Pre and post blood pressure tests in the intervention group obtained $p_{\text{value}} 0.000 < 0.05$, which means that there is an effect of the intervention group variable (systole) on blood pressure. **Conclusion:** Dhikr and prayer instructions can be a complementary therapy in overcoming mental calm and blood pressure in the elderly with hypertension. We hope that the results of this study can be used as a reference material in providing therapy to patients with hypertension in order to help lower blood pressure.

Keywords: Dhikr, Blood Pressure, Elderly, Hypertension, Peace of mind, Prayer;

Introduction

Hypertension or famously known as the silent killer is a condition where the increase of blood pressure above normal. Increased age is one factor causing the occurrence of hypertension, this is due to the increasing age of organ function decreased marked by decreased elasticity of the arteries and stiffness occurs blood vessels so vulnerable to an increase in blood pressure. Hypertension is defined as persistent blood pressure where the systolic pressure is above 140 mmHg and diastolic over 90 mmHg. [1],[2],[3] One of the major risk factors of hypertension

is stroke, heart failure, chronic kidney disease, visual impairment, and hypertension is often called the silent killer. Hypertension is a condition when a person experiences a rise in blood pressure either slowly. [4],[5],[6],[7]

The number of patients with hypertension in the world continues to increase, an estimated 2025 people with hypertension increased to 1.5 billion, and 9.4 million are estimated to die due to hypertension and its complications. Riskesdas (2018) stated the prevalence of hypertension based on measurements among people aged ≥ 18 years at 34.1%, the highest was in South Kalimantan (44.1%), while the lowest was in Papua (22.2%). The estimated number of hypertension cases in Indonesia is 63,309,620 people, while the death rate in Indonesia due to hypertension

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is 427,218 deaths. Hypertension occurs in the age group 31-44 years (31.6%), 45-54 years (45.3%), 55-64 years (55.2%).^[8]

We know that as we get older to be elderly, many changes occurred, such as the physical and psychological condition which can affect the social life and happiness in old age. With the emergence of psychosocial stressors that can lead to stress, if it is prolonged, it leads to being a more severe problem: depression. Likely, pressure and depression (which describes an unsettled mental state) in 2025 will be the number two cause of disability in the elderly worldwide. Proved that the spirituality approach capable of providing a significant relationship to hypertension. Because the spiritual program can keep one’s peace of mind, feel patient, hoping to God, love of God and blessing to God’s will.^[9]

The spiritual approach in the practice of medicine and nursing, not to change one’s faith to the religion that has believed in, but to awaken the spiritual power in the face of a disease and become a psychoreligious therapy. Most studies have shown that religious and spirituality involvement can improve health better as the ability to survive and health-related quality of life, and not show anxiety, depression, and suicide.^[10]

Based on the problems related to the prevalence

of hypertension is still a problem in society and be the target of the Ministry of Health. Based on the data, the number of elderly in PuskesmasToddopuli Makassar South Sulawesi as many as 2,454 elderly, and there are 534 elderly with hypertension by 2019. The researchers are interested in examining the effectiveness of dhikr and prayer guidance on peace of mind and blood pressure control in the elderly with hypertension.

Based on the theory and the data above, researchers consider it necessary to attempt to avoid the stress, negative emotions, anger, so it is always in a state of calm, serenity and always surrounded positive values, which can reduce the potential for high blood pressure.

Methods

The study about the effectiveness of dhikr and prayer guidance on peace of mind and blood pressure control in the elderly with hypertension conducted in June 2020. The population of this study was all elderly with hypertension in PuskesmasToddopuli Makassar. Samples in this study consisted of 24 respondents were divided into two groups: 12 elderly in the intervention group and 12 elderly in the control group. The research design used in this study is quasi-experiment with the pretest-posttest approach.

Results

Table 1. The results of difference test in a piece of mind pretest and posttest in the intervention and control groups in the elderly with hypertension in the work area of PuskesmasToddopuli Makassar

Group	Mean	P
Intervention PreTest PostTest	32.92 36.25	0.036
Control PreTest PostTest	30.42 30.83	0.906

Wilcoxon Signed-Rank test results on the peace of mind pre and post-test in the intervention group obtained $p_{value} 0.036 < 0.05$ meaning that there is a significant effect of dhikr and prayer guidance on peace of mind in elderly with hypertension. Wilcoxon

Signed-Rank test results in the control group p-value obtained $0.906 > 0.05$ meaning that there is no significant effect of dhikr and prayer by using the text on peace of mind in the elderly with hypertension.

Table 2. The results of difference test in blood pressure pretest and posttest in the intervention and control groups in the elderly with hypertension in the work area of Puskesmas Toddopuli Makassar

Blood Pressure (Intervention)	Pretest	PostTest	P
Mean of Systol	147.50	142.25	0.000
Mean of diastole	88.33	83.92	0,001
Control			
Mean of Systol	147.50	150.50	0.499
Mean of diastole	89.75	89.50	0,879

The test results of paired samples t-test on blood pressure pre and post-test in the intervention group obtained $p_{value} 0.000$ and $0.001 < 0.05$ meaning that there is a significant effect of intervention group variables (systolic and diastole) on blood pressure. The results of paired samples t-test on blood pressure pre and post-test in the control group obtained $p_{value} 0.499$ and $0.879 > 0.05$ means that there is no significant effect of control group variable (Systole and diastole) on blood pressure.

Table. 3. The results of the comparison test of blood pressure post-test in the intervention and the control group in the elderly with Hypertension in Puskesmas Toddopuli Makassar

Blood Pressure	Intervention PostTest	Control PostTest	P
Mean	142.25	150.50	0.86

Independent Test Results T-test p-value in the post test (systole) intervention and control obtained 0.86 or $p > 0.05$ means that there is no significant difference between the control group (diastolic) and the intervention group (systolic) at post-test.

Discussions

Peace and comfort are the most common expressions of happiness for people. Some of the happiness factors that have a close relationship are dhikr, the acts of worship verbal and spiritual expressed by the pronunciation of a specific sentence

in a certain way with the use of oral which could be heard by the ear, or by using the heart to mention the name of Allah without sound. Stated that the real dhikr is to forget everything, except the Almighty. Hasan al-Banna, a Muslim leader in Egypt, declared that everything closer to Allah SWT and all the memories

that make closer to Allah SWT included Dhikr. That doing dhikr therapy had a significant impact in improving peace of mind, the respondents were asked to equally istighfar 100 times simultaneously. After dhikr then respondents were asked at the end of the meeting related to the feeling you get after following dhikr therapy. The respondents said it is more manageable to control emotions and became calmer. To achieve the level of perfection and peace of mind requires education and length mental training, one of which is dhikr through meditation (concentration of consciousness) while calling name and remember God in every circumstance. The purpose of dhikr is to establish a spiritual bond (psychiatric) between the servants of God which raised the love, honor and muroqabah soul (feel close and watched over by God).^{[11],[12]}

To determine the effect of pretest and post-test of the intervention group, Wilcoxon signed rank test was carried out. The result is the $p_{\text{value}} 0.036 < 0.05$ which means no significant effect on the peace of mind of elderly with hypertension in the intervention group were who given dhikr and prayer guidance.

The effectiveness of dhikr training in promoting peace of mind of elderly patients with hypertension with the result $p 0.008 < 0.05$ means there is the influence of dhikr in improving peace of mind of elderly patients with hypertension. Based on the results of the previous study, increased mental well-being better after training dhikr. Dhikr is also able to control emotions among respondents in addressing irregularities thinking and excessive anxiety. The dhikr capable of providing peace of mind that affects as prevention and treatment of conditions of individuals with hypertension.

One of the benefits of dzikir is mental therapy. Islam as a religion rahmatanlil 'aalamiin offers a concept of the development of values in one's inner divine. Prayers, for example, in which there is full of

prayer and dhikr, can be seen as malja '(shelter) in the storms of modern life, here is the mission of Islam to soothe human hearts. Dhikr functional will bring benefits, among other things bring happiness, soothe the soul, heart disease drugs, and so on. Dhikr is a strategy that is expected to increase peace of mind. That dzikir can give a feeling of calm to the soul so that an individual who is always doing dzikir can prevent the emergence of tension (stress). The dhikr can provide a significant correlation in enhancing peace of mind. the process of dhikr by saying tawhid and istighfar, increases the disposal of CO₂ in the lungs. Yurisaldi also explained that when someone dhikr, a decrease in cerebral blood vessels occur just because of the response of the chemical. Blood supply (decreased oxygen and glucose levels) to brain tissue is decreased. This situation immediately responded by the brain with a massive yawn reflex and insert oxygen through the lungs to the cerebral with the dilated diameter of blood vessels. So that the supply of oxygen and glucose to the brain tissue increased rapidly and will revitalize all cellular and micro-scale elements impact on the strength and vitality of brain cells then supply oxygen in large quantities, as the mitochondrial cell respiration center will re-active and work out regularly.^{[13],[14],[15]}

Dhikr is the development of the relaxation response that shows a regular rhythm and the mind focuses on the creator accompanied by the rhythmic repetition of words that can lead to a relaxed state. Dhikr can affect the hypothalamus and stimulates endorphins and make things feel comfortable. Dhikr can also be said to be a human effort to get closer to Allah by remembering, reflecting His glory, unite hearts and minds only to one, Allah, the Lord of the Universe. So that dhikr therapy is one of the spiritual approaches that soothes the soul. That result of this study, there is an effect of dhikr on blood pressure changes in the elderly suffering from hypertension with a significant value of 0.008. Because of the

p-value < 0.05 means that there is an influence of dhikr on blood pressure changes. Viewed as a descriptive matter there is a blood pressure change from the first-day post-treatment until day five post-treatment, the author found that there is the effect of dhikr on blood pressure changes.^{[16],[17]}

It is proven by the test results of the analysis that the significant difference between the systolic blood pressure associated with the activity of the sympathetic nervous system. The therapeutic effect of dhikr can lower systolic blood pressure by stimulating the parasympathetic nervous system and secrete endorphins that can reduce sympathetic nervous system.

States that the whole human health shown by the four things well physically (biologically), mentally (psychologically), healthy, socially, and spiritually healthy (spiritual). A healthy human is a human whole that meets the four pillars of health. In the development of personality, man has four dimensions holistically, organo-biological, psycho-educative, socio-cultural, and spiritual. Stated that the spiritual element in humans, integrating and unifying elements of physical, emotional, and intellectual in the human body in its growth and development. Therefore, human health treatment, including the patient with hypertension should meet all four of these dimensions. The above demonstrates the importance of spiritual and religious elements in stress management.

One attempt to get closer to the creator is through dhikr. It has the power of relaxation to reduce tension (stress) and bring peace of mind. From the point of psychiatry, psychiatric therapy known dhikr is one level higher than usual psychotherapy. Dhikr is an attempt to draw closer to God by remembering. Dhikr is not a strange thing, but it is usual thing in every Muslim. Dhikr is more serves as a method of psychotherapy, because more dhikr will make the hearts of peace, quiet and peaceful, and is not easily

affected by the surrounding environment and global culture.^[18]

Based on the results of the study, paired sample t-test on blood pressure pre and post-test in the intervention group obtained $p_{\text{value}} 0.000 < 0.05$ mean that there is a significant effect of the intervention group variable (systole) on blood pressure. While the results of paired sample t-test on blood pressure prepost intervention group obtained $p_{\text{value}} 0.001 < 0.05$ means there is the effect of the intervention group variable (systole) on blood pressure.

Spirituality Conditions patients with hypertension can provide a significant effect on the condition of hypertensive individuals, well spiritual can stabilize blood pressure in patients with hypertension. Suggested that spiritual care affects the spiritual health of the cardiovascular patient. The therapeutic effect of dhikr can lower systolic blood pressure by stimulating the parasympathetic nervous system and secrete endorphins that can reduce sympathetic nervous system. ^{[19],[20],[21],[22]}

Anxiety, fear, pain, and emotional stress result in sympathetic stimulation that increases the frequency of blood, cardiac output, peripheral resistance, and the effect of sympathetic stimulation can increase blood pressure. Stress experienced by a person will arouse the sympathetic nerve that triggers the heart and increase in blood pressure. To find out the results of the comparison test between the intervention group and the control group, the Independent T-test was carried out, found that the p_{value} in the posttest intervention (systole) and control was $0.86 > 0.05$, meaning that there was no significant difference between the control group and the intervention group (systole) on the post-test.

Conclusions

Dhikr and prayer instructions can be a complementary therapy in overcoming mental calm

and blood pressure in the elderly with hypertension. We hope that the results of this study can be used as a reference material in providing therapy to patients with hypertension in order to help lower blood pressure.

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Emotional and Cognitive Impairment among Cardiac Patients - A Narrative Review

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Abstract

Cardiovascular disease is a major group of contributing 31% of global mortality rate. The lifestyle changes are the key role to develop such diseases. The cardiovascular diseases are altering the life style practices and need to change their routine life style. There are many related co morbidities that may arise after the cardiac event. In which the emotional and cognitive co morbidities are making further risk to the subsequent cardiac event. Therefore the correct and prompt treatment is needed for the prevention of the co morbidities. The main aim of this narrative review is to find out the burden of emotional and cognitive impairment and to find out the effect of relaxation technique on the reduction of the emotional and cognitive impairment among cardiac patients. Mainly the online data sources including PubMed, PubMed central, Cochrane, Medline, Google scholar were searched for the related studies. The main emotional imbalance are depression and stress. In which depression is contributing the major role among cardiac patients. The major pathophysiological changes after myocardial infarction that lead to the emotional and cognitive symptoms are due to the reduced blood supply to brain and the anaerobic metabolism. There are different complimentary therapies are there for the management of the emotional co morbidities. In which the relaxation therapy is very useful therapy in the emotional imbalance management.

Key words: *depression, cognitive impairment, stress, Acute Coronary Syndrome, heart failure, myocardial infarction*

Introduction

Cardiovascular disease is mainly focusing on the disease of the heart and the vascular system. In this disease category include coronary artery disease, cerebrovascular disease, rheumatic heart disease, valvular heart disease, peripheral vascular diseases and other related diseases. The death due to this

disease group is making the global mortality rate into 17.9 million every year which is contributing the 31% of global death. Since the burden of the cardiovascular disease is high in which four out of five death is because of the coronary artery disease and cerebrovascular accident. In which the high incidence rate is showing in the age group of less than 70 years. While considering the death rate, it is calculated that the major portion of the death (75%) in the middle and low income countries. The major risk for the development of the cardiovascular disease are modifiable and non-modifiable risk factors. In which smoking, sedentary life style, unhealthy diet, strong

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family history, high blood pressure and lipid level etc. are contributing the major part of the cardiovascular disease risk. [1]

The death due to the coronary heart disease is increasing in developing countries and is decreasing in the developed countries. [2] The important reason behind this hike is due to the rapid changes by urbanization, industrialization and life style changes and is coming under the epidemiological transition. [3] In early 20th century the transition was emerged in developed countries and 50 years later this was affected in the developing countries also. This is the major reason for the difference in the mortality rate between the developed and developing countries. [4] The transition epidemiology mainly developing through the five stages; (a) age of pestilence and famines, here in this stage the major problems facing by the countries are the malnutrition, infectious diseases, infant mortality and childhood mortality. These were contributing approximately 90% of the mortality burden of the country, and the remaining 10 and less than 10% only contributing the cardiovascular diseases. (b) age of receding pandemics, in this stage the mortality from the communicable disease were under controlled and the cardio vascular diseases started to contribute the mortality rate approximately 35%. (c) Age of degenerative and human made disease, in this stage the mortality from the cardiovascular disease are increasing to around 65%. (d) Age of delayed degenerative diseases, by here the cardiovascular related death is decreased but still it was in the rate more than 40% of all the death. (e) Age of inactivity and obesity, here the population are in sedentary life style and lack of exercise makes the population more vulnerable to the cardiovascular disease and the related mortality. In India there could be all the epidemiological transition stages can appear. [5]

In India the coronary artery disease trend is showing among the adult population over 20 years of

age have 2 times more chance to develop the disease in rural area and 6 times more in the urban area. [6] The ischemic heart disease and stroke contributing the major cause of death (83%) in India, which is slightly higher than that of the global level. The years of life lost due to the cardiovascular death is raised to 59% from 1990 to 2010. The cardiac disease burden is 7 fold more in urban than rural. [7]

Methodology

A narrative review based on the scientific research findings used as the methodology in this study. The main study objective is to find out the burden of emotional and cognitive impairment and to find out the effect of relaxation technique on the reduction of the emotional and cognitive impairment among cardiac patients. Mainly the online data sources including Pubmed, Pubmed central, Cochrane, Medline, Google scholar were searched for the related studies. We searched for the keywords depression or cognitive impairment or stress and Acute Coronary Syndrome or heart failure or myocardial infarction for the study review. We searched the article till February 2021. The inclusion criteria includes the articles related to stress and depression among cardiac patients, cognitive impairment among cardiac patients, effect of relaxation therapy among cardiac patients and the exclusion criteria includes the articles related to other emotional among cardiac patients, effect of other complimentary therapies among cardiac patients and the articles published after February 2021.

Epidemiology of Depression after MI

Globally depression and ischemic heart disease are the second and third cause of disability, after HIV/AIDS, and in the first and second position in the developed nations. The depressive symptoms after MI is very common, in which around 16 to 27% showing within two weeks of MI. among them 75% may continue with the depressive symptoms for another 3

months too. [8] Sometimes the symptoms of depression may improve or can exacerbate after that, but still it seemed to be constant for a minimum period of 18 months after MI. [9] There are many study evidence that states that depression and stress after MI may influence the prognosis after MI. A meta-analysis on depression and prognosis of MI among 16,889 patients and they were followed for a period of 18 months. The Odds Ratio of the death due to depression among the patients with depression and without depression is 2.25 (95% CI, 1.73-2.93). Another subpart analysis on the occurrence of new cardiovascular disease, they were selected 18 studies with 10,119 patients. Among them 2247 got another incidence of MI within 2 years. The Odds Ratio of the incidence of new cardiac event among depressed and non-depressed patients was 1.59 (95% CI, 1.37-1.85). [10]

Pathophysiological Mechanism for the occurrence of depression after MI

There is no exact known mechanism for the occurrence of depression after MI. There are different mechanisms have been suggested.

Biological pathway:

Disturbance in the blood clotting mechanism in the body may damage the vascular endothelium of the coronary arteries. That leads to the activation of the immune system in the body and further develops coronary artery thrombosis. It leads to the hyperactivity of hypothalamic-pituitary and adrenocortical axis. Finally there is disturbance in the cardiac autonomic tone and lower the heart rate variability and depressive stage. [11, 12]

Behavior pathway:

The patients after the incidence of Myocardial Infarction may develop depression and few of them may less likely to adhere with the dietary modification, physical activity, adhere to medication

and may increase the smoking rate. These maladaptive behaviors may leads to the further complications and poor prognosis. [13]

Poor prognostic factors

Poor social support, living alone, socially isolated people, seriousness of the cardiac disease [14], and psychological stress at work, negative life events, irritability, and low social activity [15]

Cognitive impairment after MI

The patients with cardiac disease are commonly experiencing the physical, emotional and cognitive impairments. [16] The blood supply to the heart is reducing after the occurrence of MI and leads to the production of Reactive Oxygen Species (ROS). This ROS mainly include the Hydrogen Peroxide, which is harmful to the proteins, lipids and DNA. [17-18] The antioxidant enzymes (catalase (CAT) and superoxide dismutase (SOD)) are there in the body to counteract on the effect of ROS, but these enzymes were reducing after MI. [18]

The MI not only reducing the blood supply to the heart but also reducing the blood supply to the other vital organs also. As a result there is a reduced blood supply to the brain tissue and can progress to the cognitive impairment. [19] The cognitive impairment is relatively present among the patients with congestive cardiac failure. [20] But the patients are treating with the digoxin therapy, and it can improve the cognitive impairment. [21]

Pathophysiological mechanism of occurrence of cognitive impairment after MI

A reduced blood supply after the Myocardial Infarction cause a decreased blood flow to the brain. This may leads to the ROS production, after a long term potentiation there will be an impaired ability for learning by experience and Cell damage in the brain including hippocampus, sensitive to the ischemic

reactions. [22, 23]

Hippocampus is responsible for the cognitive functions, learning and memory. The ROS, especially the hydrogen peroxide may alter the Long Term Potentiation (LTP), which is helpful for learning by experience. The Framingham Heart Study also gives clinically the supporting evidence on the relation between the heart disease and the cognitive impairment. [20] In another MI patients study revealed that there is high in hospital mortality rate in patients with NSTEMI with mild to moderate cognitive impairment (mild- OR: 1.3, 95% CI, 1.2–1.5; moderate/severe- OR: 1.7, 95% CI, 1.4–2.0). [24]

Stress after MI

Myocardial Infarction is a life threatening condition with high prevalence. Therefore it may lead to severe distress in patient's life. The patients are complaining more emotional symptoms than the somatic symptoms. It includes, helplessness, hopelessness, emotional instability, lack of support, stress on the future management etc. These emotional instability may adversely affect the cardiac health and lead to poor prognosis. [25] While assessing related study findings it showed that the perceived stress is common among the adult population. [26-27] The associate factors are the alternations in the working status and personal life, which may negatively make an impact on the adult population when compared to the old population. [25] And also when comparing the perceived stress between the men and women, the stress is more in women than men. [28]

Management of emotional and cognitive impairment

Exercise therapy: the recommended exercise duration is 150 minutes per week which can be brisk walking, or cycling, and 75 minutes of vigorous activity (cycling) with a combination mode. [29]

Cognitive behavior therapy: Relaxation therapy, stress reduction techniques, smoking cessation, acceptance strategy, dietary management, life style modification, medication adherence, regular exercise, yoga and meditation.

Pharmacotherapy: The evidence from Randomized Controlled Trial was conducted on the effectiveness of the selective serotonin reuptake inhibitors. It was found to be effective with less cardiac side effect. The founded side effect are prolonged QT interval and interaction with other medicines. The tricyclic anti-depressants are contraindicated because of the cardiac related side effects. [29]

Supportive education: Tailored Health education on the cardiovascular disease, its risk factors, prevention, management, and secondary prevention.

Relaxation therapy

Relaxation therapy is a widely acceptable alternative therapy for the patients with psychological and cognitive imbalance. It can be range from common head ache to the related disease conditions like cancer or myocardial infarction. Relaxation is defined as “a state in which the person can maintain or attain a steady hypo metabolic level with normal smooth and striate muscle functioning in a pleasant and happy mental state”. The important stages in relaxation therapy include;

a. Effective breathing: it should be deliberate, slow, deep and abdominal breathing

b. Striate muscle relaxation: preparing the striate muscle strongly to respond at the time of fight and flight reaction. This technique is described by Jacobson, and it include the progressive muscle relaxation technique.

c. Autogenic training: in this method the relaxation of the body by mentally than physically. Luthe and Schultz introduced a shortened autogenic

relaxation method including eight mental thoughts for relax the mind.

d. Mental relaxation: here the mental relaxation after the complete physical relaxation of the body. Then the individual supposed to pass through a guided visual imaginary field. And this method can stimulate the sense organs, sight, hearing, smell, touch, and/or taste.

e. Smooth muscle relaxation: the voluntary muscle relaxation is possible by any of the above methods, but it is difficult to control the muscles under the autonomic control. So it needed the visualization or the stimulation through the sense organs that can relax the smooth muscles automatically.

f. Diet and chemical stress: food also may stimulate the stress reaction in our body. Each dietary item have its own role in maintaining the homeostasis. In which the vitamin B rich foods are more helpful in reducing the food related stress.

g. Exercise: along with all the relaxation technique the regular exercise can reduce the stress. [31]

Depression after myocardial infarction is three times more than that of general population. Around 15-20% of the patients with MI is diagnosed with the depression. The depressive symptoms among the MI patients can alter their life style and it can increase the risk of develop an another incidence of MI. [32] The depression is considered as a predictor of mortality in the early phase among the patients with MI. [33]

There are many supporting evidence that the muscle relaxation have an effect on reducing the depression. A meta-analysis on relaxation therapy for rehabilitation and prevention of ischemic cardiac disease by Dixhoorn showed that a group of similar nine study result supported that relaxation technique can reduce the depression and a group

of 13 studies showed that it can reduce the anxiety among the patients. [31] Another quasi experimental study evidence showed that the relaxation therapy is effective in reducing the depression and anxiety. [29] In studies by J A. Collins et al [32] and J A. Blumenthal et al [33] reported that there is no significant change after the relaxation technique among the coronary artery disease patients. A study by Delui et al on effect of the relaxation and meditation among cardiac patients showed that there is significant reduction of anxiety and depression after meditation than that of relaxation technique. [31]

Conclusion

The emotional and cognitive impairment among the cardiac patients have a great role in the reoccurrence of the adverse cardiac events among the cardiac patients. The routine cardiac rehabilitative therapy can reduce the related impairment up to a limit. But the evidences suggests that a patient centered cognitive behavioral therapy and alternative therapies can regulate the emotional and cognitive impairment in the maximum level.

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Molecular Detection of *abaI* Genes in *Acinetobacter baumannii* Isolated from Clinical Specimens in Some Iraqi Hospitals

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Abstract

Acinetobacter baumannii is one of the most important causative bacteria of nosocomial infections. Attention turned toward this Gram negative bacterium due to its extensive resistance to antibiotics. Clinical One hundred thirty five samples sources (urine, sputum, wound burn,) were collected during the period from the between from the late September 2020 to the Mid-January 2021, from patients attending four hospitals in Anbar governorate which include (AlRamadi Genera Teaching ,Al- Fallujah Genera Teaching and Fallujah Maternity and Children's Hospital). Regarding to the age group factor, the age group (40-49) years more susceptible to the infection is constituting 6(46.1%), followed by group(30-39) years with 3(23.1%) while (20-29)(50-59) and (60-69) years with percentage at 1(7.7)%. Also, the study indicated that the *A.baumannii* was disrupted equal in male (53.8%) and female (46.2 %) with appear no significant. All specimens were cultured on culture media including blood agar and MaCconkey agar. After the growth of bacteria, the isolates were identified by microscopic examination as well as the biochemical tests including the manual biochemical tests that include(oxidase, catalase, Simmon Citrate, Motility, Indole, Urease ,haemolysin,Lactose fermentationrowth at 44 °C) .The identification of *P. mirabilis* confirmed by using the VITEK-2 system. A total of 13(9.6%) isolates of *A.baumannii* were identified, other bacteria obtained were identified as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella .pneumoniae* and *streptococcus spp.* in percentage recorded (37%) , (22%) (20.2%) and (11%) respectively. The genomic DNA of *A.baumannii* isolates were extracted using wizard genomic DNA purification kit, the extracted genomic DNA was analyzed using 1% agarose gel electrophoresis, and then the concentration and purity of the extracted genomic DNA were determined using Nanodrop spectrophotometer device, to detect *A.baumannii* isolates by molecular methods, the extracted genomic DNA of these isolates was submitted for amplification to detect the specific gene *Bap* and *abaI* by the singleplex PCR assay. At the molecular level of this study, the results of PCR reaction showed the presence of *abaI* gene in 8 (61.54%) isolates carried the gene responsible for quorum sensing.

Keywords: *abaI* Genes, *Acinetobacter baumannii*, Iraqi Hospitals

Introduction

Acinetobacter baumannii is a Gram negative, non-

lactose fermentative ,and coccobacillus bacterium (1,2). It has been considered a very important pathogen that can cause severe health care-associated infections (HAIs) of the skin and soft tissue, wound infections, urinary tract infections, pneumonia and secondary meningitis. The highest mortality rates, however, are

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seen in ventilator-associated pneumonia (VAP) and bloodstream infections especially in intensive care units (ICUs) (3). This bacterium, also well known for its ability to form biofilms, *Among the several virulence factors it have , the biofilm-associated protein encoded by the bap gene plays an important role in intercellular adhesion, accumulation of bacterial cells, and establishment of biofilm*(4). Also it has a strong environmental adaptability and the characteristics of multi-drug resistance(5). MDR *A. baumannii* strains are resistant to beta-lactams, aminoglycosides, carbapenems and fluoroquinolones. Decreased membrane permeability due to loss of porins, acquisition of extended-spectrum β - lactamase, and multidrug efflux systems are mechanisms claimed for *A. baumannii* multidrug resistance (6). The increasing prevalence of carbapenem-resistant *Acinetobacter baumannii* (CRAB) caused nosocomial infections generate significant comorbidity and can cause death among patients. Current treatment options are limited. These infections pose great difficulties for infection control and clinical treatment . It is the matter of debate globally (7). The development of drug resistant pathogens in patients is increasing day by day. It is the matter of debate globally.

Materials and Methods

Samples collection

During the period of study from beginning September 2020 to October 2020 ,one hundred thirty five specimens (Burns,wound) were taken by disposable cotton swabs, while (urine and sputum) were collected into disposable plastic containers from patients attending four hospitals in Al-Anbar governorate including: (Al-Rumadi Teaching Hospital, Al-Fallujah Teaching Hospital and Fallujah Maternity Hospital and Children's Hospital. Samples were taken from the patients under sterile conditions and immediately transferred to the laboratory to inoculate into brain heart infusion broth for 4-6 hours,

then inoculated on MaCconkey Agar and Blood agar (Hi media, India) at 37°C for 24 hrs then direct exam by Gram stain under light microscope (40x) followed by biochemical tests API20E, VITEK 2 system and molecular methods.

DNA extraction

Genomic DNA was extracted from the *A. baumannii* isolates using a commercial wizard genomic DNA purification kit according to manufacturer's instructions (Promega, USA) with some modification. Briefly, 1 ml of an overnight *A. baumannii* culture grown at 28°C in nutrient broth (Sigma, USA) was transferred to a 1.5 ml micro centrifuge tube. The microcentrifuge tube was centrifuged at 14,000 rpm for 3 minutes to pellet the cells and the supernatant was removed. 600 μ l of nuclei lysis solution (wizard genomic DNA purification kit) was added and gently pipet until the cells is resuspended. The microcentrifuge tube was incubated in water bath at 80°C for 5 minutes to lyse the cells; then cool to room temperature. 3 μ l of RNase solution (wizard genomic DNA purification kit) was added to the cell lysate and the microcentrifuge tube was inverted for 5 times to mix. The microcentrifuge tube was incubated at 37°C for 60 minutes and cool to room temperature. 200 μ l of protein precipitation solution (wizard genomic DNA purification kit) was added to the RNase-treated cell lysate and vortex vigorously at high speed for 20 seconds to mix the protein precipitation solution with the cell lysate. The microcentrifuge tube was incubated on ice for 5 minutes and centrifuged at 14,000 rpm for 5 minutes. The supernatant containing the DNA was transferred to a clean 1.5ml microcentrifuge tube containing 600 μ l of room temperature isopropanol. The microcentrifuge tube was gently mixed by inversion until the thread-like strands of DNA form a visible mass and centrifuged at 14,000 rpm for 5 minutes. The supernatant was carefully pour off and the microcentrifuge tube was drained on clean

absorbent paper. 600 µl of room temperature 70% ethanol was added and then the microcentrifuge tube was gently inverted several times to wash the DNA pellet. The microcentrifuge tube was centrifuged at 14,000 rpm for 2 minutes and the ethanol was carefully aspirated. The microcentrifuge tube tube was drained on clean absorbent paper and the pellet was allowed to air-dry for 15 minutes. 100 µl of DNA rehydration solution (wizard genomic DNA purification kit) was added to the microcentrifuge tube and the DNA was rehydrated by incubating at 65°C for 1 hour. The solution was periodically mixed by gently tapping the microcentrifuge tube and the DNA sample was stored at - 20°C until use.

DNA quantification

The extracted DNA from the *A.baumannii*

isolates was quantified spectrophotometrically at O.D. 260/ 280 nm with ratios 1.4-1.5. The sensitivity of the *A.baumannii* -F and *A.baumannii* -R primers was evaluated by PCR amplification for serial diluted concentrations (10-100 ng) of purified genomic DNA isolated from *A.baumannii*.

Primers selection

The primers for *aadA1* gene of *A.baumannii* as the target gene for this study were selected according to (5). This set of primers was designed based on the conserved region in *A.baumannii*, primers were synthesized by Alpha DNA, Kanda. The primers sequence of *aadA1* gene and their size of product are shown in (Table 1).

Table(1): The primers sequences of *aadA1* gene of *A.baumannii* and their product size

Primer Name	Seq.	Tm	Size of product
<i>abaI</i> -F	5'- GCACGACGACATCATTCGG -3'	58	~400bp
<i>abaI</i> -R	5'- ACCAAATGCGGGACAACG -3'		

Singleplex PCR master mix

The singleplex PCR reaction of *aadA1* gene detection of *A.baumannii* was performed in 25 µl volumes containing 5.5 µl of nuclease free water, 12.5 µl of GoTaq Green Master Mix 2X containing (GoTaq DNA polymerase supplied in 2X Green GoTaq reaction buffer (pH 8.5), 400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dTTP, 3 mM MgCl₂, yellow and blue dyes which function as loading dyes when reaction products are analyzed by agarose gel electrophoresis), 2.5 µl of 20 pmol *P. mirabilis* -F primer and 2.5 µl of 20 pmol *A.baumannii* -R primer and 2 µl of the genomic DNA sample. The mixes were

overlaid with 2 drops of mineral oil Table (2).

Singleplex PCR program

Singleplex PCR was carried out in a thermal cycler (Applied Biosystem, 9902, Singapore) according to the PCR program described by (14), with some modification. Briefly, the amplification of *aadA1* gene of *A.baumannii* was carried out with initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for *A.baumannii* -F and *A.baumannii* -R primers for 30 seconds, and extension at 72°C for 60 sec. The thermal cycles were terminated by a final extension for 3 minutes at 72°C Table (3).

Singleplex PCR products analysis

The analysis of singleplex PCR products of *aadA1* gene of *A.baumannii* were performed on 1% agarose gel. The 100 bp DNA ladder (Promega, USA) was

used and the gel was run at 100 volt for 45 minutes at room temperature. The singleplex PCR products were stained with ethidium bromide and visualized by an image analyzer (ChemiImager 5500, Alpha Innotech, USA).

Table (2): Singleplex PCR master mix to detect the *abaI* gene of *A.baumannii* isolates.

Component	Concentration	Amount (µl)
GoTaq Green Master Mix	2X	12.5
<i>abaI</i> -F primer	10 µM/ µl	1
<i>abaI</i> -R primer	10 µM/ µl	1
Nuclease free water	-	8.5
DNA sample	-	2
Total volume	-	25

Table (3): Singleplex PCR program to detect the *abaI* gene of *A.baumannii* isolates.

No.	Step	Temperature	Time	No. of Cycles
1	Initial denaturation	95°C	5 min.	1
2	Denaturation	95°C	30 sec.	30
3	Annealing	58°C	30 sec.	
4	Extension	72°C	60 sec.	
5	Final extension	72°C	3 min.	1
6	Storage	4°C	∞	-

Results

Conventional methods

The conventional methods include culture, Gram staining and biochemical tests showed positive results in 18(15.2%) out of 118 clinically samples diagnosed with Diabetic foot ulcer infection, on MacConkey agar *A.baumannii* colonies appeared as a pink in color

because of the lactose-fermentation, large size, round, regular edge, mucoid texture due to the presence of the heavy capsule in their outer membrane with large size (1.5-2.5 mm) in diameter (Figure 1), While, on blood agar the colonies appeared as grey-white, mucoid and non-hemolytic colonies. The Gram staining of *A.baumannii* was showed a small straight rods and arranged singly but messily in pairs

under the compound light microscope. The results of biochemical tests were used for further identification of *P. mirabilis* isolates showed positive reactions for indole, catalase, citrate utilization, urease, capsule stain, voges-proskauer (VP), and motility tests. but was negative for Kligler Iron Agar (KIA) test, motility, oxidase, H₂S production.

Analysis of extracted DNA of *A. baumannii* isolates

After performing of the DNA extraction from *A. baumannii* isolates, agarose gel electrophoresis was adopted to confirm the presence and integrity of the extracted DNA using 1 % agarose gel at 7 volt/ cm for 45 minutes (Figure 2).

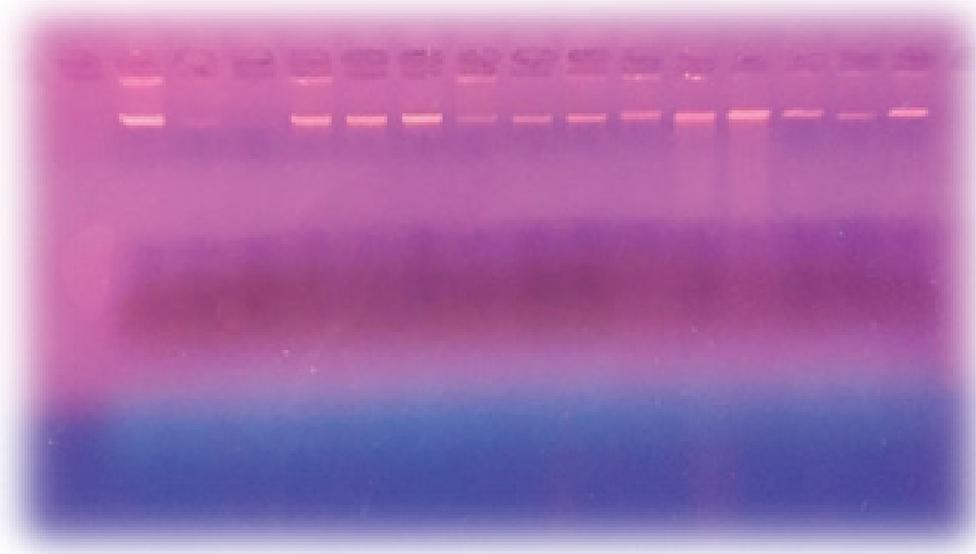
Analysis of singleplex PCR products of *abaI* gene for *A. baumannii*

On the basis of the *abaI* gene sequence, a product of ~300 bp was amplified by singleplex PCR with

A.baumannii -F and *A.baumannii* -R primers. In 50 clinically diagnosed with UTI infection, the singleplex PCR method detected positive results in 18(15.2%) out of 118 samples that were positive by the conventional methods include culture Gram staining and biochemical tests. The singleplex PCR products and 100 bp DNA ladder were resolved by electrophoresis. 5µl of the singleplex PCR product were loaded on 1.5% agarose gel and run at 100 volt/cm for 60 minutes. The gel was stained with ethidium bromide solution (0.5 µg/ ml) for 15-30 minutes; finally, bands were visualized on UV transilluminator at 740 wave length and then photographed by using photo documentation system. The singleplex PCR result was considered positive for *A.baumannii* when there was presence of ~300 bp singleplex PCR product band of *abaI* gene for the *A.baumannii* on the agarose gel electrophoresis, no amplification was observed with negative control (Figure 3).



Figure(1) Colonies morphology of *A.baumannii* culture on MacConkey agar medium at 37°C for 24hrs.



Figure(2) Colonies morphology of *A.baumannii* culture on blood agar medium at 37°C for 24hrs.

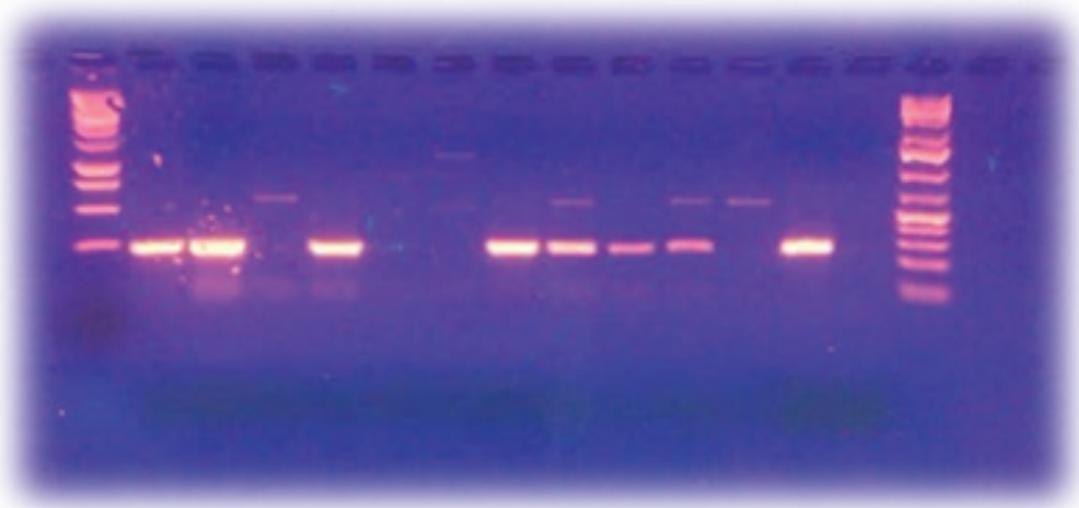


Figure (3): Agarose gel electrophoresis of PCR products for the *aadA1* gene . Lane M: 1500bp DNA ladder; lanes 1,11: positive result with positive bands of 300 bp *A.baumannii* (70V for 2hr).

Discussion

Identification of this bacteria by using the conventional methods include culture, Gram staining and biochemical tests which were go together with study conducted by ⁽⁴⁾. The other bacterial isolates bacterial isolated were *Escherichia coli*, *Pseudomonas .aeruginosa* ,*Klebsiella .pneumoniae*

and *streptococcus spp.* in percentage recorded (37%), (22%) (20.2%) and (11%) respectively..On MacConkey agar , *A.baumannii* colonies appeared as small, round, pale, a pinkish tint colonies and lactose non fermenter, such characteristics came in accordance with guide of practical medical microbiology of ⁽⁸⁾. Also, the results of *A.baumannii*

culture on blood agar appeared as gray, opaque, creamy, non-hemolytic colonies. These results came in accordance with practical medical microbiology of (4). In addition, the result of Gram staining of *A.baumannii* goes together with result of exhibited that in a typical Gram film, the organism appears as a Gram-negative coccobacilli and occasionally arranged in diplococci under the compound light microscope results as agree with (9). The results of biochemical tests were used for further identification of *A.baumannii* isolates showed positive production Catalase test, Citrate utilization test and growth at 44 °C, but was negative for oxidase test, urease test, indole test, hemolysin test and motility test. The results of biochemical tests of current study agree with study conducted by (10). In addition, the automated biochemical tests such as Api-20E and VITEK 2 system identification revealed that 13 of isolates were belonged to the *A.baumannii*. The manual biochemical tests are largely used for bacterial identification in clinical laboratories, the advantages of conventional methods were non costly but the disadvantages of those methods were consuming time, contamination present, false positive result and require a large amount of sample, while the automated biochemical tests such as Api-20E and VITEK 2 system. The API 20E system is faster still time-consuming to set up and read, requires up to 48 h of incubation, and gives results while VITEK 2 system used in many previous studies was detected bacteria faster, efficient and away from the contamination that may prevent detection of the pathogen. In addition confirmation the biochemical tests. In current study, the singleplex PCR method was used for detection of *A.baumannii* by using pair primers targeted the *abaI* gene (~293 bp) showed a positive result in 13 (61.5%) out of 118 samples. The benefits of molecular methods are more sensitive, timesaving, specific, and cost-effective ways for the identification of *A.baumannii*. These explanations made molecular methods relatively more accurate than conventional

methods(9). Conventional studies have highlighted the difficulties in identifying *A.baumannii* strains based on commercial phenotypic identification systems. Identification of *A.baumannii* has long been based on phenotypic and biochemical test, these physiological and biochemical tests were performed on selected isolates using API20E, Vitek 2 system and PCR method(11). This explains that the molecular diagnosis of *A.baumannii* by the singleplex PCR method was more sensitive and efficiency than the diagnosis of these bacteria by conventional methods. The *abaI* gene is a quorum-sensing-regulating gene, encoding an auto inducer synthase. The auto-inducer synthases gene, *abaI*, was present among the isolates that produce QS signaling molecules and a mutation in *abaI* influenced *Acinetobacter* sp. biofilm-forming capabilities(12). This data agrees with the study by (13) who confirms the efficacy of the PCR assay compared to conventional methods of diagnosis in the clinical setting.

Conclusion

Many evidence indicate that *A.baumannii* are based upon inaccurate isolate identification, resulting from inadequate identification conventional methods include culture, Gram staining and biochemical tests that lack the resolution needed to discriminate *A.baumannii* isolates, on the other hand, *aadAI* gene appeared to be useful genetic marker for determination of *A.baumannii* and singleplex PCR using species-specific primers could be represented rapid, sensitive and specific molecular method for detection of this bacteria in different human infections.

Conflict of Interest: None

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Ethical Clearance: Not required

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Wild Strawberry *Fragaria vesca* L. extracts Hepatoprotective Activities Against Paracetamol-Induced Hepatotoxicity in Male Wistar Rats

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Abstract

Background: Administration of high-dose paracetamol had been indicated to induce several molecular and biochemical cascades of hepatotoxicity. The abundant amount of ROS formation following the uptake of the medication exceeds the physiologic antioxidant capacity of the body, leading to the occurrence of oxidative stress. Wild strawberries (*Fragaria vesca* L) extracts are thought to have hepatoprotective activities regarding the high activity of antioxidants contained.

Method: Aspartate Transaminase (AST) activity was used as a marker of hepatotoxicity. In the 10 days trial, randomized samples of 24 male Wistar rats (*Rattus Norvegicus*) were used and divided into three groups: 1 group without treatment, 1 group receiving oral paracetamol 1750 mg/kg in CMC-Na suspension, and 1 group receiving oral paracetamol 1750 mg/kg following administration of strawberry extract 400 mg/kg. The strawberry extract was administered from day 1 to 10, while high-dose paracetamol was administered on day 9. AST activity analysis was done on day 10 of the trial. Significant results were found showing an increased AST activity in the paracetamol-only group ($p=0.001$) and a decreased AST activity in the group receiving strawberry extract ($p=0.001$).

Conclusion: *Fragaria vesca* L extracts were proven *in vivo* to have hepatoprotective activities against paracetamol-induced hepatotoxicity.

Keywords: *Fragaria vesca* L, paracetamol, liver, AST, SGOT.

Introduction

Increasing trends in self-medication had reached a quite worth-considering activity. A recent study showed a prevalence of 89.6% of self-medication

among the locals¹. Over-the-counter (OTC) medication purchases were reported to be the most source to obtain drugs for self-medication, with NSAIDs being the most purchased medication. Fever was reported to take a major part as symptoms triggering this medication habits². This unrestricted access to medications leading to bizarre doses, frequencies, and durations in drug usage; a habitual use that commonly taken place when symptoms persisted but there was a lack of capability to afford some medical helps³.

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Paracetamol is one of the most frequently prescribed and purchased antipyretic agents. Paracetamol (N-acetyl-p-aminophenol, Acetaminophen) is a para-aminophenol derivative that served as a non-steroidal analgesic and antipyretic agent. Although it appeared that Paracetamol seems like a non-harmful medication with a broad range of therapeutic doses and a minimum number of adverse effects reported, Paracetamol is often associated with hepatotoxicity and acute liver failure, particularly in high-doses and frequent unintentional administrations, or in simultaneous ingestion with precipitating substances such as metoclopramide, anticholinergics, and several CNS (central nervous system) medications, which increased the absorption or decreased the rate of metabolism of Paracetamol⁴.

The adverse effect of hepatotoxicity of Paracetamol is thought to occur mainly through two mechanisms of molecular interactions, covalent and non-covalent. Covalent bonds between NAPQI and hepatocytes' proteins were established due to lack of the supposed binder cytosol GSH following administration of high-dose Paracetamol, leading to destructions of the hepatocytes. Non-covalent interaction, on the other hand, involving formations of free radicals such as N-Acetyl-p-semiquinones (NAPSQI), triggers of reactive oxygen substances (ROS) and superoxide anions, and disruption in Ca²⁺ homeostasis. All those cascades ended up with an occurrence of oxidative stress and hepatocytes destructions⁵.

Many chemical substances had been suggested to have hepatoprotective activities. Extracts of wild strawberries, *Fragaria vesca L*, were found to contain substantial amounts of antioxidants that are hoped to have the ability to neutralize the over-accumulation of free radicals⁶. It is a family of *Rosacea* plants and is a vastly cultivated agricultural plant. It has a high ability to adapt to various geographic and climate settings that it could be accessed without further difficulties⁷.

The extract of these wild strawberries was reported to have a high activity of ascorbic acid, ellagic acid, and several forms of flavonoid, including anthocyanin, catechin, quercetin, and kaempferol⁷. Qualitative analysis of phytochemical substances in *Fragaria vesca L* showed accumulation of phenols, flavonoids, anthocyanin, and terpenoids. These substances were suggested to have antioxidant activities particularly through *in silico* dockings. Maximum potencies were reported to be achieved via oral or rectal administration with antioxidant properties 2 to 11 times more potent than those in apples, peaches, pears, grapes, tomatoes, or even oranges⁸.

With the basic pathophysiology of hepatotoxicity being a depletion of physiologic antioxidant capacity and an over-exceeding amount of free radicals, *Fragaria vesca L* with its antioxidant properties could be an organic reversal of the cascades. Thus, this study aims to analyze the significance of *Fragaria vesca L* hepatoprotective activities against Paracetamol-induced hepatotoxicity.

Materials and Methods

Study design

Measurable and controllable results in this study could be obtained by conducting true experimental research in a laboratory setting with controlled samples and treatments. The design used was the Randomized, post-only control group design.

Sampling method

Simple random sampling was used to randomized the result probabilities in each study animal both in the control group and the treatment group. The sample size was determined using statistical consideration.

Paracetamol-induced hepatotoxicity

Paracetamol was administered in adjusted human doses. The daily therapeutic dose of 15 to 20 g per

day of Paracetamol for humans weighed about 70 kg was adjusted for rats weighed about 200 g⁹. The adjustment dose of 1750 mg/kg was administered to the second (II) and third (III) sample groups.

***Fragaria vesca L* extract**

Fresh fruits of wild strawberries *Fragaria vesca L* were obtained from the Argo Wisata plantation in Malang, Indonesia. Initial preparation was done. The fruits were rinsed with clean tap water, dabbed with a soft cloth, leaves-removed, then cut into small fragments. The cut was then dried in a 45° Celsius degree oven for 2 to 3 days and ground into powder. 10 g of *Fragaria vesca L* powder was dissolved in 100 ml 70% Ethanol (1:10 ratio) then continuously stirred for 6 hours and left in for a night. The solvent was filtrated then evaporated using the vacuum rotatory evaporator to remove the ethanol. Extracts of *Fragaria vesca L* were obtained through an extraction process using a suspension of 1:50 70% Ethanol in 0.5% CMC-Na. The extract was administered to the third (III) sample group for 10 days in 200 mg/kg daily doses.

Animals

24 male Wistar rats (*Rattus norvegicus*) aged 10 to 12 weeks and weighted 150 to 200 g were included. All ethical considerations had been cleared and approved by the Ethics Committee. The selection of healthy rats was done. Rats with shiny-coated hair, active movements, and no scar, was cared for and adapted in the Biochemical Laboratory, Faculty of Medicine, Hang Tuah University, Surabaya, Indonesia. Standard laboratory condition was maintained at 23±20 Celsius degree temperature, 45 to 55±10% humidity, and 12 by 12 light and dark cycles. The samples were then divided into three groups as followed.

(I) Negative control group: the samples were given filtrated water via NGT for 10 days and CMC-Na 0.5% on day 8. CMC-Na 0.5% was given 2 hours

after administration of filtrated water on the same day.

(II) Positive control group: the samples were given filtrated water for 10 days and oral Paracetamol with 1750 mg/kg doses on day 9. Paracetamol was given 2 hours after administration of filtrated water on the same day.

(III) Treatment group: the samples were given *Fragaria vesca L* extract in 200 mg/kg doses for 10 days and oral Paracetamol with 1750 mg/kg doses on day 9. Paracetamol was given 2 hours after administration of *Fragaria vesca L* extract on the same day.

All three groups were given euthanasic anesthesia on day 11 of the trial using ketamine hydrochloride 40-60 mg/kg through intramuscular injection on femoral quadriceps or triceps.

Hepatotoxicity biochemical marker

Aspartate transaminase (AST) activity is extensively used as an indicator of liver injuries. It is a glycolysis and electron transport facilitator, NAD⁺/NADH balancing enzyme, in cellular metabolism, particularly in malate-aspartate shuttle where cytosol-NADH are oxidized and mitochondrial NAD⁺ are reduced. The early release of AST in liver injuries and the later release of the mitochondrial isoform made this enzyme suitable as a hepatotoxicity marker, respectively to determine the onset of the injury and the extent of liver necrosis¹⁰. The early appearance of AST following a liver injury, even before any presence of clinical signs or symptoms, added the superiority of AST as a marker¹¹.

Liver cell destruction in hepatotoxicity raised a specific pattern concordant to the vasculature flows. The injury would mainly occur in the 3 central zones of the hepatic acinus which receive the least oxygen supply from the triad portal. In the benefits of using AST as a marker, these areas are happened

to have the highest AST activity. It makes any hepatocytes destruction due to toxicities would be able to be sensitively screened¹². Serum AST activity was observed using the Cobra Integra method then analyzed through photometric measurements using 340 nm wavelength light with results in U/L unit.

Statistical Analysis

A descriptive analysis on the activity of serum AST activity was done. Mean and standard deviation was calculated. The significance of the results was analyzed using one-way Analysis of Variance (one-way ANOVA) followed by post-hoc Least Significance Difference (LSD) for normally distributed data. Kruskal Wallis analysis was done for non-normal distributed data. The value of $p < 0.05$ is considered significant.

Results

Descriptive analysis of serum AST showed a mean level of 187.23 ± 33.69 U/L (Table 1) in the group given only 0.5% CMC-Na, served as a negative control group (I). In accordance with the hypothesis, the activity of the enzyme was increased in the group given only Paracetamol (II). There was a significant reduction of serum AST activity from 269.48 ± 45.52 in the group given only high-dose Paracetamol (II) to 185.69 ± 21.27 in the group given *Fragaria vesca* L extract (III). The activity in the intervention group (III) was found even lesser compared to the group without Paracetamol induction (I).

Table 1. Mean and Standard Deviation of Serum AST Between Groups.

Group	Mean \pm SD (U/L)
Ia	187.23 ± 33.69
IIb	269.48 ± 45.52
IIIc	185.69 ± 21.27

^astudy group without treatment; ^bstudy group receiving high-dose Paracetamol; ^cstudy group

receiving high-dose Paracetamol and *Fragaria vesca* L extract; SD = standard deviation

A normality test was done to ensure that all data in each group were normally distributed before determining the significance of the results. Shapiro-Wilk test was chosen as the normality test method due to the limited size of the sample in each group (Table 2). All data in each group were found normally distributed, except for the positive control group (II).

Table 2: Normality Test of Serum AST Distribution in Each Group.

Group	p-value	Interpretation
Ia	0.453	Normal distribution
IIb	0.039	Non-normal distribution
IIIc	0.939	Normal distribution

^astudy group without treatment; ^bstudy group receiving high-dose Paracetamol; ^cstudy group receiving high-dose Paracetamol and *Fragaria vesca* L extract; p -value > 0.05 considered normally distributed

Kruskal-Wallis test was then chosen as the significance test due to the presence of non-normal distribution data. A significant result was obtained ($\chi^2 = 15.440$, $df = 2$, $p = 0.001$) showing that there were significant differences in the serum AST activity between the study groups. A further significance test was done using a post-hoc analysis, the Mann-Whitney test, to locate specifically the differences between the study groups (Table 3). It appeared that a significant difference was found between the group without Paracetamol induction (I) and the group given Paracetamol (II) [$p = 0.001$]. A significant difference was also found between the group given only high-dose Paracetamol (II) and the group given *F. vesca* L extract following Paracetamol induction (III) [$p = 0.001$]. Serum AST activity in subjects receiving *Fragaria vesca* L extract (III) interestingly showed

no significant difference compared to the negative control group (I) [$p = 0.674$].

Table 3: Mann-Whitney Test of Serum AST Between Groups.

Group	I ^a	II ^b	III ^c
I ^a		0.001	0.674
II ^b			0.001
III ^c			

^astudy group without treatment; ^bstudy group receiving high-dose Paracetamol; ^cstudy group receiving high-dose Paracetamol and *Fragaria vesca L* extract

Discussion

The significant increase in the serum AST activity between the group without intervention (I) and the group given only Paracetamol (II) showed that the Paracetamol-induced hepatotoxicity had been successfully recreated in the Wistar rats in this study. The presence of additional reaction in the liver metabolism, particularly due to Acetaminophen, would positively correlate to an increase of the serum AST activity¹⁴. The massive elevation, nearly 1.5 times higher, had also proven that there was a presence of liver damage or necrosis to the extent of liver injuries. It has been demonstrated in several studies that administration of Paracetamol particularly in high doses would cause drug-induced hepatotoxicity in a form of liver damage or necrosis¹¹.

High-dose Paracetamol would saturate the glucuronidation and sulfation pathway of liver metabolism, thus increasing the utilization of the Cytochrome P-450 oxidation pathway. The oxidation pathway would produce destructing end-products including free radicals and toxic metabolites as NAPQI (N-acetyl-p-benzoquinone imine). Supposedly, these

NAPQIs would have been bound by the GSH enzyme (glutathione sulfhydryl) and turned into hydrophilic cysteine and mercapturic metabolites. Yet in a high dose administration of Paracetamol, the slow nature of GSH production along with increased NAPQIs formation would both exhaust the endogenous enzyme. These toxic metabolites would accumulate in hepatocytes, underwent nucleophilic reactions with the cells' macromolecules such as protein, leading to necrosis of the cells^{14,15}.

On the other hand, the significant reduction of serum AST activity from the group given only high-dose Paracetamol to the group given *Fragaria vesca L* extract following Paracetamol-induction supported our hypothesis that administration of the extract could neutralize the hepatotoxicity that occurred due to administration of a high-dose Paracetamol. Escalation of serum AST activity indicated a presence of cellular leakage and functional disintegration of the hepatocyte membranes⁹. Thus, this reduction of serum AST activity might occur due to the ability of *Fragaria vesca L* extract to stabilize the cell membrane of hepatocytes following the injury which prevented any leakages of cytosol enzymes¹⁶.

Anthocyanin, particularly the pelargonidin-3-glycoside contained in *Fragaria vesca L*, is thought to be the main substance that provides the antioxidant capacity of the extract. The work might center on preventing the free radicals from oxidizing the macromolecules of the hepatocytes. These findings are in accordance with the suggested pathophysiology of Paracetamol-induced hepatotoxicity. These exogenous antioxidants would assist the GSH enzyme in binding NAPQIs preventing the exhaustion of the endogenous antioxidants⁷.

Furthermore, there was no significant difference in serum AST activity between subjects receiving *Fragaria vesca L* extract (III) and subjects without intervention (I). Through this finding, we can roughly

infer that there was a nearly complete reversal of the hepatic condition from Paracetamol-induced hepatotoxicity, including hepatocytes restoration and hepatic regeneration¹⁶, following the administration of *Fragaria vesca L* extract.

Fragaria vesca L extracts hepatoprotective and antioxidant capacities in reversing the Paracetamol-induced hepatotoxicity had been proven. Nonetheless, it still should be noted that hepatotoxicity is a complex process and may occur through different pathophysiology. Thus, we suggest conduction of further studies in discovering other hepatoprotective pathways and agents.

Certain boundaries had given several limitations in this study to observe the hepatoprotective activities of *Fragaria vesca L* extract. Administration of various doses of the extract would give us a better view of the optimum amount and frequency that should be given to evoke the antioxidant properties. Multiple administration of Paracetamol with various doses, rather than fixed-dose single administration, would give us the ability to explore the maximum antioxidant capacity of the extract. Analysis of more hepatic markers should also provide a more holistic evaluation. Regarding several limits that we'd foreseen, we hope that this study could be a help in acknowledging the significance of *Fragaria vesca L* as a hepatoprotective agent.

Conclusion

Fragaria vesca L fruit extract is a promising alternative as a hepatoprotective agent particularly in reversing the hepatotoxicity following an administration of high-dose Paracetamol.

Ethical Clearance: All ethical consideration had been cleared and approved by the Ethics Committee of Hang Tuah University.

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Conflict of Interest: None should be declared.

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Successful Treatment with Rectal tube Placement and Barium Enema in Uncomplicated Acute Sigmoid Volvulus: A Case Report

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Abstract

Sigmoid volvulus is a condition in which sigmoid colon wraps around itself and its own mesentery, which then causes a closed-loop obstruction. This condition accounts for 50-90% cases of twisted large intestine and often affects adults and are commonly found in males. The classic triad signs and symptoms are abdominal pain, distention, and constipation. Although the emergency resection seems to be the right procedure in treating acute sigmoid volvulus, there is still non-surgical treatment often done in several conditions. We reported a 52-years-old male with uncomplicated acute sigmoid volvulus who underwent non-surgical treatment due to his refusal of surgical therapy. Non-surgical treatment was successfully done and no recurrence found within one year. The aim of this report was to emphasize that non-surgical therapy only could be successfully done without surgical treatment.

Keywords: Sigmoid Volvulus, Non-Operative Management Of Volvulus, Conservative Treatment

Introduction

Acute sigmoid volvulus (SV) occurs when there is an obstruction due to the torsion of the colon around itself, including its mesentery¹. It may cause ischemia and leads to gangrene, or even perforation². SV accounts for more than half (60-75%) of total cases as the most susceptible part of colonic volvulus². Some predisposition factors considered are old age, dietary of high fibre, prolonged constipation, anatomical factors, living in the highland, diabetes, and neurological disease³.

Surgical treatment is needed for unsuccessful non-surgical treatment or recurrent volvulus, peritonitis,

and perforation⁴. Whereas for uncomplicated SV, an early non-surgical detorsion followed by elective sigmoid resection is preferable⁴. However, high recurrent rates (43-75%) were observed in patients who undergo non-surgical treatment without followed by surgical intervention². Those who underwent surgery after a recurrence of SV had a greater mortality rate than patients who underwent elective surgery after their initial volvulus episode⁴.

We report a case of a 52-years-old male who suffered from uncomplicated acute SV who was treated non-surgically using rectal tube insertion. We intend to report our experience in managing SV without surgical intervention which was successfully done with no recurrence within one year.

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Case

We present a 52-years-old man who came to the emergency department Dr. Soetomo Hospital on January 13, 2019 with ten days history of abdominal distention and intermittent abdominal pain all over his abdomen. He felt nausea and vomit 30 minutes right after eating. Five days prior, he had constipation with no fevers. He couldn't flatus one day before his admission to hospital. Within the last three years, he has been suffering from mild stroke, hypertension, and diabetes. In the past six months, he tended to depressed because of the economic problem.

Patient was hemodynamically stable. The physical examination revealed an increase of bowel sounds, abdominal distention with tympanic percussion, and tenderness while liver dullness was missing. There

was approximately 7 cm soft and painless palpable mass on the left lower quadrant, no abnormality in Rectal Toucher examination. We found a wound at the plantar foot that has existed for two months and made him spent more time in bed.

His complete blood count, liver and renal function tests were within the normal limit. Blood glucose level showed an increment to 323 g/dl and the HbA1c was 9,9%. Meanwhile, other laboratories values did not show any change.

Abdominal X-ray (Fig. 1) showed a dilated intestinal gas shadow ('coffee bean shaped') projected as high as VL 2 to sacrum ostium, mixed with bulging fecal material. An elongated pathological step ladder image was found. There was no free air outside the intestinal contour.

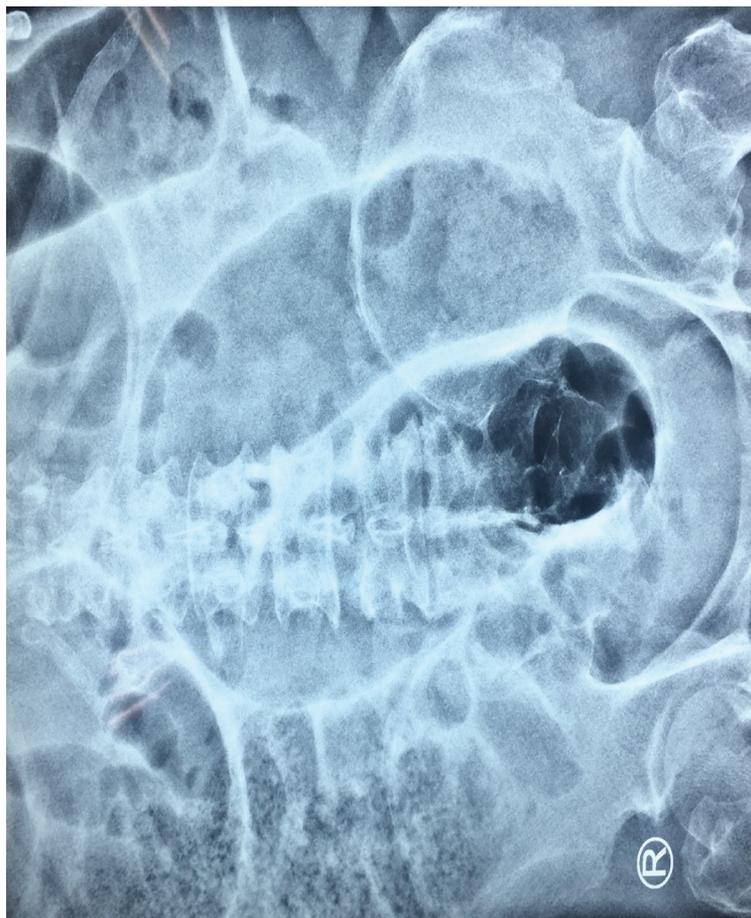


Figure 1. Abdominal X-ray of the Patient (coffee bean shaped)

Patient was instructed to fast while undergoing a nasogastric tube insertion. Rectal tube insertion was performed slowly to release the SV because surgical treatment was refused. Therefore, His general state improved and the abdomen was decompressed. He was evaluated each hour for signs of perforation. The X-Ray evaluation was done after six hours of rectal tube insertion (Fig. 2). The fecal material was

still bulging but no coffee bean-shaped image was obtained. Based on the evaluation results of rectal tube insertion, the SV was released. The rectal tube was retained for the next two days to prevent the recurrence of volvulus while waiting for the Colon in Loop procedure to be prepared. Furthermore, Digestive Surgery Department suggested to perform sigmoidectomy surgical procedure, but the patient still refused.



Figure 2. Abdominal X-ray evaluation of the Patient after rectal tube insertion.

Due to the bulging fecal material, the patient was given 100 ml glycerin enema every 8-12 hours and oral macrogol solution before performing the Colon in Loop. After that, Colon in Loop examination was done with barium enema using water-soluble contrast for diagnostic and reduction therapy procedures. The contrast appeared to fill almost all parts of the colon. However, there was a narrowing of sigmoid colon lumen, forming a 7.5 cm napkin ring sign (Fig. 3). There was no visible filling defect nor contrast

leakage. Thereafter, the tube was removed. The patient was evaluated for the next two days. Glycerin enema was given once in 24 hours. It shaped and softened the fecal consistency. Furthermore, the patient had no more complaints nor recurrence of volvulus event. The patient was discharged with an uneventful outcome from the hospital on the 5th day of treatment. He was prescribed a glycerin enema, used if the patient could not defecate for two days.

After one week discharged, the patient could defecate normally without any complaints. There were no distended stomach, abdominal pain, nor abnormal bowel sounds discovered. Based on the evaluation of the disease, it concluded that there was no recurrent

volvulus. The patient routinely had medical follow-up once a month for a year. Furthermore, there was no complaint from the patient. Any further supervision was not performed due to the refusal from the patient and family for further examination.



Figure 3. Colon in Loop examination of the Patient (napkin ring sign).

Discussion

SV is the common site of colonic volvulus obstruction, which accounts for more than half of the total cases². It particularly occurred in man with elderly age but could be suffered by anyone in any age. Besides old age, some predisposition factors are varied, such as anatomical factors, dietary of high fibre, prolonged constipation, living in the high

altitude, diabetes, and neurological disease². In this case, the predisposing factors were old age, high fibre diet (agar), chronic constipation (immobilization due to diabetic food and depression).

Nowadays, the diagnosis of SV often done very quickly by doing some imaging examinations⁵. The plain abdominal X-Ray is often done to diagnose the SV in the emergency room. It shows a “coffee bean-

shaped” which represents the dilatation of the colon due to the torsion loop of the sigmoid colon³. For more accurate results, an abdominal CT scan could be performed in several conditions. The bird beak appearance and the whirl sign could be represented as the torsion of the pedicle colon³.

We performed an abdominal X-ray and there was a ‘coffee bean shaped’ image projected as high as VL 2 to sacrum ostium, mixed with bulging fecal material. It is highly pathognomonic picture of SV. An elongated pathological step ladder image was found with no free air outside the intestinal contour suggesting no free air in the abdominal cavity. Besides, a Colon in Loop examination using Barium Enema was also done because surgical therapy was refused by patient. There was no visible filling defect, indicate there was no perforation. This patient was in the stable condition with no complication detected.

The goals of the SV decompression are intended to resolve the obstruction and to prevent a recurrence. There are surgical and non-surgical treatment¹. Unstable patients with ischemia, peritonitis, perforation, or recurrent unsuccessful non-surgical treatment are the suitable conditions for urgent surgical treatment, while the non-surgical treatment done in the stable patients⁴.

The mortality and morbidity of uncomplicated patients that following surgical intervention were low, 0% and 12% respectively². Therefore, surgical treatment is frequently suggested in SV patients during the initial admission or as an elective treatment⁴. One of the surgical techniques is the Hartmann procedure. It is suggested for patients with non-viable or perforated colon².

Non-surgical treatment could be done in uncomplicated patients. In patients with viable colon, the endoscopy procedure is the first-line therapy for sigmoid colon detorsion³. The effectiveness could

achieve approximately 60-95% rates². Even if the success rate is high, the recurrence after endoscopy detorsion is up to 90%³. The SV often recurrent in more than half patients after three months from the prior treatment and almost in the first year³. Unfortunately, the mortality rate in recurrent SV is also higher than patients who had an elective surgery thereafter². Therefore, subsequent elective surgery after initial decompression is needed.

This patient was categorized as an uncomplicated acute SV. In this case, endoscopy detorsion and elective surgery were suggested, considering that the patient was in stable condition: no signs of peritonitis, ischemia, nor perforation. Nevertheless, the patient and family refused the colonoscopy procedure and surgical therapy due to the mentality problem and cost. Therefore, the Colon in Loop as reduction therapy procedures and the rectal tube insertion was chosen to be performed to release the SV. Overall, his general state improved and the abdomen was decompressed due to the elimination of gas and faeces through the tube. If the non-surgical procedure was not effective, urgent surgery would be necessary. In this case, the patient has not presented any recurrence after one year of follow-up and is under observation.

Conclusion

To identify SV, a clinical examination and simple abdominal radiographic findings are frequently performed. Early diagnosis and therapy are critical for the disease’s prognosis. Although surgery is recommended, there is still a chance that the patient will decline surgical treatment for several reasons. This report included an instance in which the patient declined surgery or even non-surgery method. As a result, the Colon in Loop and rectal tube were chosen and were successfully completed with no recurrence discovered within one year in this patient.

Ethical Clearance- Nil

Source of Funding- Nil

Conflict of Interest - Nil

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The Contribution of Family Cohesion And Self-Efficacy on the Mental Health of Older Adults: A Survey Conducted in Aceh, Indonesia

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Abstract

Background: Mental illness is recognized as a critical global issue and is a critical aspect of health problems, especially in older adults. Many studies analyze the correlation between self-efficacy and mental health. Still, studies that analyze the correlation between family cohesion and mental health among older adults in Aceh remain limited.

Methods: This study employed a survey method. The survey was carried out on older adults aged 60 years or more by applying a cross-sectional design with the number of samples was 483. At the same time, the data collection technique was done for four months in 2019. A logistic regression test was conducted for statistical analysis to answer the research hypothesis.

Results: The finding of this study proved that family cohesion ($OR > 3, p < 0.001$) and self-efficacy ($OR > 2, p < 0.001$) were correlated with the mental health of older adults.

Conclusions: The researchers conclude that families with good cohesion and older adults with high self-efficacy can support and improve the mental health of older adults in Aceh.

Keywords: Family Cohesion, Mental Health, Self-Efficacy, Older Adults.

Introduction

Nowadays, mental illness is recognized as one of the major issues in the world. It is considered a critical aspect of health problems, especially in the elderly population¹. Overall, health illness is also considered the leading factor for disease burden, disability, and welfare^{2,3}. There are worse effects experienced by people with mental health problems than the general population. For instance, people who suffer from clinical depression and schizophrenia have a 40 to

60% risk of death premature⁴. At the same time, the impact of mental illness on social and economic conditions is poverty^{5,6}. In addition, mental illness also results in loss of productive time of the sufferers and their family members.

The prevalence of people with depression in the world is estimated at 264 million or more⁷. In Indonesia, based on Basic Health Research (Riskesdas) in 2018, the prevalence of people with depression in the age group of 55 to 64 years is 6,5%, age of 65 to 74 years is 8,0%, and age above 74 years is 8,9%. Whereas, in Aceh Province, the prevalence of people who suffer from depression is 6,16 in the age group of 55 to 64 years, 7,40% in the age group of

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65 to 74 years, and 7,82% in the age above 75 years⁸. Basic Health Research indicates that the older age group has a higher risk of experiencing mental issues than the younger age group.

There are many reasons why the elderly are at higher risk of mental health problems, including illness, isolation, lack of self-reliance, weakness, and separation¹. WHO (World Health Organization) explain that in addition to personal factors, mental health is also determined by social, economic, political, and environmental⁴. The results of previous studies showed that self-efficacy is one of the individual attributes associated with mental health. Furthermore, family cohesion which is considered a social factor also correlates with mental health.

Self-efficacy is defined as people's beliefs about their capabilities to produce designated levels of performance that exercise influence over events that affect their lives⁹. Emotion, thought, motivation, and behavior or action can be explained and predicted through self-efficacy^{10,11}. People with high self-efficacy can deal with various obstacles and work harder to achieve the desired condition, including realizing their behavior and health. On the other hand, family cohesion is considered a positive element in the family because it refers to the emotional bonding among family members. Also, cohesion is an essential dimension for understanding family function¹² and interactions among family member¹³. When a family has a well-balanced level of cohesion, family members are free to express their differences, feel more safe and secure in their family.

Therefore, this study aims to assess the correlation between family cohesion and self-efficacy with the mental health of older adults in Aceh Province, Indonesia. Besides, the research hypothesis is revealed that there is a contribution of family cohesion and self-efficacy towards the improvement and maintenance of older adults' mental health from both variables.

Materials and Methods

Design and sample

By limiting the population in the age group of 60 years or more, data collection was carried out through a survey with a cross-sectional design approach. Additionally, this study employed the rule of thumb formula to determine the sample size. The result of multiplying 43 indicators (observed variables) with 5-10 found the minimum sample size was 215-430 respondents. Further, the researchers determined that the sample size in this study was 500, yet 17 questionnaires did not have complete data, so they were not included in the analysis. Thus, the research samples in the statistical analysis were 483.

Variable measurement

The independent variables in this research were family cohesion and self-efficacy, whereas the dependent variable was mental health. The assessment of family cohesion was done by using Likert scales with four answer choices, include every rarely; rarely; often and very often. Whilst, there were seven questions regarding family cohesion (Cronbach's alpha = 0,88) namely: my family members enjoy spending free time with each other; family togetherness is a very precious time for my family; my family members feel very close to each other; my family members have a sense of loyalty to each other; my family members support each other; my family members participate together if there is a family event; decision making for the family interest is done together in my family. Whereas, the assessment of self-efficacy used eight questions (Cronbach's alpha = 0,85), as follows: I believe I can maintain my physical activities properly; I believe I can manage myself to eat healthy foods; I believe I can manage myself not to smoke; I believe I can manage myself to maintain a healthy sleep pattern; I believe I can take care of my self without the help of others; I believe I can manage myself to do activities

based on my hobbies in my leisure time; I believe I can manage my self to control emotion; I believe I can manage my self to maintain religious activities.

Furthermore, the measurement use the Likert scale that was carried out with four answer choices: strongly disagree; disagree; agree and strongly agree. Besides, mental health was measured using the geriatric depression scale (GDS) developed by Yesavage and Sheikh¹⁴. The 15 questions developed by them have been translated into Indonesian with

dichotomous choice questions (Yes or No). This GDS is recommended by the Ministry of Health of the Republic of Indonesia to be used as an instrument to assess the mental health conditions of older adults¹⁵.

Statistical Analysis

The descriptive analysis was carried out to determine the characteristics of respondents. Whilst, the correlation between family cohesion and self-efficacy with mental health was analyzed by logistic regression test with the help of SPSS version 21.

Results

Table 1. Demography Characteristics of Respondents

Respondents Characteristics (n=483)	N	Mean/%	SD
Age		69.76	8.41
Age 60-69	273	56.5	
Age ≥ 70	210	43.5	
Gender			
Male	150	31.1	
Women	333	68.9	
Employment			
Not Working	281	58.2	
Farmer/ Laborer/Fisher	133	27.5	
Pensionary	24	5.0	
Private Employee	45	9.3	
Income		520559.01	856194.59
No Income	198	41.0	
IDR < 1,000,000,-	184	38.1	
IDR 1,000,000,- to <2,000,000,-	50	12.4	
IDR ≥ 2,000,000,-	41	8.5	

The previous table (Table 1) shows the overview of respondents' demographic characteristics, which indicates that the average age of the respondents is 69,76, and the age group of 60 to 69 years is higher

(56,5%) than the age group of 70 years or more. Meanwhile, the number of female respondents is higher (68,9%) compared to male respondents. Based on the employment, it is found that unemployment

(not working) respondents are higher (58,2%) than those who work as farmer/laborer/fisher; pensionary; and private employee. In addition, from the aspect of

income, it is found that the numbers of respondents who have no income are higher (41,0%) compared to those who have income for IDR <1,000,000,-; IDR 1,000,000,- to < 2,000,000,-; and IDR ≥ 2,000,000,-.

Table 2. Logistic Regression Analysis

Variables	B	SE	Wald	p-value	Adj OR	95%CI	
						Lower	Upper
Family Cohesion	1.193	.224	28.302	<0.001*	3.299	2.125	5.120
Self efficacy	.839	.216	15.106	<0.001*	2.314	1.516	3.534
Constant	-2.525	.363	12.764	<0.001	.080		

Abbreviations: B = Beta coefficient, SE = Standard Error, CI = Confidence Interval, Adj OR = Adjusted odds ratio, * = significant p-value

The result of logistic regression analysis, as shown in Table 2, denotes that family cohesion ($p < 0,001$ and OR = 3.299) and self-efficacy ($p < 0,001$ and OR = 2.314) have a positive and significant correlation with mental health.

Discussion

A strong family bond has the potential to protect its members from the impact of life and health crisis¹⁶. There are many indications of family cohesion, namely strong emotional bond and closeness, support, care, and affection among family members^{17,18,19,20}. Moreover, the emotional connection and affectionate of each family member that is well-established create a relaxed, comfortable, and harmonious atmosphere in the family. Good cohesion in the family represents the quality of emotional bond among family members is good. It is considered as the primary protector of someone’s mental health²¹, involving older adults.

On the contrary, conflict among family members can increase stress and anxiety levels in older

adults since it can hinder the support that family members give to them, which is potentially bad for their mental health²². Family failure to resolve conflict among family members is considered a strong predictor of death and disease progression, especially in older adults with depression²³. The finding of this study discloses that family cohesion has a positive and significant correlation with mental health. The elderly who live in a family with better cohesion have better mental health than older adults with lower family cohesion.

This finding is supported by a number of previous studies that have shown a correlation between family cohesion and mental health in the older adult population. For instance, a study on Asian and Latino

people aged 65 years or more in the US showed a correlation between family cohesion and depression rates. It was found that the risk of depression was decreased in those who lived in families with better cohesiveness²⁴. While in China, it was evident that better family cohesion played a significant role in the great and good mental health of Chinese Americans aged 55 years or more who lived in the Phoenix metropolitan area²¹. Equally important, the closeness and warmth of relationships among the family members positively impact mood, reduce the effect of stress, and improve prosocial behavior^{25,20}. This is also in line with the finding of Guo et al., which asserts that there is a correlation between family cohesion and mental health (anxiety and mood disorder) in people aged 60 years or more²². Another different study obtained that family mealtime is closely associated with depressive symptoms in older adults²⁶. This is because when eating together, family members have the opportunity to strengthen their emotional relationship while enjoying the food^{27,28,26}. This condition is a form of family cohesion.

In addition, the contribution of family cohesion is not only shown on the mental health of older adults because studies in different populations have also found the role of family cohesion against mental health. For example, the result of a study that was conducted on HIV-infected adolescents in Uganda unveiled that there was a correlation between family cohesion and depression²⁹. A systematic review and meta-analysis revealed that family dysfunction, including low family cohesion, was correlated with symptoms of depression and this correlation (family dysfunction and depression) was strong³⁰. Family cohesion also denotes a significant contribution to the prevention of suicide attempts in adolescents³¹. In adolescents with higher levels of family cohesion, depression, anxiety, and anger levels are lower, while life satisfaction is increased²⁰. Referring to the several pieces of evidence found, thus the effort to improve

mental health can be started from the family. The family can increase its role through better family cohesion and reduce conflict within the family.

Aside from family cohesion, as previously discussed, one protective factor against mental illness that is consistently found is self-efficacy³². Likewise, the finding of this study has proved a significant and positive correlation between self-efficacy and mental health. Older adults with higher self-efficacy have better mental health than those with lower self-efficacy. Self-efficacy is required in order that the older adults can control themselves in dealing with various weaknesses and limitations that may be experienced in the aging process³³. Although enthusiasm and motivation are not only determined by self-efficacy, people with high self-efficacy can struggle and try persistently in various situations that hinder them from achieving certain goals³⁴. Self-efficacy is a personal resource that can foster a sense of optimism to face and solve many problems in life. Meanwhile, optimism is considered the center of mental health and is associated with the perception of someone who assesses him/herself as capable and effective in facing various obstacles³⁵. For this reason, self-efficacy has a significant role in psychological or mental health conditions. What is believed by someone to do, then s/he would do it with the skills and abilities s/he may have¹⁰.

Correspondingly, there have been several studies that have shown the same result. In India, it was found that self-efficacy was a notable predictor in determining mental health in older adults in the age group of 60 years or more³⁶. Additionally, Cheng et al. had conducted a study on the elderly with chronic pain. Their study concludes that self-efficacy plays a crucial role and is considered a significant protective factor against psychological endurance by reducing depressive symptoms³². Another survey of people aged 50 years or older also showed a

positive relationship between self-efficacy and mental health, in which self-efficacy is regarded as a strong predictor of their mental health³⁷. Apart from the older adult population, studies conducted on students showed the same results, i.e., there was a powerful relationship between self-efficacy and mental health, both in women and men.³⁸ Some of the evidence clarifies that the low level of self-efficacy, including in older adults, has a significant role in mental health. The older adults who believe that they can manage the difficult aspects will not avoid the complex and challenging situation, whereas those who do not believe will be afraid to deal with these situations³⁶.

Moreover, self-efficacy is a feeling of confidence about self-ability that can be changed, obtained, increased from a number of sources that form self-efficacy. One of the sources of self-efficacy is social persuasion. This social persuasion can be carried out by deliberately providing interventions that can increase older adults' self-efficacy. This effort aims to strengthen their belief that they are able to face a difficult situation and carry out an activity, despite some obstacles. Consequently, programs to maintain and enhance self-efficacy perhaps become the targeted interventions helpful in improving mental health³⁸.

Conclusion

In conclusion, this study proves that family cohesion and self-efficacy have an essential contribution to the mental health of older adults in Aceh-Indonesia. Family cohesion and self-efficacy can maintain and improve the mental health of older adults. For this reason, family members need to improve and maintain emotional relationships, communicate with each other, as well as appreciate and give love to each other. In addition, interventions to increase self-efficacy with a social persuasion approach can be carried out.

Conflict of Interest—Nil

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Ethical Approval: The study was approved by the Ethics committee of the institute.

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Soluble Antimicrobial Peptide Pyocin of *Pseudomonas aeruginosa* and its Therapeutics: A Review Article

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Abstract

Pyocin is a bacteriocin produced by a group of Gram-negative bacteria that belongs to *Pseudomonas* species. Pyocin is classified as two distinct families of pyocins: (i) S-type pyocins (colicin-like bacteriocins), (ii) Tailocins (high-molecular weight bacteriocins that resemble phage tails). The structure of S-type pyocin is similar to that of colicin except that many S-type pyocins have three domains. Under normal conditions, the expression of prtN is repressed by PrtR. Upon exposure to stress conditions, such as DNA damage by chemicals or ultraviolet irradiation, an activated RecA triggers autoproteolytic cleavage of PrtR, which abrogates prtN repression and leads to pyocin production. The outer membrane receptors for three pyocins have been identified. These are FpvAI, FpvAII and FptA, all of which are involved in the uptake of iron-siderophore complexes. Before being translocated through the membrane and killing their target. This is becoming increasingly important as microbial imbalances in the natural gut flora have been suggested to play a role in a range of chronic diseases such as inflammatory bowel disease, diabetes, obesity and rheumatoid arthritis. Pyocin has now been shown to have antimicrobial activity against bacteria in a biofilm is a limiting factor in the successful treatment of a range of chronic infections.

Key Words: *P. aeruginosa*, Bacteriocin, S-type pyocin, Structure, Genetics, therapeutics.

Pyocin

Pyocins are ribosomally synthesized bacteriocins that appear to comprise of a heterogeneous group of

substances ranging in size from a small low molecular weight protein to a high molecular weight protein with complicated structure and composition, but the part responsible for killing activity seems to be protein invariably¹.

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P. aeruginosa is the quintessence of microbiological arms depot, living in all settings from aquatic to terrestrial, from dirt to distilled water, from plants to people. To protect itself against other fungus and distantly related bacteria, it creates a wide spectrum of secondary metabolites². In order to compete with other *Pseudomonads* and closely related bacterial species for shared habitats³, *P. aeruginosa*

produces a wide spectrum of bacteriocins known as pyocins, which are produced by all strains. *P. aeruginosa* produces two distinct families of pyocins. (i) S-type pyocins (colicin-like bacteriocins), (ii) Tailocins (high-molecular weight bacteriocins that resemble phage tails)⁴.

The differ by their morphology and mode of killing. Their bactericidal activities are strain specific and have been used as a typing tool for *P. aeruginosa* strains, along with other typing schemes such as serotyping and phage typing⁵, secreted by over 90% of *P. aeruginosa* strains⁶. Which are used by bacteria to compete for resources by killing competitors, usually of the same bacterial species⁷, Which have shown action against embryonal ovarian cancer, human hepatocellular carcinoma, and cervical adenocarcinoma⁸.

Several authors study pyocins, characterized of the diversity of R- and F- pyocins and bacteriophages generated by diverse *P. aeruginosa* strains so as to identify pyocins of therapeutic value⁹, Structure and Analysis of R1 and R2 Pyocin Receptor-Binding Fibers¹⁰. Susceptibility to R-pyocins of *Pseudomonas aeruginosa* clinical isolates from cystic fibrosis patients^{11,12} suggest that the combined effects of dispersal limitation among sites and competitive exclusion within them maintain diversity in pyocin inhibition and susceptibility phenotypes, and that additional processes such as local adaptation and effects of phylogenetic distance could further contribute to spatial variability. Pyocins produced no adverse effects when injected alone into mice and showed good in vitro antipseudomonal activity. In an invertebrate model of sepsis using *Galleria mellonella*, both pyocins significantly prolonged survival¹³. Role pyocin in protecting *p. aeruginosa* and their resistance to antibiotics¹⁴.

History of pyocins

In 1952, researchers in Paris, Jacob discovered

bacteriocin normally produced by *P. pyocyanea* (the alternative species epithet for *Pseudomonas aeruginosa*). Japanese team in 1960 found pyocin of the type¹⁵. In the late pyocin R type describe produced by *P. aeruginosa* strain R¹⁶.

Finally, a third type of pyocin was described in 1970, called S¹⁷. It has been suggested by some researchers that the gene coding for pyocin R2 originated from a common ancestor of phage P2 and pyocin F2 originated from of phage lambda inserted the bacteria's chromosome via transduction¹⁸. The S type (soluble) pyocins, including S1, S2, S3, and AP41, possess the structures and the modes of action similar to those of colicins, bacteriocins produced by *Escherichia coli*¹⁹.

Types of Pyocins

The majority of findings related to natural pyocin synthesis have come from research of *P. aeruginosa* strain PAO1. It is possible to classify (on the basis of their structure and mode of action). A single strain of *P. aeruginosa*, on the other hand, can create many types of pyocin at the same time. More than 90% of *P. aeruginosa* strains generate R- and F-type pyocins, while 70% of *P. aeruginosa* strains may create at least one S-type pyocin. Pyocins of the R/F type have a large molecular weight. Pyocins of the S-type have a lower molecular weight²⁰.

R-type (rod-like) is a protein particle which morphologically resembles a bacteriophage of the Myoviridae family, the particle is composed of a core, sheath, baseplate and tail fibers. They induce a depolarization of the cytoplasmic membrane in relation with pore formation²².

F-type (flexible and non-contractible) pyocins also resemble phage tails, but with a flexible and non-contractile rod-like structure. R- and F-type pyocins are particles evolutionary related to bacteriophage tails, hence representing²³.

S-type (soluble) pyocins are Colicin-like bacteriocins (CLBs) protease-and heat-sensitive²⁴.

More recently, a fourth type of pyocin, the **M-type** pyocins are lipid II-degrading bacteriocins that share homology with colicin M²⁵, and fifth type The lectin-like bacteriocins (**L-pyocins; Llb**) are comprised of one or two monocot mannose-binding lectin domains (MMBL) and may kill at the OM surface by blocking the function of BamA, a protein of the β -barrel assembly machinery^{25,26}. Discovered a new pyocin called **pyocin G** (PyoG), that PyoG has broad killing activity against a collection of clinical *P. aeruginosa* isolates and is active in a *Galleria mellonella* infection model.

Pyocins S

One class of molecule that readily translocates across the impervious outer membrane of *P. aeruginosa* to deliver a cytotoxin is the S-type pyocins, which are 40- to 90-kDa protein bacteriocins made by *P. aeruginosa*²⁷. These antibacterial are secreted as binary protein complexes consisting of a large protein that harbors the killing function and a smaller immunity protein that remains tightly bound to the cytotoxic domain of the former²⁸.

Structure of pyocins S

The structure of S-type pyocin is similar to that of colicin except that many S-type pyocins have three domains²⁹ domain I is N-terminal that recognizes the cell surface receptor, domain II has unknown function and domain III translocate and penetrates pyocin, C terminal domain carries out the killing activity³⁰.

One pyocin structure has been solved to date. The crystal structure of pyocin M shares structural similarities with colicin M, namely a short N-terminal T-domain, followed by a central globular -helical R-domain and a C-domain incorporating a half-barrel fold¹⁸. Early characterization of pyocins S2 and AP41,

using analytical ultracentrifugation and gel filtration to estimate their molecular weights and shapes, suggested that these pyocins have elongated structures like those of colicins E3 and Ia. Like colicins, pyocins are predicted to be structurally diverse. Therefore, further structural characterization of pyocins would be extremely useful³¹.

Pyocins Stranslocation

Pyocins are translocated across the outer membrane. The exact mechanism remains unknown though the use of the ferrisiderophore receptors suggests that S-type pyocins are translocated in a similar way as the pyoverdines and pyochelin, energized by the TonB system³².

Little is known about the translocation of pyocins into sensitive cells. Pyocin AP41 is predicted to utilize the Tol system³³, similar to many colicins, as introduction of the tolQRA genes in a pyocin AP41 tolerant mutant restored killing by AP41. Pyocins S2, S3, S4 and S5 are predicted to utilize the TonB system for translocation due to their binding to TBDTs. However, colicins A and E1-E9 bind the TBDT BtuB and use the Tol system for translocation, so this assumption may not be valid³⁴.

PyocinsS receptors

The outer membrane receptors for three pyocins have been identified. These are FpvAI, FpvAII and FptA, all of which are involved in the uptake of iron-siderophore complexes^{35,36}. Before being translocated through the membrane and killing their target, the order of the receptor recognition domain and translocation domain is generally reversed: the N-terminal domain is involved in recognition of the cell surface receptor¹⁰, domain II (not present in pyocin S1) has an unknown function and is dispensable for killing activity, the third domain is responsible for pyocin translocation and penetration, and the C-terminal domain carries the lethal activity³⁶.

Pyocin S killer protein

Soluble pyocins have an N-terminal receptor binding domain, a translocation domain, and a C-terminal killing domain³⁷. The majority of S-type pyocins (AP41, S1, S2, and S3) cause cell death by DNA breakdown due to an endonuclease C-terminal domain³⁵, while pyocin S4 is predicted to have tRNase activity and S5 is predicted to have pore-forming activity³⁸. This motif constitutes the core of the catalytic site of the endonuclease and can chelate a single metal ion, required for hydrolysis of the dsDNA strand³⁹.

Self-Immunity of pyocins S type

This entry represents the DNase domain found in some colicin/pyocin bacteriocins, including colicin E2, E7, E9 and pyocin S1 and S2. In colicin E7, this domain has been described as a novel alpha/beta fold containing a Zn²⁺ ion binding site⁴⁰. Bacteriocin production can be lethal to the producer strain if specific protection mechanisms are not employed, leading to the employment of self-immunity mechanisms⁴¹. Immunity proteins from pyocins S1, S2 and AP41 share homology (approximately

44% to 99%) as they protect homologous H-N-H nuclease domains, whereas the pyocin S3 immunity protein shares little homology. Pyocins S1 and S6 are almost identical in their R- and T-domains, with their C-domains differing to alter nuclease activity²⁶. The other RNase pyocin is pyocin S4, which has an almost identical R-domain to pyocin S2. The C-domain of pyocin S4 is similar to the tRNase colicin E5 (31% amino acid identity), while sharing little homology with the tRNase colicin D1. The only pore-forming pyocin identified to date is pyocin S5, which shares greater than 75% amino acid identity between amino acids 217-307 with the unknown domain of pyocin S2 (amino acids 207-312) and less than 30% homology in the other domains. The C-domain shares 30% amino acid identity with that of colicin Ia and is predicted to be structurally homologous to colicins Ia, B, S4 and N⁴². Pyocin S5, like the pore-forming colicins, does not form a complex with its cognate immunity protein prior to release⁴³. The putative S immunity (SI) protein confers resistance to pyocin S in *P. aeruginosa*. Figure(1) show that SI prevents cellular lysis in cells expressing SI protein. This paves the possibility of learning more about its involvement in cellular adaptability

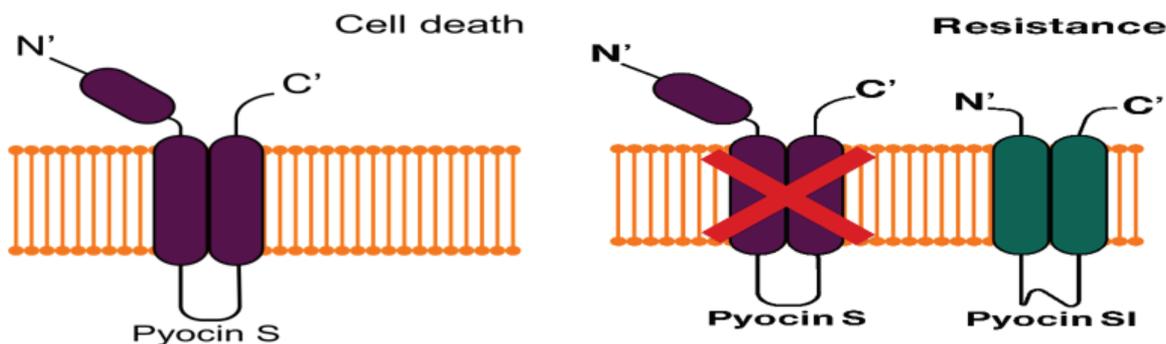


Figure 2. SI immunity protein resistance to pyocin S. Adapted from⁴⁴.

Regulation of pyocins synthesis

Pyocin production is highly energy cost and the release of pyocin is through cell lysis⁴⁵. The synthesis of pyocin involves genes *recA*, *prrR* and *prrN* and proteins RecA, PrtR and PrtN. The gene *recA* encodes a protein

RecA that is responsible for the repair of damaged DNA, gene *prtR* encodes protein PrtR which is a repressor of gene expression and gene *prtN* encodes a protein PrtN which is an activator⁴⁶. During normal conditions or in the absence of a mutagen, the *recA* gene produces very small amounts of RecA protein⁴⁷. PrtN is a transcriptional activator that binds to the P boxes located approximately 60-100 bp upstream of the ribosome-binding site. The P-box consists of a 10-12 nt consensus sequence - ATTGnn(n)GT-nn(n). PrtR is a transcriptional repressor protein, which binds to PrtN, preventing its binding to the P-box. Under conditions of stress, such as DNA damage RecA cleaves PrtR, releasing PrtN. The binding of PrtN to the P box induces transcription of pyocin genes⁴⁸.

Genetics of Pyocins S

The genes for bacteriocins are encoded on chromosomes, plasmids, and/or mobile elements such as transposons. Pyocins were reported to be chromosomally located⁴⁹. Bacteriocins genes encoding are often found in clusters, which include a toxin, immunity, and lysis genes. The immunity gene produces a protein that protects against the toxin, while the lysis gene produces a protein that helps in the removal of the toxin from the cell, which also results in cell death⁵⁰. The immunity protein and killing proteins are co-transcribed at a similar rate to prevent DNA degradation within the producing cell.

P.aeruginosa PAO1 produces multiple S-type pyocins, which contains S2, S4, and S5, containing only a short region with high homology to pyocin S2, in addition to R2 and F2⁵¹. S-type pyocin loci are found scattered in the genome, three loci encode S-type pyocin complexes: PA1150-1151 (pyocin S2 and immunity gene), pyocin S4 (PA3866 plus non-annotated immunity gene), and PA0984-0985 (immunity gene and pyocin S5 gene). Unlike the tail-like pyocin loci that are always often present between *trpE* (PA0609) and *trpG* (PA0649) on the

chromosome⁵².

The typical S-pyocin operon spans an approximately 2 kb region of DNA and includes two genes: the toxin gene and the immunity gene. Operon of pyocins S contains two open reading frames (ORF). The first ORF encodes large protein, while the small component is encoded by the second ORF⁵³.

Pyocins S as therapeutics

Bacteriocins from Gram-negative bacteria, and the pyocins of *P. aeruginosa* in particular are suited to therapeutic development for a number of reasons. They are a source of readymade antibiotics that are extremely potent (as low as pM affinity) and are amenable to protein engineering⁵⁴. The modular composition of pyocins means that chimeric pyocin proteins can be constructed that contain R-, T- and C-domains from different pyocins, broadening the number of therapeutic candidates. This technique was used with pyocins S2 (T- and C-domains) and S5 (central R-domain) to identify the R-domain of pyocin S5 and was also demonstrated with pyocins S1 and AP41⁵⁵. The modular composition and conserved toxin activity of pyocins and colicins means that active pyocin/colicin chimeras can also be constructed⁵⁶. This is becoming increasingly important as microbial imbalances in the natural gut flora have been suggested to play a role in a range of chronic diseases such as inflammatory bowel disease, diabetes, obesity and rheumatoid arthritis. The inability of antibiotics to kill bacteria in a biofilm is a limiting factor in the successful treatment of a range of chronic infections, including *P. aeruginosa* infection in the CF lung⁵⁷.

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Anthropometric Measurement of Nasal Parameters in Adult Malay Population

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Abstract

Objectives: Anthropometry is notable for its suggestions in, human anatomy, forensic science, and physical human studies. Human nose is an unmistakable rule in close to recognizable proof and is an impression of uniqueness of each person. The information on nasal estimation is essential to clinicians like maxillofacial specialists, craniofacial specialists empowering them in identification of typical or strange changes, help with repair and reconstruction. Prosthetic recovery additionally requires the capacity to envision the position and measurement of the nose inside the facial closeness. In this way, the admittance to nasal information for every populace are worthwhile. The main intension was to quantify the parameters of outer nose of adult Malay populace and to decide the critical distinction in nose measurement among races.

Materials and Methods: The cross-sectional study was led on 420 people (80 Malay male, 80 Malay female, 60 Indian male, 70 Indian female, 60 Chinese male and 70 Chinese female) in the age range 18-45 years. In this study digital caliper was utilized to identify the nasal landmarks. This research was conducted that the mean of variables were statistically significant differences in nose measurement scores among adult Malay population. All the average independent variables were measured and compared whether differs at the same time among races and ethnic groups.

Results: It was discovered that nasal measurement were remarkably larger in Indian and Malay than in Chinese for nasal tip protrusion, nasal height and nasal length. There were larger in Chinese than in Malay for nasal width and alar base root. There were larger in Indian than in Malay for nasal width, nasal height and nasal length. But there was no significant difference in nasal parameters among genders of Malay populace.

Conclusion: The statistically significant difference in nasal anthropometric nasal parameters were found among three ethnic groups of adult Malay population. But there was no significant difference in anthropometric nasal values among genders of Malay populace.

Keywords: Anthropometry; ethnic group; nasal landmarks, nose parameters

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Introduction

Anthropometry has been utilized in forensic science, to comprehend human physical variety, in palaeoanthropology and in different endeavours to

correspond physical, ethnic and mental qualities. Anthropometry is the trademark technique that manages the investigation of body extent and total measurements that differ broadly with age, sex and racial groups.¹Over the hundreds of years, there have been noteworthy changes in Anthropometric estimations because of topographical, social, hereditary and natural factors²just as overall blending of races. In this manner, confinement of unadulterated races has end up being a troublesome issue. Be that as it may, anthropometric investigations keep on assuming a significant job in recognizing unadulterated race and neighbourhood blending of races.³The human face is generally perceived as the include which best recognizes an individual.⁴ It is the foremost piece of the head that incorporates the eyebrow, eyes, nose, mouth and jawline. The state of the nose is a mark demonstrating the nationality, race, age and sex . The human nose can likewise be influenced by socioeconomic status, climate and area.⁵As an outcome of human development, the smaller noses are supported in cold and dry environments while more extensive noses in hotter, moister ones. The size, shape, and extents of the nose give a visual premise recommending the personality of the individual.⁶

Farkas studied widely in numerous ethnic gatherings in the field of facial anthropometry. To treat intrinsic or then again post-horrendous facial deformations in individuals from a specific ethnic group effectively, specialists expect access to craniofacial databases dependent on precise anthropometric estimations. Regularizing information were basic for exact assurance of the level of inherent or post-awful facial deviations from the typical.⁷An anthropometric investigation of the Malay ethnic group in Malaysia had been led by Ngeow and Aljunid.⁸The outcome was contrasted with the

Singaporean Chinese from Farkas' investigation. They presumed that three highlights, to be specific the stature of the head, intercanthal width and distension of the nasal tip might be helpful to separate a Malay face from that of the Singaporean Chinese.⁹ The nose was one of the primary segments of the facial feel, and the investigation of its structure is critical in plastic surgery procedure and forensic facial reproduction.^{10,11}Powell NB and Humphreys B examined the contrasting of facial proportions characterized by the canons with 50 Italian models. They noticed a relative decrease in nasal dimensions, and an increment in the width of the eyes and mouth.¹²

Materials and Methods

Study design

This was a cross sectional study involving the collection and analysis. The location of the study was Management and Science University in Malaysia located in Shah Alam, Selangor. This study was conducted from 2019 to 2021.

Sampling and sample selection

Subjects were Malaysian adults of Malay ethnicity. This was a cross-sectional examination with 420 subjects were participated in this research, 200 male and 220 female students: (130 Malaysian Chinese, 160 Malaysian Malay and 130 Malaysian Indian), aged 18±45 years old. Inclusion criteria were Malay, Chinese and Indian. The subjects confirmed their nationality by means of a self-managed survey. The Age was in the range of 18 and 45 years. Exclusion criteria were age lower than 18 years, and older than 45 years, blended or dubious unsure group of races, past history of craniofacial injury, nasal scars, history of nasal or facial surgical procedure interventions on the nose.

Fig. 1: Landmarks of the nasal measurement from an anterior view

The following landmarks nasion were displayed on the nose as per fig.1, 2 and 3.

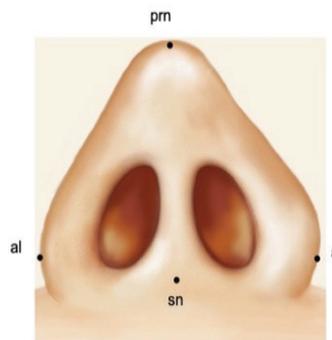
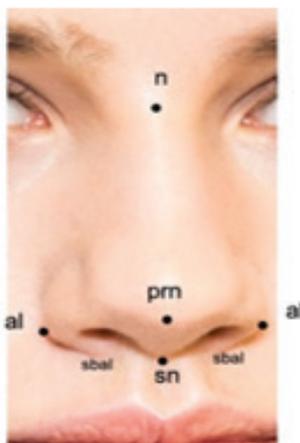


Fig. 3: Landmarks of the nasal measurement from an inferior view

The linear measurement definition using landmarks on the nose were assessed for nasal tip protrusion (sn-prn), nose width (al-al), nose height (n-sn), nasal bridge length (n-prn) and alar base root width (sbaR±sbaL).

Statistical Analysis

These data were analysed by utilizing Statistical Package for the Social Sciences (SPSS) version 25.0. Depending on its accurate statistical analysis, this software interpreted the findings. The data were investigated to be normality assumption by histograms and Q-Q plots. Independent t Test was used to determine whether there was a significant difference between the means of two unrelated groups of races. It was also to find out the correlation between dependent and each of the independent variables of ethnicity. Significance was considered as p values smaller than 0.05 and the confidence interval was considered as 95%. The investigation, One way ANOVA was completed utilizing the log-changed information to explore contrasts among races as the independent variable, and the variables of interest as the dependent variable factors. The investigation demonstrated measurably noteworthy contrasts ($p < 0.05$) between ethnics for the factors concerned. The analysis demonstrated the mean, standard deviation, and mean differences, as well as the comparison of

Nasion (n)	Deepest concavity point on the nasolabial suture
Alare (al) (alR, alL)	The most lateral point on each alar contour
Pronasale (prn)	Most prominent midline point on the nose tip, identified on the lateral view
Subnasale (sn)	intersection point of the upper lip and nasal septum



Fig. 2: Landmarks of the nasal measurement from a lateral view

the variables among ethnics. The clinically critical contrasts, a cut-off minimum value of 3mm was set.

Results

The histograms for nasal parameters revealed normal distribution curve for ethnics. The normality assumption of nose phenotypes was conducted by Q-Q plots and box plots for race. There was a statistically significant in a person correlation of concerned calculation of mean scores of nose among three ethnic groups.

The independent T test revealed that there was a statistically significant differences ($p < .001$) among races for nasal tip protrusion, nasal width, nasal height, nasal length and alar base root. Table 1 exhibited statistics for race including mean and standard deviation. Table 2, Table 3 and Table 4 exhibited the mean differences, 95% confidence interval of the differences and significant difference ($p < .001$) in concerned variables among Malay, Indian and Chinese.

Table: 1 Statistics of ethnic group

		Descriptive							
		N	Mean	Standard Deviation	Standard Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Nasal tip protrusion	Malay	160	20.41	2.035	.161	20.09	20.72	16	24
	Indian	130	20.50	1.982	.174	20.16	20.84	18	24
	Chinese	130	14.54	.545	.048	14.44	14.63	14	16
Nasal width	Malay	160	37.38	2.632	.208	36.96	37.79	31	41
	Indian	130	40.41	1.173	.103	40.20	40.61	39	42
	Chinese	130	42.99	.564	.049	42.89	43.09	42	44
Nasal height	Malay	160	49.14	1.900	.150	48.85	49.44	45	51
	Indian	130	54.52	3.570	.313	53.90	55.13	48	62
	Chinese	130	42.49	1.228	.108	42.28	42.71	40	45
Nasal length	Malay	160	49.20	1.422	.112	48.98	49.42	46	52
	Indian	130	53.82	2.487	.218	53.38	54.25	49	58
	Chinese	130	41.35	.567	.050	41.25	41.44	40	42
Alar base root width	Malay	160	16.84	.592	.047	16.75	16.93	16	18
	Indian	130	17.65	.644	.056	17.54	17.77	17	19
	Chinese	130	20.70	.631	.055	20.59	20.81	20	22

N=number of respondents

Table: 2 Independent T test for Indian and Chinese

		F	t	df	Significance	Mean Difference	Standard Error Difference	95% Confidence Interval	
								Lower	Upper
Nasal tip protrusion	Equal variances assumed	180.983	33.075	258	.000	5.962	.180	5.607	6.316
Nasal width	Equal variances assumed	155.653	-22.649	258	.000	-2.585	.114	-2.809	-2.360
Nasal height	Equal variances assumed	137.413	36.307	258	.000	12.023	.331	11.371	12.675
Nasal length	Equal variances assumed	118.453	55.748	258	.000	12.469	.224	12.029	12.910
Alar base root width	Equal variances assumed	.429	-38.548	258	.000	-3.046	.079	-3.202	-2.891

As per table 1 and 2, for nasal tip protrusion, there was a notable distinction between Indian (M=20.50,SD=1.582)and Chinese (M=14.54, SD=.545); t (258)=33.075, p=.000

For nasal height, there was a remarkablecontrast in the mean scores for Indian (M=54.52, SD=3.570)) and Chinese (M=42.49, SD=1.228); t (258) = 36.307, p= .000

Fornasal length,therewasahugedistinctionbetween Indian (M=53.82, SD=2.487)) and Chinese (M=41.35, SD=0.567); t (258) = 55.748, p= .000

For alar base root, there was a notabledifference between Indian (M=17.65, SD=.644) and Chinese (M=20.70, SD=.631); t (258)=-38.548, p=.000

Table: 3 Independent T test for Malay and Chinese

		F	t	df	Significance	Mean Difference	Standard Error Difference	95% Confidence Interval	
								Lower	Upper
Nasal tip protrusion	Equal variances assumed	97.221	31.946	288	.000	5.868	.184	5.506	6.229
Nasal width	Equal variances assumed	269.303	-23.885	288	.000	-5.617	.235	-6.080	-5.154
Nasal height	Equal variances assumed	24.675	34.491	288	.000	6.651	.193	6.272	7.031

Cont... Table: 3 Independent T test for Malay and Chinese

Nasal length	Equal variances assumed	66.147	59.244	288	.000	7.854	.133	7.593	8.115
Alar base root width	Equal variances assumed	5.722	-53.648	288	.000	-3.863	.072	-4.004	-3.721

As per table 1 and 3, the nasal tip protrusion was remarkable distinction between Malay (M=20.41,SD=2.035) and Chinese (M=14.54, SD=.545); t (288)=31.946, p=.000

for nasal width, there was a notable difference between Malay (M=37.38, SD=2.632)) and Chinese (M=42.99, SD=0.564); t (288) = -23.885, p= .000

For nasal height, there was a remarkable distinction between for Malay (M=49.14,

SD=1.900)) and Chinese (M=42.49, SD=1.228); t (288) = 34.491, p= .000

For nasal length, there was a significant contrast between Malay (M=49.20, SD=1.422)) and Chinese (M=41.35, SD=0.567); t (288) = 59.244, p= .000

The alar base root was a huge contrast between Malay (M=16.84,SD=.592) and Chinese (M=20.70, SD=.631); t (288)=-53.648, p=.000

Table: 4 Independent T test for Malay and Indian

		F	t	df	Significance	Mean Difference	Standard Error Difference	95% Confidence Interval	
								Lower	Upper
Nasal tip protrusion	Equal variances assumed	.582	-.395	288	.693	-.094	.237	-.561	.374
Nasal width	Equal variances assumed	103.283	-12.188	288	.000	-3.033	.249	-3.522	-2.543
Nasal height	Equal variances assumed	72.229	-16.392	288	.000	-5.372	.328	-6.017	-4.727
Nasal length	Equal variances assumed	35.083	-19.828	288	.000	-4.615	.233	-5.074	-4.157
Alar base root width	Equal variances assumed	9.214	-11.228	288	.000	-.816	.073	-.959	-.673

As per table 1 and 4, for nasal width, there was a notable difference between Malay (M=37.38, SD=2.632) and Indian (M=40.41, SD=1.173); $t(288) = -12.188, p = .000$

for nasal height, there was a huge difference between Malay (M=49.14, SD=1.900) and Indian (M=54.52, SD=3.570); $t(288) = -16.392, p = .000$

For nasal length, there was a notable contrast between Malay (M=49.20, SD=1.3422) and Indian (M=53.82, SD=2.487); $t(288) = -19.828, p = .000 (p < 0.05)$.

All confidence interval of the difference do not include zero for nasal tip protrusion, nasal height, nasal length, nasal width and alar base root in table 2, 3 and 4.

ANOVA test was for overall differences between races. ANOVA was F test to determine significance of a factor. The F value in ANOVA was based on a comparison of the factor variance to the error variance. The F ratio is a measure of variance between groups divided by variance within groups. There were two sources of variance. These were variance between and within. But ANOVA did not determine exactly which means differ. Post-Hoc test was a follow up test that determined exactly which means differed after statistically significant result were found. It compared each individual mean against the other means for three or more groups. Tukey HSD was used to determine if the relationship between two sets was statistically significant.

There was a remarkable contrast among Malay, Indian and Chinese at the level of $p < 0.05$ for the three conditions in ANOVA as following:

Nasal width $F(2, 417) = 361.268, p = 0.0000$, nasal height $F(2, 417) = 815.504, p = 0.000$

nasal length $F(2, 417) = 1862.249, p = 0.000$, nasal tip protrusion $F(2, 417) = 543.279, p = 0.000$ and alar base $F(2, 417) = 1487.729, p = 0.000$.

Post-Hoc test displayed that there was not a significant difference in nasal tip protrusion, at the level of $p = .887$, between Malay and Indian.

In this study, there was no significant difference in nasal parameters among genders of Malay, Indian and Chinese of Malay populace.

Discussion

In the present research, there were remarkable distinction in the mean difference of nasal tip protrusion, nasal width, nasal height, nasal length and alar base root across the three ethnics of Malay populace. It was discovered that nasal measurement were remarkably larger in Indian and Malay than in Chinese for nasal tip protrusion, nasal height and nasal length. There were larger in Chinese than in Malay for nasal width and alar base root. There were larger in Indian than in Malay for nasal width, nasal height and nasal length.

The various research discovered the mean scores for parameters of nose morphometry in various populaces. Varieties in the nose morphology emerge through number of components which incorporate sex, nationality, dietary, environment, and climate.¹¹

The consequences of the investigations directed in certain ethnic groups may not be appropriate to the populaces somewhere else. This distinctive finding may be because of contrast of nationality as Malay has a place with the Ongoloid while Chilean has a place with Caucasian group.¹³

The investigation conducted by Farkas working independently across the world, with little examples of the populace, was obviously fundamental in nature and degree. He examined though about fourteen standardizing estimations of the face across various ethnic and demic groups. The five primary districts of examination included: Africa, Asia, Europe, Middle East, and North America. The nose height and nose

width differentiated strongly corresponding to North America whites nose was entirely wide in both genders of Asian and Black ethnic group. Among Caucasians, the nose height significantly contrasted from North America whites in three ethnic groups. In the Middle Eastern groups the nose width was indistinguishable from those of North America whites however the height was fundamentally more prominent.^{14, 15}

Some study showed the racial differences of the external nasal anthropometric values in Egyptian males and additionally provided a database for anthropometric average values of the external nose in healthy adult Egyptian males.¹⁶

Some researchers compared the nasal measurement between Oriental and Caucasian nose. the oriental nose projected less from the face, the alar base and tip projection than Caucasian nose. When compared with the Oriental nose, Caucasian nose was more greatly projected at tip and nasion. The Oriental nose was wider at alar base but not at bony base.¹⁷

Some researchers studied the Caucasian nose that the men were higher tip projection than women in comparing the genders.¹⁸

Conclusion

There was a strong and positive correlation between nasal parameters in the adult population among Malay, Indian and Chinese but not in genders.

Ethical Clearance: Taken from Research Management Centre, Management and Science University

Source of Funding: Self

Conflict of Interest: Nil

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Evaluation of Blood Parameters When Infected with *Candida Albicans* and Its Recovery Using Probiotics

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Abstract

Purpose: To study, blood components and some indicators of liver function were measured and *Candida albicans* was isolated and identified from infected cows' vagina with inflammatory signs then experimentally in rabbits treated with estradiol hormone.

Introduction: Candidiasis is an infection caused by yeast, *Candida* can multiply and cause an infection if the environment inside the vagina changes in a way that induces its growth.

Materials and Methods: Isolation and identification of *Candida albicans* isolate from cow' vaginitis (40 samples), duration in November 2020 till May 2021 then treated with *Saccharomyces cerevisiae*. *Candida albicans* inoculums at 1.5×10^8 cell /ml, in non-pregnant vaginal' Rabbits a weight (2 kg) after treated with estradiol hormone for several days before the infection occurred. The experiment was carried out in the microbiology laboratories in College Veterinary Medicine- the University of Baghdad, the blood, liver parameters, and rabbits' vaginal histology was studied.

Results: The Hb parameters in G2 showed higher significant differences (8.31 ± 0.75107) compared with G1 (12.80 ± 2.42782), G3 (13.26 ± 1.465), G4 (12.46 ± 1.987), G5 (12.75 ± 2.128) respectively, the effectiveness of the treatment with *S. cerevisiae* and its ability to return hemoglobin and liver functions to its normal value and the treatment cause inhibitory effect of *C. albicans* infection, the vaginal histological section in G4 showed narrow folding of mucosa thrown with mild MNCs infiltrate LP and submucosa, no inflammatory cells seen in a muscular layer or in the serosa.

Conclusion: The study proved the effectiveness by using *Saccharomyces cerevisiae* and treating rabbits vaginal infection with pathogenic *Candida albicans*, that Injected estradiol hormone S\C then, recovery the blood and liver parameters to their normal condition, in addition, the treatment of vaginal tissue and its return to its normal state.

Key words: *Candida albicans* , Blood parameters, probiotics.

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Introduction

Candidiasis is an infection caused by a yeast (a type of fungus) called *Candida*, it is normally living inside the body (in places such as the mouth, throat, gut, and vagina) and on the skin without causing any problems, sometimes *Candida* can multiply

and cause an infection if the environment inside the vagina changes in a way that induces its growth. Candidiasis in the vagina is commonly called a vaginal yeast infection, other names for this infection are vaginal Candidiasis, vulvovaginalCandidiasis, or candidlyVaginitis¹. Candidiasis is one of the fungal diseases occur saprophytically but only *Candida albicans* is commonly associated with diseases in humans and animals found commensallyof mucocutaneous areas particularly of intestinal and genital tracts². Also, there are many microflora in the vagina like *E.coli*, *Klebsiella*, and *Streptococcus faecalis*³.

Among *Candida* spp., *Candida albicans* is the most common infectious agent, this dimorphic yeast is a commensal that colonizes skin, the gastrointestinal and the reproductive tracts, and the most frequent manifestations of genitourinary Candidiasis include Vulvovaginal candidiasis (VVC), and it the most prevalent vaginal infection worldwide, and *Candida albicans* is major agent⁴. VulvovaginalCandidiasis is characterized by disruption of the vaginal microbiota composition, following large spectrum antibiotic use. Recent studies support the effectiveness of oral and local probiotic treatment for prevention of vulvovaginal candidiasis, demonstrate that vaginal administration of probiotic *Saccharomyces cerevisiae* live yeast used as therapeutics, this effect was likely due to multiple interactions of *Saccharomyces cerevisiae* with *Candida albicans*, induced co-aggregation of *Candida* and inhibited its adherence to epithelial cells, however, only the probiotic yeast was able to suppress some major virulence factors of *Candida albicans* such as the ability to switch from yeast to mycelia form and the capacity to express several aspartyl proteases⁵*Saccharomyces cerevisiae* is a species of yeast (single-celled fungus microorganisms, yeasts are an important source for obtaining products with probiotic activity, yeast, strains are more well-known in their use as probiotics⁶*Saccharomyces cerevisiae*

has developed as a model because it is a single-cell organism, small with a short generation time (doubling time 1.25–2 hours at 30 °C), can be easily cultured, it is a strong economic driver, as a result of its established use in industry⁷Also used as a feed additive in livestock that Contains; proteins, vitamins (vitamin B6, thiamin (B1), biotin, riboflavin (B2), nicotinic acid pantothenic acid (B5) and enzymes that improve weight gain and growth performance⁸. lowers mortality rates, increases hematological parameters decreases serum cholesterol levels, improves the fertility of female⁹.

Materials and Methods

Isolation of *candida albicans* from cow' vaginitis (40 samples), and Preparation of *saccharomyces cerevisiae* according to ¹⁰.and

Candidaalbicans inoculum's concentration 1.5 ×10⁸ cell /ml according to¹¹, Experimental animals (Rabbits eight for each group).

Group1: untreated (control).

Group2: Inject female rabbits S/C with 5mg estradiol in 50µl sesame oil for 5 days prior to injecting vaginal inoculation (2 ml/ rabbit *Candida albicans*) for 7 days (daily intake) / Intravaginal.

Group3 : Same group2 + 2ml /kg B.W (orally) of *Saccharomyces cerevisiae* for 7 days (daily intake) at the same time.

Group4: Same group 2 + 2ml /kg B.W (orally) of *Saccharomyces cerevisiae* for 10 days, daily intake treat after final doses of *Candida albicans*.

Group5 : Treated with *Saccharomyces cerevisiae* 2ml /kg B.W (orally) for 7 days only (daily intake).

Methods:

Preparation of culture media:

Sabouraud dextrose agar (SDA), Cornmeal agar

(CMA), Glucose Peptone Yeast Broth Medium (GPYB) The medium was prepared according to the manufacturer's directions.

Candida albicans were described based on morphological characteristics of the culture medium, the development of germ tubes, and the production of Chlamydo spores^{12,13}

Histopathological Examination

Vaginal Rabbits tissues have been prepared for histopathological examination according to¹⁴

Statistical Analysis

The results data were analyzed statistically by using the Microsoft Program (SPSS). Statistical analysis of data was performed on the basis of Analysis of Variance (ANOVA) and specific group differences were determined using least significant differences (L.S.D), as described by¹⁵

Results and Discussion

Candida albicans identification by typical and rapid methods: -

Candida albicans were described based on morphological characteristics of the culture medium, the development of germ tubes, and the production of Chlamydo spores.

Cultural Characteristics on Sabouraud's dextrose agar:

Candida albicans was cultivated on Sabouraud dextrose agar for 48-72 hours, and colonies appeared as thin, smooth white-cream, glistening round and curved colonies at 37 °C for 2-3 days after inoculation; these results are consistent with those obtained using Sabouraud dextrose agar for 48-72 hours¹⁶. *Candida albicans* colonies on sabouraud dextrose agar revealed white creamy opaque pasty colonies under the microscope. as in figure (1).



Figure (1): *Candida albicans* colonies on sabouraud dextrose agar showed white creamy opaque pasty .

Microscopical Characteristics:

Using Lactophenol Cotton Blue stain, microscopically analysis revealed the presence of pseudohyphae with clusters of budding cells. These

morphological features of isolates were *Candida albicans*.fig(2)

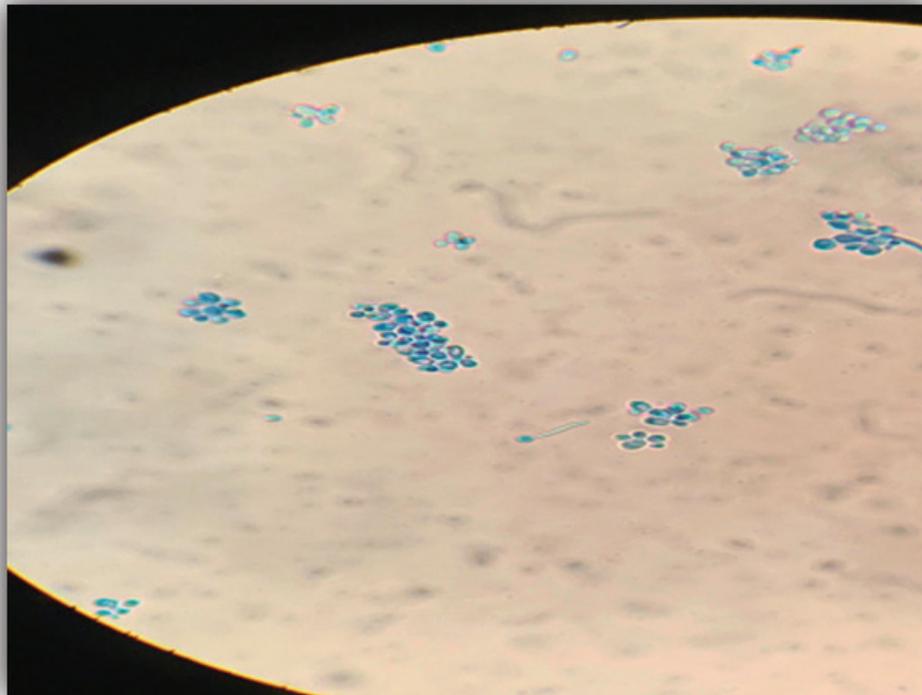


Figure (2): Microscopically appearance Chlamydospores of *Candida albicans*.

Germ tubes formation:

Forming long tube-like projections from yeast cells, these tubular extensions are an early stage in the formation of hyphae in the hyphae formation process. The germ tubes developed within 2-3 hours of incubation at 37 °C when *Candida albicans* was inoculated in the human serum, which is a distinct diagnostic feature of *Candida albicans* that distinguishes it from other *Candida* species this study is agree with ¹⁷Fig(3).



Figure (3): Germ tube production after 3 hours of growth in fresh human's serum at 37°C. (40X).

Chlamyospores production

Another measure for the detection of *Candida albicans*, the ability to produce Chlamyospores was tested using corn meal agar, and the results were consistent¹⁸. When the isolates are cultured on corn meal with 1% of tween-80 agar and incubation at 30 °C for 48 hours (Dalmau plate technique), The growth was then stained with lacto phenol cotton blue stain and analyzed under a microscope with a 40X objective, which agree with¹⁹. The hematologic parameters showed the HB is higher significant differences at level ($P \leq 0.05$) in G2 (8.31 ± 0.75107) when the vagina's rabbit infected by *C.albicans* compared with G1 (12.80 ± 2.42782), G3 (13.26 ± 1.465), G4 (12.46 ± 1.987), G5 (12.75 ± 2.128) respectively. the results showed the effectiveness of the treatment with *S.cerevisiae* and its ability to return hemoglobin to its normal value and its inhibitory effect on the growth of *C.albicans* isolates, this result is similar to the researcher's study an agreement with²⁰, they found in their research that the Hemolysin are involved in *Candida albicans* virulence because they are responsible for the destruction of red blood cells and the acquisition of iron. Iron and other inorganic elements are required for the development of *Candida* cells and the establishment of the infection process, the synthesis of haemolysin by *Candida albicans* could be associated with reductions in red blood cell and hemoglobin levels throughout the infection period²¹.

The PCV parameter showed the higher significant differences at level ($P \leq 0.05$) in G2 (26.78 ± 3.98) when the vagina's rabbit infected by *C.albicans* compared with G1 (38.33 ± 8.09), G3 (38.58 ± 7.54), G4 (38.86 ± 8.12), G5 (43.73 ± 8.96) respectively. the results showed decrease in PCV level this results were an agreement with²² when he found a PCV less than 30 percent indicating anemia with infected specially particularly if hemoglobin levels are low.

The WBCs count parameters showed the higher significant differences at level ($P \leq 0.05$) in G2 (29.35 ± 3.46) when the vagina's rabbit infected by *C.albicans* compared with G1 (8.24 ± 2.20), G3 (10.91 ± 1.77), G4 (7.37 ± 1.27), G5 (8.67 ± 2.61) respectively. Laboratory investigations have shown increase in the total number of circulating leukocytes in rabbits injected with bacteria or yeast. An agreement with²³.

The cholesterol counts parameter showed the higher significant differences at level ($P \leq 0.05$) in G2 (90.22 ± 4.73) when the vagina's rabbit infected by *C.albicans* compared with G1 (58.98 ± 15.75) G3 (71.86 ± 10.19), G4 (47.41 ± 19.46), G5 (45.78 ± 16.99) respectively. Metabolic effects of *S. cerevisiae* lower the cholesterol levels, this results were an agreement with²⁴.

The ALT test counts parameter showed the higher significant differences at level ($P \leq 0.05$) in G2 (361.21 ± 65.21) when the vagina's rabbit infected by *C.albicans* compared with G1 (96.30 ± 24.83), G3 (95.34 ± 28.17), G4 (129.60 ± 52.15), G5 (85.34 ± 25.23) respectively, indicated high significant differences in group 2 the results an agreement with Kretschmar²⁵ who found that Alanine Aminotransferase (ALT) is abundant in many tissues, it is of limited use in rabbit liver disease, however, if ALT levels are abnormally high, it could suggest liver inflammation and necrosis.

The AST test counts parameter showed the higher significant differences at level ($P \leq 0.05$) in G2 (180.85 ± 61.62) when, the vagina's rabbit infected by *C.albicans* compared with G1 (55.67 ± 20.39), G3 (61.61 ± 13.23), G4 (83.60 ± 13.79), G5 (44.46 ± 17.92) respectively. The enzyme aspartate aminotransferase (AST) is present in a number of tissues in the rabbit, including the liver, heart, pancreas, and muscle. Elevations in this enzyme can be caused by liver cell necrosis²⁶(Table 1).

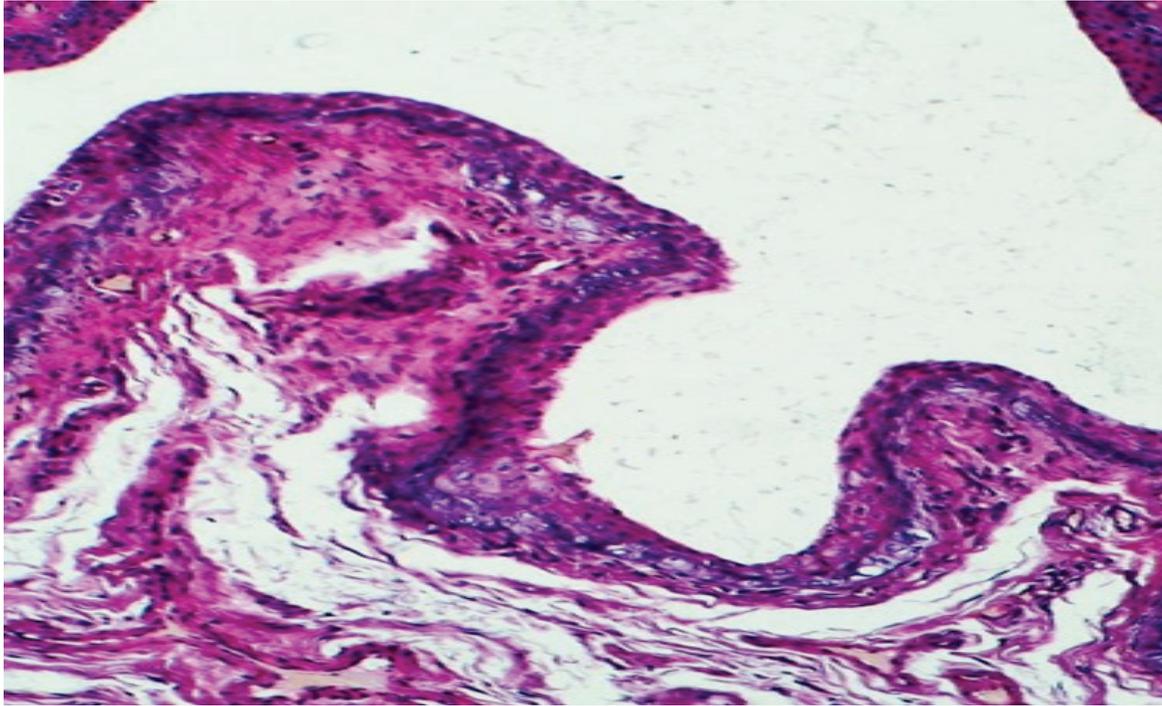
Table (1): Blood parameters in rabbits infected with *Candida albicans* and treatment with probiotics.

Group Parameter	GROUP 1 Control negative	GROUP 2 Control positive infected with <i>c.albicans</i> 7 days	GROUP 3 Infection with <i>C.albicans</i> 7 days & treatment with <i>S.cerevisiae</i> 7 days in the same times	GROUP 4 Infection with <i>C.albicans</i> 7 days Then treated with <i>S.cerevisiae</i> 10 days	GROUP 5 Only Treated with <i>S.cerevisiae</i> 7 days	NO.	LSD
Hb	12.80 ± 2.42782A	8.31±0.75107 B	13.26 ± 1.46574 A	12.46 ± 1.98778 A	12.75 ± 2.12804 A	8	1.863
PCV	38.33 ± 8.09 A	26.78 ± 3.98 B	38.58 ± 7.54 A	38.86 ± 8.12 A	43.73 ± 8.96 A	8	7.62
WBC	8.24 ± 2.20 C	29.35 ± 3.46 A	10.91 ± 1.77 B	7.37 ± 1.27 C	8.67 ± 2.61 BC	8	2.40
Cholesterol	58.98 ± 15.75 BC	90.22 ± 4.73 A	71.86 ± 10.19 B	47.41 ± 19.46 C	45.78 ± 16.99 C	8	14.58
ALT	96.30 ± 24.83 BC	361.21 ± 65.21 A	95.34 ± 28.17 BC	129.60 ± 52.15 B	85.34 ± 25.23 C	8	42.87
AST	55.67 ± 20.39 BC	180.85 ± 61.62 A	61.61 ± 13.23 BC	83.60 ± 13.79 B	44.46 ± 17.92 C	8	31.56
Different Capital letters mean significant differences horizontally (P ≤ 0.05) between groups							

Histological Examination

In G1 the microscopic examination of control animals revealed the vaginal wall by light microscope, in lower 1/3 of vaginal duct the mucosa consist from 2 to 3 layers of stratified squamous epithelium and

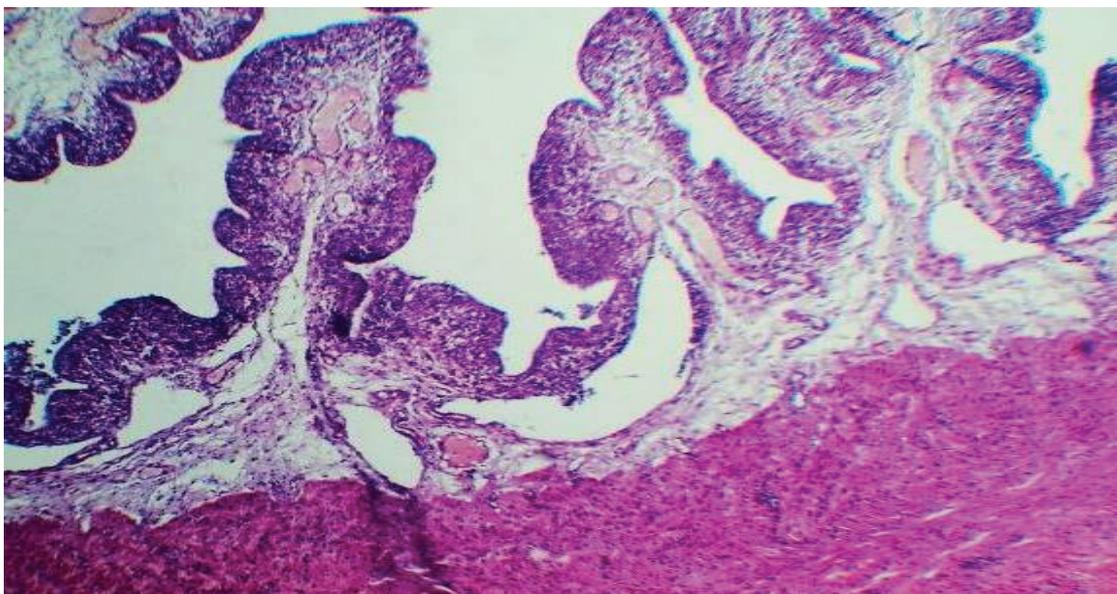
numerous vascular sinusoids lined by endothelial cells in muscularispropria the muscular layer consist from compact arrangement of muscle strands (inner longitudinal and cross external layer) without sinusoidal vascular in muscularispropria.



Figure(4) : Histopathologic section in vaginae of rabbit in G1 showed normal architecture of vaginae wall lined by stratified columnar epitheliumvascular sinusoids in muscularispropria. (H&E stain, 100X).

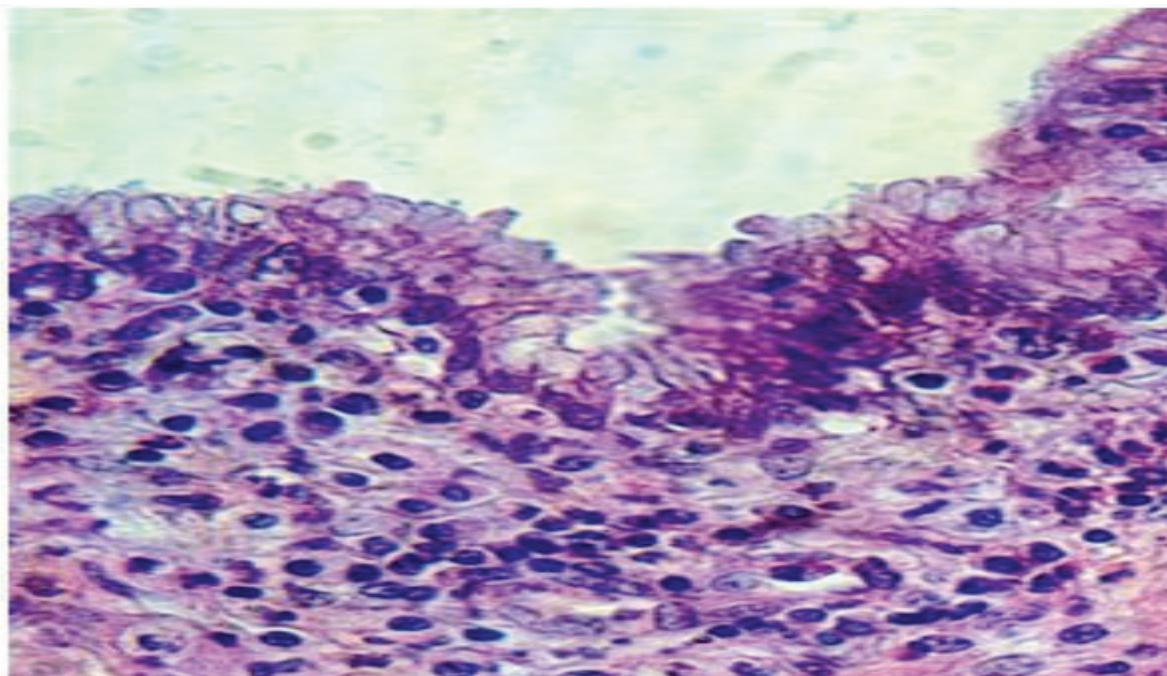
In G2 there was marked acute Vaginitis described as severe vacuolar degeneration and necrosis of squamous epithelium in mucosa with heavy infiltration of inflammatory cells hetrophiles and few mononuclear cells mainly macrophages and few lymphocytes , also infiltrated in submucosa

few perivascular in the outer muscular layer extend to serosa with dilation of serosal blood vessels, fibrinousexudates as fibrils network precipitate in submucosa with congestion of blood vessels dilated and contained few inflammatory cells.



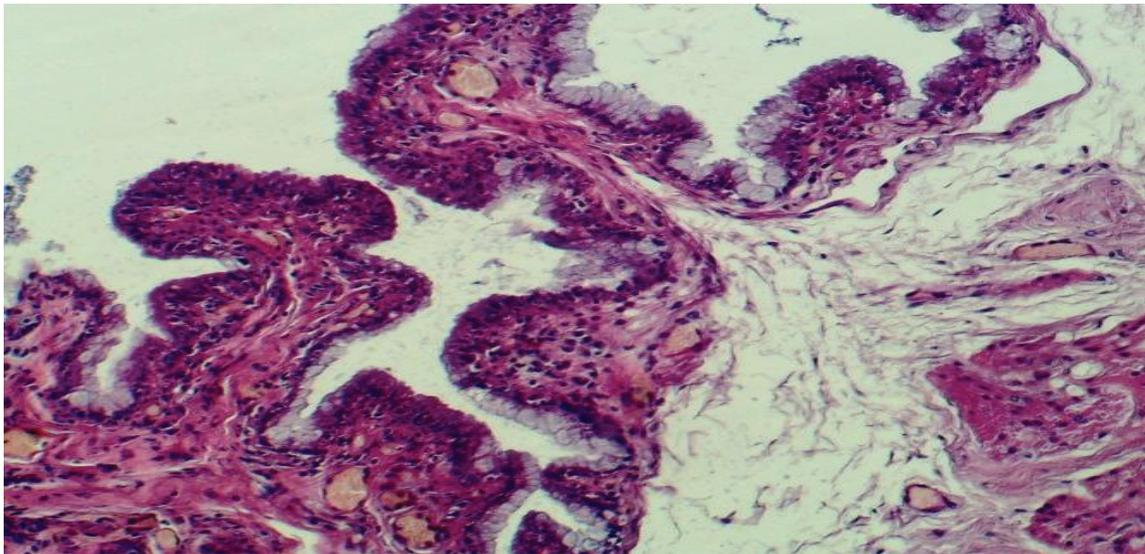
Figure(5) : Histopathologic section in vagina of rabbit in G2 shows severe vaginitis as thickening of mucosa protrude like papillae in lumen. (H&E stain, 100X) .

In G3 moderate to mild degeneration of squamous epithelial cells (vacuolar degeneration) and moderate mononuclear cells infiltrate lamina propria in mucosa. None inflammatory cells seen in muscular layer and serosa.



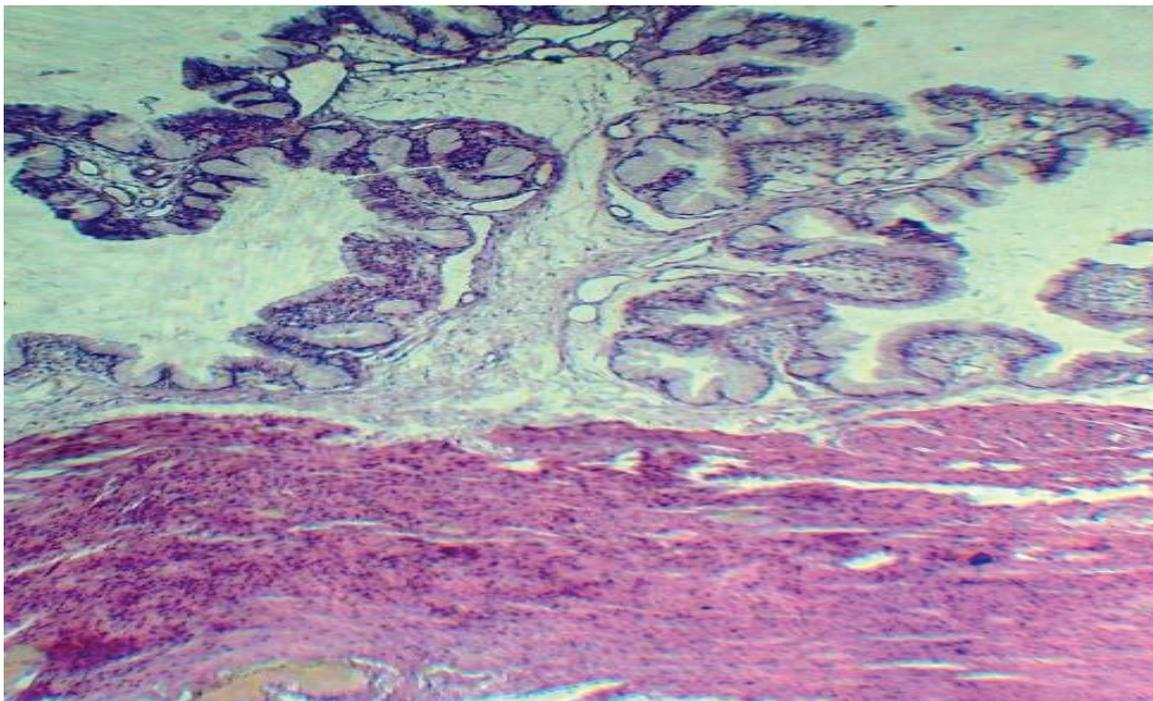
Figure(6) : Histopathologic section in vagina of rabbit in G3 showed: mild to moderate vacuolar degeneration of squamous epithelium, and mononuclear cells infiltration in lamina propria and in muscular layer perivascular, B shows normal villi and mucosal epithelium. (H&E stain, 400X).

In G4 narrow foldings of mucosa thrown into with mild MNCs infiltrate LP and submucosa, no inflammatory cells seen in muscular layer or in serosa.



Figure(7) : Histopathological section in vaginal of rabbit in G4 shows mucosal folding ,few mononuclearcell infiltrate lamina propria. (H&E stain, 100X).

In G5:the architecture of mucosa appeared as normal histology, in lamina propria very mild mononuclear cells seen, no congestion of vascular sinusoids in muscular layer. (Figure 8)



Figure(8): Histopathological section in vagina of rabbit in G5 shows: numerousinfoldings in mucosa, infiltrate lamina propria with lymphocytic cells. (H&E stain, 400X).

The overall summary the probiotic treatments are useful for managing common vaginal infections²⁷. The present study confirms the results of other reports in a quantitative manner, namely that probiotics as a supplement to conventional pharmacological treatments are effective in the short term for the treatment of common vaginal infections in non-pregnant adult females. However, high-quality evidence for the effectiveness of probiotics alone in recurrent or curative vaginal infections is limited. Further high-quality clinical trials are necessary to identify the most effective probiotic strains, the most effective treatment regimens (with or without antibiotics, and females (e.g. pre-menopausal vs. post-menopausal) that may benefit the most from probiotics.

The beneficial effect of both live and inactivated *S. cerevisiae* was due to a co-aggregation of *Candida* and consequently to its inability to adhere to the mucosal surface, protecting the vaginal epithelium from the fungus induced damage²⁸. However, only the live and not the attenuated yeast strongly suppressed some of the crucial virulence factors of *C. albicans*, such as its capacity to switch from the yeast to the hyphae form and the ability to express aspartyl proteases. These effects were related to the ability of the live yeast to significantly inhibit the expression of two important hyphae growth-associated genes, in particular the hyphae wall protein and extent of cell elongation, as well as the two secretory aspartyl proteinases, which play a key role in the immunopathogenesis of vaginal Candidiasis²⁹.

Conflict of Interest: None declared.

Ethical Clearance: Taken from institutional ethical committee.

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Impact of Dietary Ginger and Ginseng Powder in Broiler Diet on the Physiological Traits and Immune Response of Avian Influenza

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Abstract

The study aimed to investigate the synergistic effect of dietary ginger (Gn) and ginseng (Gs) powder on performance and evaluate the immune response to Avian Influenza (AI) vaccine in broilers. A total of 160 unsexed broiler chicks (Ross 308) at one day-old were randomly divided to four equal groups with two replicates. The chicks of the 1st group (control) were fed on a basal diet. The 2nd group fed on a basal diet with adding Gn at (5 gm/ kg feed). The 3rd group was fed on a basal diet with adding Gs (5 gm / kg feed). While the chicks of the 4th group were fed on a basal diet with adding Gn and Gs at (2.5 gm/kg feed) of each. Immune response against AI was measured at aged (5, 15, 25 and 35) days, while hematological and biochemical tests were measured at the end of the experiment. The results showed significant differences ($P \leq 0.05$) in 2nd group in the total protein and globulin concentrations as compared to other groups and control at 35 days old. No significant difference in albumin concentration, liver enzymes (ALT, AST). Also, the 2nd and 4th groups recorded a significant increase in WBC, RBC, Hb and PCV%, as well as, the superiority in the Ab against the AI at 35 days of age as compared to the other groups. Finding of the current study indicated that groups which receiving dietary Gn and Gs powder showed better health and higher Ab against AI disease vaccine.

Keywords: *Ginger, Ginseng, Avian Influenza.*

Introduction

The poultry industry in many regions of the world faces great challenges represented in finding new ways to confront epidemic diseases. There is no doubt that avian influenza is the biggest and most important of these challenges, and this disease does not only because heavy economic losses to the poultry sector, but also harms human health⁽¹⁾. Therefore, it is necessary to search for new means to curb this deadly

disease. The use of feed additives and nutritional herbs to enhance the health status and immune response of birds that are susceptible to infection may be one of those means, including the use of ginger⁽²⁾, ginseng⁽³⁾, vitamins⁽⁴⁾ and minerals⁽⁵⁾.

Ginger (*Zingiber officinale*) is a major crop, grown primarily in Central Asia. Ginger is a well-known plant that is widely used as a spice and in traditional medicine to cure a variety of ailments⁽⁶⁾. The main important compounds in ginger are gingerol, gingerdiol and gingerdione which have the ability to stimulate digestive enzymes, affect the microbial activity, and having antioxidant activity

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(7).Ginseng (*Ginseng panax*) also known as Asian ginseng, is one of the most well-known medicinal plants in the world, especially in Asian countries, and has been used for thousands of years to maintain bodily homeostasis and increase vital energy (8). Ginseng extract contains a number of important bioactive constituents, namely ginsenosides and flavonoids. It is regarded as an adaptogenic element that aids in the promotion of vitality, improved production performance, and metabolic mission (9). In this study, we investigated the synergistic effect of Ginger and Ginseng powder on some productive performance and some physiological characteristics of broiler chickens, as well as, evaluate the immune response to influenza disease vaccine.

Materials and Method

A total of 160 1-day-old broiler chicks (Ross 308) were randomly assigned to four experimental groups (40 chicks)each having tworeplicate. The experimental diets were prepared by adding certain dietary ginger(Gn),ginsengpowder (Gs) and both together that provided 0 (control), 5 gm, 5gm and (2.5gm+2.5gm) /kg feed in the basal ration. Starter and grower diets were formulated according to the recommendations (NRC, 1994). Water and feed were supplied *ad libitum* throughout the experiment. Immune response against AI was measured at ages (5, 15, 25, and 35) days, while hematological and biochemical tests were measured on the last day of the trial at (35 days) after randomly selected five broilers from each replicate to get the blood for tests.Data

was analyzed with SAS software (10) using LSD and ANOVA to compare between groups.

Results and Discussion

The results showed significant ($P \leq 0.05$) increased in WBCs, Hb and PCV% of the 2nd and 4thgroups as compared with the 3rd and control, while the count of RBCs recorded highest values in 2nd group than other groups (table 1).This increase in WBC count might be dietary ginger (Gn) and ginseng (Gs) powder were affected on the immune response of the vaccinated chicks with AI vaccine, also, the increase in RBC count, PCV and Hb concentration of birds fed the Gnare indication of improved oxygen carrying capacity of the cells which translated to a better availability of nutrient to the birds consequently affecting their well-being. Hematological constituents reflect the physiological responsiveness of animal to its internal and external environment, which includes feed and feeding.

Esonu et al., (11) who referred that hematological profile index a clear picture of the activity of the blood-forming organs, which are used as tools to assess the health and physiological status of farm animals.The blood profile in the body was influenced by nutrition, environment, breed, and the clinical conditions of the animal (12). Olafedehan et al.,(13) said the blood serves as a pathological reflector of the condition of an animal exposed to toxicants and other environmental stressors it is a means of assessing clinical and nutritional health of animals in feeding trials.

Table (1) Synergistic effect of dietary ginger and ginseng powder on blood picture of different groups at 35 days (mean \pm SE).

Groups Parameters	G1 (Control) Basal diet	G2 Ginger	G3 Ginseng	G4 Ginger with Ginseng
WBC(cell \times 103/ μ l)	28.00 \pm 1.90B	36.20 \pm 1.84A	32.80 \pm 0.69A	34.70 \pm 1.27A

Cont... Table (1) Synergistic effect of dietary ginger and ginseng powder on blood picture of different groups at 35 days (mean \pm SE).

RBC(cell \times 106 / μ l)	1.38 \pm 0.02B	1.61 \pm 0.03A	1.40 \pm 0.02B	1.43 \pm 0.04B
PCV%	33.00 \pm 1.34B	39.73 \pm 0.46A	34.41 \pm 0.55B	37.65 \pm 1.00A
Hb(gm/dl)	11.55 \pm 0.19B	13.38 \pm 0.16A	11.53 \pm 0.40B	13.08 \pm 0.34A

Means with a different letter in the same row are significantly different ($P \leq 0.05$).

Ginger is highly rich in protein and other nutrients like vitamin B and C, minerals in particular iron, and the sulphur containing amino acids, methionine and cysteine⁽¹⁴⁾. The current results agreed with results observed by Kehinde et al.,⁽¹⁵⁾ who reported that Gn supplementation on broiler diet had significantly ($P < 0.05$) higher PCV and HB concentration. In addition, our results confirmed that there is no toxicity associated with the consumption of Gn and Gs combination. Habibi et al.,⁽¹⁶⁾ found that Gs was not toxic, and hence had no negative effect on the health and physiological condition of the birds. Ginseng had positive significant effect on hematological profile due to the specific effects of saponins which is the main bioactive ingredient in ginseng⁽¹⁷⁾. Amaefule et al.,⁽¹⁸⁾ who observed that ginseng had a positive effect on packed cell volume of broilers fed ginseng and may have accelerated red blood cell formation from the bone marrow.

The results recorded significant ($P \leq 0.05$) increase in the 2nd and 4th group in Total protein (TP) and Globulin concentrations as compared with 3rd and control at 35 days of age as shown in table (2). Also, the data revealed that no significant ($P > 0.05$) difference among the groups in the Albumin and liver enzymes (AST and ALT) concentrations. The increased TP concentration in the serum of broilers supplemented with ginger is consistent with the enhanced antioxidant enzyme activity. Ginger has been shown to contain a high level of plant proteolytic enzyme⁽¹⁹⁾. Ginger could help birds in digest dietary protein upon ingestion^(20,21).

These results were confirmed by Zhang *et al.*,⁽⁶⁾ that referred that supplementation of Gn powder at the level of 5 gm/kg feed tended to increase growth rate and enhanced oxidative stability, increased TP in the serum of broilers. This positive effect is due to Gn powder being rich in bioactive compounds that improve the antioxidant status of the bird and improve protein metabolism.

Table (2) Synergistic effect of dietary ginger and ginseng powder on Total Protein, Albumin, Globulin and Liver enzymes concentrations for different groups (Mean \pm SE)

Groups Parameters	G1 (Control) Basal diet	G2 Ginger	G3 Ginseng	G4 Ginger with Ginseng
Total protein(gm/dl)	3.51 \pm 0.09C	3.92 \pm 0.06A	3.62 \pm 0.05BC	3.72 \pm 0.04B
Albumin(gm/dl)	2.30 \pm 0.07	2.39 \pm 0.06	2.32 \pm 0.04	2.35 \pm 0.05
Glubulin(gm/dl)	1.19 \pm 0.03D	1.65 \pm 0.03A	1.34 \pm 0.05C	1.46 \pm 0.03B
ALT(U/L)	26.06 \pm 1.17	27.40 \pm 1.30	27.40 \pm 0.40	28.60 \pm 1.71
AST(U/L)	47.77 \pm 1.10	46.67 \pm 0.41	49.14 \pm 1.94	48.02 \pm 1.19

Means with a different letter in the same row are significantly different ($P \leq 0.05$).

Generally, the increase in TP and globulin concentrations could be due to the role of ginger constitutes (Polyphenolic and flavonoids) the major sources of the antioxidant compounds. These constitutes prevent lipid hydro peroxide formation through their ability to quench free radicals ⁽²²⁾, thus G will protect hepatic cells from any oxidative damage. Khan et al. ⁽²³⁾ indicated that adding Gn to bird diets contributed to protecting the body cell membranes from damage caused by free radicals as a good antioxidant, and since the liver is an important organ in regulating body proteins and thus protecting liver cells from any damage, they contributed in not change protein concentration in the body. Also, table (3) showed that no significant ($P > 0.05$) difference in the liver enzymes concentration (AST and ALT) among the groups at 35 days age. Ginger has active biomolecules such as phenols and flavonoids that fight oxidative stress and subsequently protect the liver cells ⁽²⁴⁾. These results confirmed with Dias et al., ⁽²⁵⁾ and Emadi, and Kermanshahi, ⁽²⁶⁾ who demonstrate profound antioxidant, and hepatoprotective actions of ginger. There had no adverse effects of ginseng on the liver enzymes and health status of the chicks at (2.5% and 0.5%).

Antibody titers (Ab) against Avian Influenza disease (AI) vaccine measured by ELISA test, data of table (3) showed a significant ($P \leq 0.05$) higher Ab of the 2nd group than the 3rd, 4th and control. In addition, the 4th group increases antibody titer as compared with the 3rd and control at age 35 day. Increasing the immunoglobulin attribute to role of ginger in rising immunity response in the body by stimulates lymphocyte production, these results confirmed with results of ⁽²⁷⁾. Additionally, Ginseng inclusion in poultry diets enhances the immune function by increasing lymphocyte levels ⁽²⁸⁾. Ginger is associated with properties like antioxidant, antimicrobial, hepatoprotective, immunomodulatory, these positive results indicated that the effect of ginger in the preservation of the immune cells by inhibiting free radicals formation as a product due to cellular immune response during the phagocytosis process against microorganisms when the body attacks ⁽²⁹⁾. Rasha et al., ⁽³⁰⁾ concluded in a study that addition ginger in broiler diets at the level of 0.4%/kg had increased the serum concentration of total protein, albumin and globulin which is responsible of the humoral immunity. Supplementation of any antioxidants boosted endogenous antioxidants against oxidative stress and prevent lipid and protein oxidation ⁽³¹⁾.

Table (3) Synergistic effect dietary ginger and ginseng powder on antibody titers for Avian Influenza Vaccine measured by ELISA for different groups (Mean \pm SE).

Groups Age	G1 (Control) Basal diet	G2 Ginger	G3 Ginseng	G4 Ginger with Ginseng
5 days	5255.10 \pm 160.07 Aa	5205.40 \pm 110.07 Aa	5235.10 \pm 119.99 Aa	5267.10 \pm 133.75 Aa
15 days	1104.00 \pm 21.93 Ad	1050.00 \pm 7.38 Ad	730.00 \pm 44.95 Bd	728.00 \pm 21.60 Bd
25 days	1979.40 \pm 47.23 Bb	2685.00 \pm 66.08 Ac	1128.90 \pm 9.76 Cc	1322.60 \pm 40.75 Cc
35 days	1633.80 \pm 45.87 Dc	3410.80 \pm 144.45 Ab	1907.20 \pm 91.98 Cb	2198.00 \pm 104.73 Bb

Means with a different capital letters in the same row are significantly ($P \leq 0.05$) different. Means with a different small letters in the same column are significantly ($P \leq 0.05$) different.

Nawal et al.,⁽³²⁾ concluded that Gn powder have positively effect on the immune system by increase the antibody titers against viral diseases. Ginger enhances the serological response and had an antioxidant activity (both in vivo and in vitro) mainly due to pungent active principles such as gingerols and shogaols⁽²³⁾. Carrasco et al.,⁽³³⁾ revealed that Gn showed improvement in humoral and cell mediated immune response in immune suppressed mice. The adding of Gn and Gs may have helped in maintaining the cells of the immune system as good antioxidants, as well as enhancing the auto-antioxidants against vaccines and immune stress that occur due to them, as well as preventing the oxidation of fats and proteins in the body.

Ginseng is one of the most valuable medicinal herbs, its most notable features are modulation of the immune system, and activities against stress effect^(34,35). Several studies have demonstrated that the immunostimulatory functions of Gs could be due to the effects of polysaccharides⁽³⁶⁾. Xi et al.,⁽³⁾ reported that dietary maternal Gs polysaccharides supplementation would affect the immune function of sows and then improve the immune function and growth of pigs. The saponins and polysaccharides derived from Gs could enhance immunity in vitro and perform a variety of functions, including immune-modulation, anti-tumor, and anti-oxidant actives^(37,6).

Kim,⁽³⁸⁾ suggested that Gs may improve physiological function and immunity because this herb is rich in monosaccharides that meet most of the requirements and functions of the immune system. While, Ao et al.,⁽³⁹⁾ concluded that influence of fermented red Gs extract has positive effect on immune system of broilers and laying hens, that effects may

attribute to the saponins the main bioactive ingredient in ginseng⁽¹⁷⁾. Experiments of the farm animals indicated that Gs had adjuvant effects in stimulating antibody responses to immunization against various pathogens in cattle and pigs^(40,41). The results of this study indicated that dietary supplementation of ginger and ginseng improves performance and may be utilized as an immunostimulant, therefore, they can be considered as a potential functional ingredient to improve immune response against AI disease of broilers.

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Pharmaceutical Nano-Delivery Systems: A Review

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Abstract

Nanotechnology is the science that deals with the processes that occurs at molecular level and of nano-length scale size. Reduction of drugs toxicity and enhancement of their bioavailability, biocompatibility, stability and appropriate release are of high opportunities to be accomplished by size reduction, which make pharmaceutical field highly interesting in nanotechnology. Formulation and preparation of highly advanced nano-materials as drug delivery systems achieve the desired therapeutic objective. Current review will discuss in brief types of pharmaceutical nano-sized drug delivery systems by focusing on the specificity and the nature of each type.

Keywords: nanotechnology, pharmaceutical nanotechnology, nanoparticles delivery systems.

Introduction

Nanotechnology is still wide new science with advanced technologies and applications of different fields, nanomedicine and nano based drug delivery systems are very interesting applications of nanotechnology^{1,2}. Nano is used as a scientific units to denote one-billionth part (10^{-9}) of the base unit, where the size of materials start from 1nm to 100nm highly utilized or applied by nanomedicine such as biosensors and nano drug delivery such as liposome, nano suspension, dendrimers, carbon nanotubes, metal nano delivery systems and others^{3,4,5}. Nanoparticles have unique structural,

chemical, physical and biological properties. Nano technology expected to have substantial applications in medicine in disease diagnostics and therapeutics. nano delivery systems have become well appreciated in the last years where utilizing of nanostructures as delivery agents through incorporation of the drug in the nanocarriers or adsorption on them which promote high specificity and selectivity for drug localization and targeting^{6,7}. The first generation of nanoparticle-based drug delivery systems which are lipid based systems such as solid-lipid nanoparticles, liposomes and micelles^{8,9,10}. Nanostructures delivery systems help to deliver drugs of low water-solubility and/or even low drug oral bioavailability and targeting it to the specified site action which lead to reduce dose required, enhance drug efficacy, decrease toxicity, decrease drug resistance, decrease patient to patient drug effect variability and increase the stability of the drug and formulation¹¹. Nanostructures stay in the blood circulatory system for a prolonged period and enable the release of drug as per the specified dose. Thus, they cause fewer plasma fluctuations with reduced adverse side effects; hence, they directly

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interact to treat the diseased cells with improved efficiency and reduced or negligible side effects¹².

Types of pharmaceutical Nanotechnology based on the systems:

Nano-materials and nano-devices play a key role in pharmaceutical nanotechnology. Nano-materials are also further classified into nano-crystalline and nano-structured materials¹³. Nano-crystalline materials are pure crystals of the drug which readily manufactured and then stabilized by thin coating of surfactant; their nano-sized lead to highly enhance the surface area to the volume ratio also lead to enhance the bioavailability of low solubility or insoluble drugs¹⁴. On the other hand; nano-structured are processed from nano-materials that provide special shapes and functionality in which at least external dimension is in the range 1-100nm¹⁵. Those multifunctional nano-materials can be broadly sub classified by their most applications as follow:-

1-Lipid-based Nano-Materials

Lipids based nano-materials are highly attractive choice nano delivery system due to their natural components and easily scaled up syntheses processes.

Liposomes:

Liposomes were the first nano-materials where it was applied as a drug delivery system. Liposomes are artificial vesicles composed from amphiphilic phospholipids and cholesterol which give self-association forming bilayer encapsulate an aqueous interiorly, liposomes still the most popular nanocarrier which used due to their ability to incorporate hydrophilic, hydrophobic and also amphiphilic drugs; where hydrophilic drugs incorporated in the aqueous compartment where the hydrophobic drugs are localized in the lipid membrane while the amphiphilic drug substances located at the lipid-aqueous interface, liposomes are biocompatible and biodegradable

components^{16,17}. Doxorubicin (Doxil[®]) was developed by Janssen Company is the first liposomal formula approved by FDA¹⁸.

Nanoemulsion:

Nanoemulsion is the dispersed modern colloidal system having isotropically clear or transparent dispersion of two immiscible liquid phases as oil and water where their stabilization can be done using an interfacial film of surfactant molecules; the dispersed phase droplets size is about 50-200nm, nanoemulsion preparations used to deliver drugs to enhance their solubility, stability and also bioavailability such those preparations as vitamins nanoemulsionpreparations¹⁹. Taha et al. reported a two folds enhancement in the bioavailability of vitamin A through self-emulsifyingnanoemulsion in compare with the free vitamin A²⁰. Propofolnanoemulsion (Diprivan[®]) developed by Astra Zeneca where it was approved in 2001 by FDA²¹.

Solid lipid Nanoparticles:

Solid lipid nanoparticles (SLN) are nanocarriers developed as a substitute colloidal drug delivery systems parallel to liposomes and lipid emulsion delivery systems, SLN are colloidal particles composed of solid lipid core which may contain triglycerides, glycerides mixtures or waxes, which being as solid in both human body temperature and room temperature, SLN are show distinctive features such as low toxicity, large surface area, prolonged drug release, superior cellular uptake in compare with the traditional colloidal carriers as well as compatibility to improve solubility and bioavailability of drugs. SLN can also further produced in powder form where it can be loaded in pellets, capsules or tablets for further enhancement of drug delivery^{22,23}. Pharmatec Company developed in 2006 the first cyclosporine SLN formulation for oral administration²⁴.

Nano-Structure Lipid Carriers:

Nano-Structured Lipid Carrier (NLC) referred to the second generation of SLN where some limitations of SLN such low drug loading efficiency can be overcome by NLC, NLCs are biocompatible systems distinguished by a rigid morphology that contributes their unique properties compared to other lipid-based formulations. NLCs can prepare by blend of solid-phase and liquid phase lipids; which generally in solid state at temperature above 40°C. The lipid matrix of NLC has an imperfect crystal or amorphous structure which allow for loading in both molecular form and in clustered aggregates form at lattice imperfections²⁷. SLN and NLC have the ability to protect liable molecules those are susceptible to hydrolysis such as peptides and proteins²⁵.

2-Polymer-Based Nano-Materials***Dendrimers:***

Dendrimers are nano-sized radially symmetric molecules with well-defined, homogeneous and monodisperse structure consist of tree-like arms or branches three dimensional 3D structures of synthetic polymers²⁶. These hyperbranched molecules were first discovered by Fritz Vogtle in 1978, by Donald Tomalia and co-workers in the early 1980s²⁷. Dendrimers are nearly monodisperse macromolecules that contain symmetric branching units built around a small molecule or a linear polymer core²⁸. Polyamidoamine dendrimers were developed first in 1985; which produced by controlled polymerization and became the most popular one. Dendrimers can be used as nano-delivery system of drugs, vaccines and gene delivery²⁹.

Polymeric Nanoparticles:

Polymeric nanoparticles are colloid solid particles (nanospheres and nanocapsules) prepared by biocompatible and biodegradable polymers

which offer complete drug protection. Drugs can be incorporated into nanoparticles by dissolution, adsorption entrapment, attachment or encapsulation; which provide controlled and sustained release of drugs for longer period of action as days or weeks. Stealth and surface modified polymeric nanoparticles can be used for passive and active delivery of bioactive³⁰. Eligard[®] developed by Tolmar Company is leuprolide acetate and poly lactide co glycolide nanoparticles formulation approved by FDA³¹.

Polymeric Micelles:

Polymeric Micelles (PMs) are nanocarriers that are formed by spontaneous arrangement of amphiphilic block copolymers in aqueous solutions. These nanoparticles have a hydrophobic core-hydrophilic shell architecture that facilitate the loading of hydrophobic drugs in the core lead to improve the solubility of these water insoluble drugs; PMs offer high entrapment efficiency, payload, stability in physiological condition, long circulating and target site accumulation³². Estrasorb[®] developed by Novavax Company is micellar estradiol formulation approved in 2003 by FDA³³.

Polymer-Drug Conjugates:

Polymer-drug conjugates act as a prodrug, site-specific drug delivery nanocarriers formed by the conjugation of low molecular weight drugs with polymer which cause drastic change in pharmacokinetic of drug in whole body and at cellular level. They are designed to increase the overall molecular weight which facilitate their retention in cancer cells as well as enhance drug activity of multidrug resistance cells^{34,35}, also these conjugates offers drug protection from degradation and reduce the premature drug release and harmful side effects on healthy cells. Oncaspar[®] developed by Sigma-Tau Company is polyethylene glycol pegaspargase conjugate approved by FDA in 1994³⁶.

3-Carbon-Based Nano-materials

Carbon Based Nanomaterials (CBNs) have drawn significant interest in diverse areas due to their distinctive structural dimensions and physicochemical properties, based on the structure of CBNs are categorized as carbon nanotubes, graphene, mesoporous carbon, nanodiamond and fullerenes, these materials demonstrated higher drug-loading capacity, improve biocompatibility and lower immunogenicity. CBNs are extremely useful in various biological applications as novel carriers to deliver drugs or vaccines with loading capacity and extended blood circulation time³⁷. Yang et al. developed epirubicin-loaded carbon nanoparticles, which have the ability to release the drug slowly and found that significantly ($p < 0.001$) enhanced the drug concentration in the metastatic lymph nodes in patients with breast cancer³⁸.

4-Inorganic-Based Nano-Materials

Ceramic Nanoparticles:

Ceramic nanoparticles are primarily made up of oxides, carbides, phosphates and carbonates of metals and metalloids such as calcium, titanium, silicon, etc. ceramic nanoparticles are mesoporous particles have high mechanical strength with highly resistant to environmental changes. Ceramic nanoparticles are biocompatible in nature and easily engineered to desired size and porosity in addition to high heat resistance and chemical inertness that make ceramic nanoparticles have a wide range of applications³⁹. Ceramic nanoparticles are considered to be excellent carriers for drugs, genes, proteins, imaging agents etc. which provide the complete protection to these entrapped molecules against the denaturing effects of external pH and temperature. The first successful ceramic drug loading was performed by Mattie et al. in 1988 the study reports the delivery of several drugs such as (danazol and dihydrotestosterone) loaded

aluminocalcium phosphorous oxide nanoparticles, which demonstrating that the nanoparticles can release the steroidal drug uninterruptedly for one year⁴⁰.

Quantum Dot:

Quantum dot nanoparticles are semiconductor nanomaterial's with intrinsic chemical and physical properties, these nanoparticles have particular optical and electronic properties such as size-tunable absorption bands and emission colors due to quantum confinement effect. Quantum dot nanoparticles can artificially synthesized from II to IV and III to V elements such as Cd, Te, Zn, Se, etc. these nanoparticles have physical dimension of 2-10nm that make them a more reliable and influential candidate in most of industrial applications also these nanoparticles are widely used in biological applications that require fluorescence including DNA array technology, cell biology and immuno-fluorescence assays, particularly in immuno-staining of proteins, actins, microtubules and nuclear antigens for cancer cell detection⁴¹.

Metallic Nanoparticles:

Metallic Nanoparticles are particulate materials have novel electrical, optical, physical chemical and magnetic properties such as gold, silver, copper, iron, platinum, cobalt, nickel and palladium⁴². Metal nanoparticles have prime importance for various biomedical applications due to unique Plasmon absorption peak at visible region, surface functionalization ability and high stability; which can be used for delivery of drugs and genes, diagnostic assay with high sensitivity, magnetic resonance imaging, radiotherapy enhancement, protein bioseparation and thermal ablation. Recently metal nanoparticles are universally considered as a promising multifunctional platform for wide purposes particularly in the case of cancer⁴³. INFed[®] developed by Sanofi Aventis Company, is the first nano-structured material approved by FDA in 1974 for the

iron deficiency in chronic renal disease⁴⁴.

Conclusion

Since the discovery of nanotechnology and development in nano-scale products enable highly growth in several fields especially pharmaceutical nanotechnology and drug nano delivery systems which lead to enhance the therapeutic efficacy of the drug with reduction of their toxicity, also observed a significant improvement for drug selectivity and specificity at the targeted site of action of numerous diseases and infections from cancer to other several diseases and gene therapy and vaccination.

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The Legal Responsibility to the Family for Shackling People with Mental Illness During the Covid-19 Pandemic

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Abstract

During the COVID-19 pandemic until June 2020, there were 277,000 mental health cases in Indonesia. The number increase compared to 2019. The number of shackling due to mental illness increased from 5,200 in 2019 to 6,200 in 2020. Shackling is strictly prohibited because it violates the law and human rights. Criminal sanctions in Article 333 of the Criminal Code are prepared for those who shackle people with mental illness. The punishment stipulated in this article is also applied to people who intentionally give space to the deprivation of other people's freedom. The program of "Indonesia Shackle-Free" was launched in 2010, the target was forced to be pushed back until 2019 and remains challenging to achieve. The number of shackling increases due to a lack of public understanding of mental health. This paper aims to explore further the relationship between the COVID-19 pandemic and the condition of people with mental illnesses (ODGJ) in Indonesia. Another impact of the COVID-19 pandemic on ODGJ is drug withdrawal because of the disruption of drug circulation during the pandemic. The allocation of health financing is also focused on dealing with the pandemic. Promotive efforts are expected to eliminate stigma, discrimination, and human rights violations for ODGJ in the community. Whereas, preventive efforts by developing parenting styles that support mental growth and development, communication, and education in the family are expected to provide appropriate mental health services for ODGJ when entering the rehabilitation stage.

Keywords: *Shackling, People with Mental Illnesses (ODGJ), COVID-19*

Introduction

The Coronavirus disease (COVID-19) pandemic raises new problems for people's mental health. One that is closely related and affects mental health is social restrictions aimed at minimizing transmission of

the disease. Before the pandemic, as social creatures, people interacted directly with other people, but the current situation causes someone to limit direct interaction with others suddenly. It is complicated matters to some extent, especially in uncertain situations, which creates anxiety, worry, fear, stress, and even depression.¹

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As the Ministry of Health noted, during the COVID-19 pandemic, there are 277,000 mental health cases in Indonesia. The number of mental health cases has significantly increased compared to 2019, with only 197,000 cases. The community's limited access and social problems experienced by the community

during the pandemic, which leads to depression, become the leading cause of mental health cases increasing number. Besides affected economically, people also experience stress and mental pressure due to layoffs and social restrictions.²

The Indonesian Psychiatric Association (PDSKJI) surveyed mental health through an online self-examination. The survey involved 1,552 respondents regarding the three psychiatric problems. It was anxiety, depression, and trauma. Based on the result, most respondents were women (76.1%), aged ranging from 14 to 71 years. Further examinations were carried out on 2,364 respondents in 34 provinces, and the results were not much different from the previous examination, with 69% of respondents had psychological problems, 68% had anxiety, 67% had depression, and 77% experienced psychological trauma.³

The family plays a crucial role in caring for people with mental illnesses (hereinafter referred to as ODGJ). The role of the family, among others, is related to treatment, such as delivering treatment, getting medicine, ensuring medicine consumption, and fulfilling daily life such as controlling personal hygiene and supervising daily activities. However, some families abandon them on the grounds since the status of ODGJ gives a bad image and creates discrimination by the community. The family feels ashamed and tries to cover up the existence of ODGJ in the family by ostracizing, expelling, and even taking shackles on them.

The stigma given by the surrounding community to the family members harms the recovery of ODGJ. It causes feelings of sadness, shame, irritation, shock, restlessness, and blaming each other, ultimately affecting the quality of treatment and daily life of ODGJ. Sometimes, the family will hide and even shackle ODGJ to avoid the lousy stigma and disturbing behaviors of ODGJ itself.⁵

The result of Moh. Abdul Hakim's survey has shown the impact of the COVID-19 pandemic on psychosocial conditions in Indonesia. It showed that 27% of the 1,319 participants experienced acute stress due to social restrictions, lack of basic needs, threats of infection, and behavioral adjustments. A similar survey conducted on the community and ODGJ by Ipsos MORI (Holmes et al., 2020) also showed concerns about social isolation or restrictions, increased anxiety, depression, stress, and other negative feelings, including financial difficulties, recurrence of pre-existing mental disorders, and difficulties in accessing mental health support and services.⁶

The increasing number of families who use shackles in caring for ODGJ becomes the critical point why it is necessary to write a paper outlining the legal responsibilities of the families who practice Shackling. This explanation needs to be studied to make the community, especially family, aware that shackling is included in violating human rights against ODGJ.

Discussion

Right of ODGJ to Receive Health Services and Free from Shackling

The arrangement of legal protection towards ODGJ in health services is included in Article 70 paragraph (1), Law No. 18 of 2014 on Mental Health (Mental Health Law), which describes ODGJ rights. These rights include the right to obtain mental health services in easily accessible health care facilities and to obtain protection from any form of neglect, violence, exploitation, and discrimination.⁷

Guarantees for ODGJ to have the same rights as citizens are also stated in Law No. 36 of 2009 on health, article 144 paragraph (1) and 148 paragraph (1) and (2). Based on this law, ODGJ receives equal treatment in every aspect of life, including health

services in easily accessible healthcare facilities according to mental health service standards. ODGJ's daily life should be protected from every form of neglect, violence, exploitation, and discrimination. All their social needs are met according to the level of mental illness through the family's involvement. As the government's hope, the ODGJ can achieve the best quality of life and enjoy a healthy mental life, free from fear and imprisonment.⁸

Director of Prevention and Control of Mental Health and Drug Problems, SitiKhalimah, explained an increase in shackling cases due to mental illness from 5,200 in 2019 to 6,200 in 2020. This increasing number is another impact of the COVID-19 pandemic. Shackling is strictly prohibited because it violates human rights (HAM). Therefore, the government has targeted Indonesia to be free from Shackling starting in 2010. However, because it is challenging works with much effort, it was postponed to 2014, then 2016, and the last to 2019.⁹In many cases, once the shackle is released, patients do not receive any further treatment, in the worst case, sometimes the patients are abandoned by their families. It is terrible since it triggers the recurrence of patient problems such as angry, threatening, even violent behavior towards themselves, others, and the environment. This situation occurs because of the lack of support from the family in the patient's ongoing treatments. Family support refers to attitude, action, and acceptance of the family towards the patients. In the view of family members, supportive people are always ready to provide help and assistance if needed.

Treatment for ODGJ is Costly and Continued

According to a study conducted by The Conversation in collaboration with the Center for Excellence in Science and Technology for the Pharmacy Service Innovation University of Padjadjaran, the average medical cost per patient per year was around Rp 3,307,931 for schizophrenia, Rp

17,978,865 for bipolar disorder, Rp 1,601,850 for depression, and Rp 1,190,563 for anxiety disorders. If it is assumed all patients get treatment for one year, then the highest direct cost nationally is the treatment for bipolar disorder, which reaches Rp 1.5 trillion, and the total cost for all cases of mental disorders can reach Rp 87.5 trillion. Despite the cost of treating ODGJ is covered by the Health Care and Social Security Agency (BPJS), in practice, patients and their families still have to bear the costs of transportation and other costs.¹⁰

The central government, regional governments, and the community not only must work hand in hand to prevent and strengthen health services through promotive, preventive, curative, and rehabilitative approaches. It must also be conducted in a comprehensive and integrated way, including the financing issues. All efforts need the collaboration of all parties since ODGJ needs continuous treatment, and it is costly.¹⁰

Shackling in Indonesian Laws

Shackling is contrary to human rights and legislation. The problem of shackling arises because ODGJ often goes on a rampage and endangers the environment and family. It is also supported by the lack of family knowledge about the legal impact of shackling. The shackling matter is regulated in Article 28 of the Constitution of the Republic of Indonesia. As stated, the right of everyone to have freedom of thought and conscience is not to be tortured or deprived.¹¹ The shackling of ODGJ is an act that makes them tormented since it forces ODGJs to stay in one place with their legs kept in chains. It is torturous because their freedom for regular activities as human beings is taken away. Shackling is also classified as an act of not respecting the personal rights of ODGJ. Therefore, the rights of ODGJ must be respected by the law even though they cannot state their rights.

According to Article 42 of Law No 39 of 1999 on Human Rights, ODGJ has the right to receive exceptional care, education, training, and assistance at the state's expense. The statement clearly explains that keeping ODGJ in shackles is a form of deprivation of human rights of freedom.¹² The act of deprivation includes giving no attention to the special care and treatment required by ODGJ. Moreover, the state guarantees the financing spent for exceptional care and assistance for ODGJ so that families should not carry out Shackling for ODGJ.

As stated in Article 147, Article 148 paragraph (1), and Article 149 of the Health Law, the central government, regional government, and the community are responsible for curing ODGJ by authorized health workers and are still respecting human rights. Shackling is contrary to the above provisions. It is not an attempt to cure and is not treated by authorized health workers, violating human rights. Treatment of ODGJ requires special and standardized health facilities and is performed by trained health workers instead of keeping the patient in Shackling. Article 148 paragraph (1) of the Health Law stipulates that ODGJs have the same rights as citizens, so they must be free from Shackling. Similarly, Article 149 of the Health Law clarifies that ODGJs must receive treatment in health care facilities rather than keeping them shackled.^{7,13}

The state has guaranteed all treatment and care for ODGJ. There is no more space for the family to do Shackling. Shackling, as previously described, is a violation of human rights. It has the same meaning as deprivation of liberty which makes the condition of ODGJ get even worse. The state has declared Indonesia free from Shackling in 2019, but unfortunately, the program faced many obstacles and has not been realized. To support the program, Indonesia stipulates criminal sanctions against families who practice Shackling.¹³

Article 333 of the Criminal Code (KUHP) states, "Anyone who intentionally and unlawfully robs someone's freedom, or continues to deprive someone's freedom is threatened with a maximum imprisonment of eight years."¹⁴ The punishment provided in this article is also applied to people who intentionally and unlawfully provide space for deprivation. The deprivation of ODGJ means taking someone's freedom to have daily activities without restrictions. Shackling can also result in serious physical injuries to ODGJ, and for this case, the threatened punishment is nine years. Moreover, if the Shackling result in death, the punishment for the perpetrator will be heavier; the threatened punishment is twelve years.¹⁴ By seeing the criminal threats faced by families, the family should think twice about putting ODGJ in Shackling. Unfortunately, the lack of socialization and education to the public regarding the threat of criminal punishment against those in Shackling has made Shackling is still practiced.

The Role of the State and Families in Dealing with Shackled-ODGJ

Human rights are a dimension of human life. It is inherent in a person not because it is given by society or the state's goodness, but it is based on his dignity as a human being. The right to health is a fundamental right, and it is a basic human right inherent as the dignity of a human being. Fulfillment of the right to health for ODGJ is one of the main focuses for the government. The behavior and recurrence of ODGJ must be controlled to place them no longer discriminated against families and communities as they are giving lousy luck and cursed.¹²

Article 3 of the Mental Health Law states that mental health efforts aim to provide and guarantee mental health services for ODGJ based on human rights. Meanwhile, As stated in Article 3 letter G, mental health efforts aim to provide opportunities for ODGJ to obtain their rights as Indonesian citizens.

Referring to Article 3 letters C and G, Shackling is in big contrary to what is written in the two articles. Shackling is identical to depriving a person of human rights, negating guarantees of protection from the state, and violating someone's rights as an Indonesian citizen.⁷ Regarding the Shackling, the government, through the Ministry of Social Affairs, had launched an Indonesia Shackle-Free in 2019. A noble vision considering the ODGJ should be treated like humans, in general, They are citizens whose rights must be protected by the government.

Integrated health efforts by the government, community, and family are needed in dealing with shackles. Several steps as follow:

1. The government needs to disseminate information on mental health actively. Eliminate the negative stigma against ODGJ as cursed individuals. It is undeniable that a lack of understanding of ODGJ creates various perceptions in society, stigma, and rejection. As explained by Nxumalo in his research report conducted in Kwazulu-Natal, stigma in ODGJ is a severe problem, has significant consequences for these ODGJs and their families.¹⁵ Various stigma can afflict families with ODGJ, including isolation from the family, exploitation, neglect from the community, and labeling and stereotyping.¹⁶ It is necessary to educate the public regarding myths and facts related to ODGJ to reduce the stigma experienced by families and ODGJ. Support from the community, basic education, and various government and private sectors are very much needed. Promotive efforts aimed at eliminating negative stigma, discrimination and violations against ODGJ as part of the community are contained in Article 7 letters b and c on the Mental Health Law.^{7,17}

2. Shackles on ODGJ still occur due to some reason. First is the public's lack of understanding about mental health diseases. Then, one fact of the shackling is that the families do not know what they

have done by keeping the patients in chain robs the patient's rights. Third, the families do not know the legal consequences of doing shackling. Promotive efforts should be made to increase public knowledge about ODGJ. With a promotive effort, the community will handle ODGJ properly and know what to do, so there is no option to do shackles. It can be internalized in the family, educational institutions, workplaces, communities, health service facilities, mass media, religious institutions, places of worship, even in prisons and detention centers. In the family, the promotive effort focuses on parenting and communication patterns to support the growth and development of a healthy soul. In this case, it is very crucial to understand the risks of handling ODGJ with shackling, including the legal risk. The family must know all the risk as to the closest community to ODGJ.^{7,15}

3. The family of ODGJ and the community are actively involved in eradicating shackling in Indonesia. The community now needs to report the shackling that occurred for ODGJ in their immediate environment to the authorities to avoid more cases of shackling. The role of the community is stated in Article 85, letter b of the Mental Health Law, which is to report the ODGJ in need.⁷ ODGJ in shackling are included as a person in need. If the family does not have the awareness to free the shackled ODGJ, the community must report the incident of shackling to the authorities. It is expected that the shackled ODGJ can be released immediately. ODGJ has the right to have free life as citizens are the view the family must understand. A persuasive approach to families is needed. Another view the families must understand is that the state is responsible for protecting the right of ODGJ with the issuance of a law that regulates it. The family should be at the forefront of maintaining mental health and the first party to provide psychological help once mental health illness symptoms appear instead of carrying out shackling that violates the law and

human rights. As stated in Article 86 of the Mental Health Law, any person who intentionally detains, neglects, abuses, and induces others to detain, neglect, and violent the ODGJ or other actions that violate the ODGJ's human rights be punished following the provisions of the legislation.⁷

Conclusion

Shackling is contrary to the provisions of laws and regulations. It has been regulated regarding the prohibition of shackling on ODGJ. Something to improve is the implementation, and it is expected that a sense of justice is obtained for all ODGJ, and the target for Indonesia free from shackling is achieved. The responsibility for the families of ODGJ is to increase understanding that shackling is an unlawful act and can be criminalized. Families also need to provide accurate and fast information to health service providers to provide appropriate care for ODGJ. Therefore, the ODGJ becomes productive and independent human beings like others.

Ethical Clearance: Nil

Conflict of Interest: Nil

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Molecular Study Using Real Time PCR to Detect Influenza Viruses (Flu A, Flu B and RSV) in Patients at Ramadi Hospital

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Abstract

Background: Because of the increasing incidence of infections with different types of influenza of various types, type A, B, and SRV, this study included investigating the type of infection in influenza in women and men in different age groups and making a comparison between them. **Methodology:** 90 blood samples were collected, including 30 samples for men 30 samples for women showing clinical symptoms of infection, and 30 samples from people with clinical symptoms under control. These samples were placed in tubes containing an anticoagulant to ensure that blood does not clot DNA/RNA extraction kit, CerTest, Spanish Company and detection by Flu A, Flu B and RSV, CerTest, Spanish Company by using real time PCR. **Result:** Table 1 shows the investigation of some types of influenza in women who showed symptoms of infection and who are between 20 to 40 years old, where the frequency of infection in women between the ages of 20-30 years was high with types Flu A, SRV compared with control and did not show infection with type B for the same Age group . As for women aged between 30-40 years, the frequency of type B infection was high compared to control, and there were no infections with Flu a and SRV for the same age group .

Keyword: Real time PCR , Flu A, Flu B and RSV

Introduction

There are three sorts of seasonal influenza A, B, and C. Type A influenza viruses additionally department into subtypes in accordance to combos of two unique proteins, haemagglutinin (H) and neuraminidase (N), placed on the floor of the virus. (1,2) Among the many influenza A virus subtypes,

the two subtypes A (H1N1) and A (H3N2) are currently circulating in humans. (3,4) The communicable influenza virus A (H1N1) is referred to as A (H1N1) pdm09 as it brought about pandemic influenza in In 2009 it due to this fact changed the seasonal influenza A (H1N1) virus that used to be circulating earlier than 2009. Influenza viruses by myself are recognized to purpose pandemics (5,6) Influenza B viruses can be divided into two most important training (strains) referred to as the B / Yamagata stress and the B / Victoria strain. Type B influenza viruses are no longer categorized as

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subtypes^(7,8) Influenza viruses of kind A and B flow into and motive outbreaks and epidemics. ^(9,10) For this reason, the applicable lines of influenza viruses A and B are covered in seasonal influenza vaccines. Influenza C virus is detected solely in uncommon instances and typically motives slight infections and for this reason has much less extreme public fitness effects.^(11,12) Seasonal flu is characterised by way of a unexpected excessive physique temperature, cough (usually dry), headache, muscle and joint pain, extreme nausea (malaise), sore throat, and runny nose. You can have a extreme cough that lasts two weeks or more. Most sufferers unravel their fever and different signs and symptoms inside one week besides desiring scientific attention. However, influenza can reason extreme sickness or even dying if it impacts one of the high-risk businesses (see below).^(13,14) The duration between the acquisition of the contamination and the onset of the disease, recognized as the incubation period, lasts about two days. ⁽¹⁵⁾

Methodology

Samples collection

90 blood samples were collected, including 30 samples for men 30 samples for women showing clinical symptoms of infection, and 30 samples from people with clinical symptoms under control.

These samples were placed in tubes containing an anticoagulant to ensure that blood does not clot

RNA Extraction and Detection

DNA/RNA extraction kit, CerTest, Spanish Company and detection by Flu A, Flu B and RSV, CerTest, Spanish Company by using real time PCR ..⁽¹⁶⁾

Reagents and equipment to be supplied by the user The ⁽¹⁷⁾

Creating PCR test programmer for VIASURE Flu A, Flu B & RSV Real Time PCR Detection kit . ⁽³⁾

Result and Discussion

All flu subtypes contain different strains of the influenza virus. Not all strains infect people. Subtypes of influenza A viruses that currently appear in people are H1N1 and H3N2 strains. Each year the influenza vaccine contains a variety of these two strains and influenza B. In most cases, the body’s immune system will fight the virus itself. But some people will experience additional complications. These complications are more common in older adults and those with conditions that affect their immune systems. Taking immunosuppressant medications may also increase the risk of complications.

Table (1) Screening for the three types of influenza (Flu A, Flu B, SRV) in women whose ages range from 20 to 40 years old

Descriptive Statistics				
Dependent Variable: Number of Virus copy				
age of woman patient	type of Flu	Mean	Std. Deviation	N
20-30 year	FLU A	34.60	8.081	5
	FLU B	3.40	1.140	5
	RSV	35.00	5.874	5
	Total	24.33	16.238	15

Cont... Table (1) Screening for the three types of influenza (Flu A, Flu B, SRV) in women whose ages range from 20 to 40 years old

30-40 year	FLU A	3.80	.837	5
	FLU B	39.60	16.682	5
	RSV	3.00	.707	5
	Total	15.47	19.799	15
control	FLU A	2.80	.837	5
	FLU B	3.20	.837	5
	RSV	3.40	1.140	5
	Total	3.13	.915	15
Total	FLU A	13.73	15.890	15
	FLU B	15.40	19.845	15
	RSV	13.80	15.848	15
	Total	14.31	16.917	45

Table 1 shows the investigation of some types of influenza in women who showed symptoms of infection and who are between 20 to 40 years old, where the frequency of infection in women between the ages of 20-30 years was high with types Flu

A, SRV compared with control and did not show infection with type B for the same Age group . As for women aged between 30-40 years, the frequency of type B infection was high compared to control, and there were no infections with Flu a and SRV for the same age group.

Table (2) ANOVA table for Screening of the three types of influenza (Flu A, Flu B, SRV) in women whose ages range from 20 to 40 years old

Tests of Between-Subjects Effects					
Dependent Variable: Number of Virus copy					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	11058.444a	8	1382.306	32.457	.000
Intercept	9216.356	1	9216.356	216.403	.000
age	3400.844	2	1700.422	39.926	.000
Flu type	26.711	2	13.356	.314	.733
age * Flu type	7630.889	4	1907.722	44.794	.000
Error	1533.200	36	42.589		
Total	21808.000	45			
Corrected Total	12591.644	44			

a. R Squared = .878 (Adjusted R Squared = .851)

Table 2: Analysis of variance for the detection of the three types in women whose age ranges between 4-20 years, as it was noted that there are significant differences between influenza type A for women within the age group 20-30 years compared to control and compared to the age group between

30-40 years while the second type From influenza B, there were clear significant differences in the large age groups, located between 30-40, compared to control and also compared to the younger age groups.

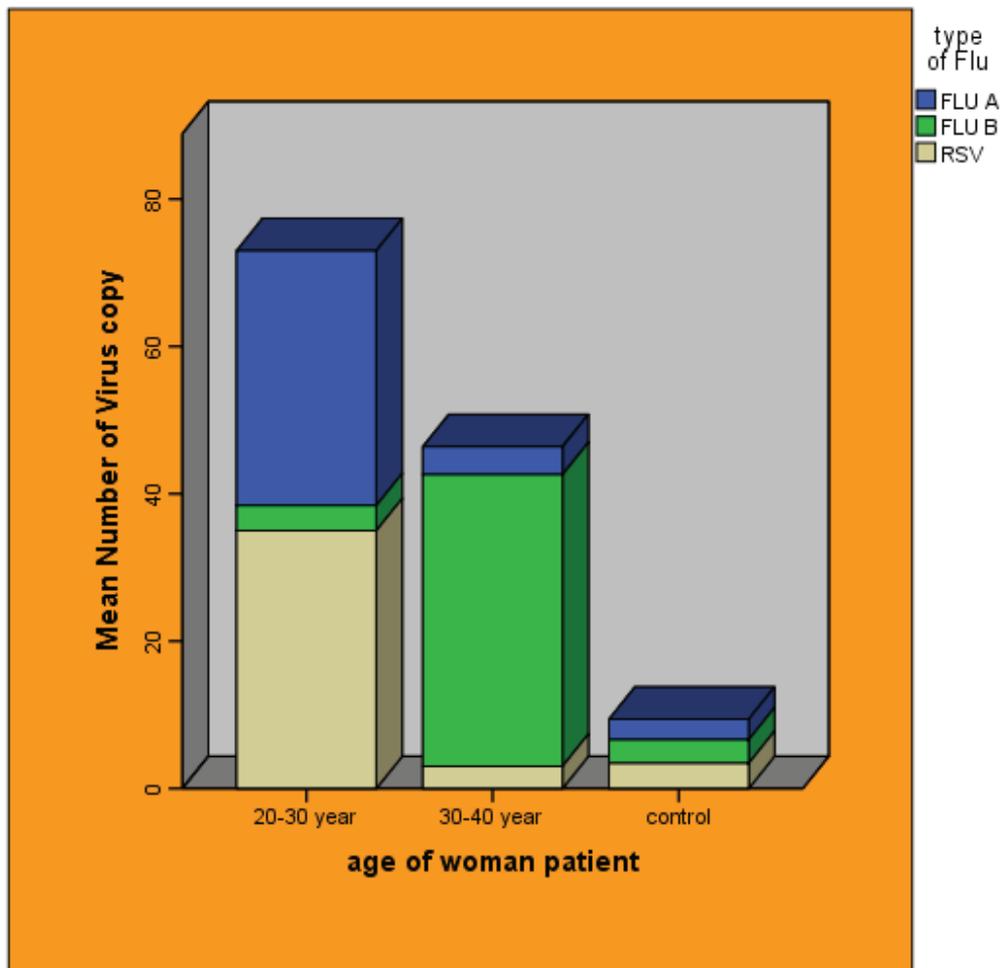


Figure 1 detection of three types of influenza (Flu A, Flu B, SRV) in women whose ages range from 20 to 40 years old

Table (3) Screening for the three types of influenza (Flu A, Flu B, SRV) in men whose ages range from 20 to 40 years old

Descriptive Statistics				
Dependent Variable: Number of Virus copy				
age of men patients	type of Flu	Mean	Std. Deviation	N
20-30 year	FLU A	27.00	5.958	5
	FLU B	3.80	.837	5
	RSV	4.40	1.517	5
	Total	11.73	11.659	15
30-40 year	FLU A	33.80	7.791	5
	FLU B	3.60	1.140	5
	RSV	4.00	1.581	5
	Total	13.80	15.256	15
control	FLU A	4.60	1.517	5
	FLU B	6.40	1.140	5
	RSV	2.80	.837	5
	Total	4.60	1.882	15
Total	FLU A	21.80	13.960	15
	FLU B	4.60	1.639	15
	RSV	3.73	1.438	15
	Total	10.04	11.589	45

Epidemiological data from Hong Kong showed that adult men are more at risk of hospitalization due to influenza. An American study revealed that men died more often from influenza compared to women of the same age, regardless of other underlying diseases such as heart, cancer, and organs. It was found that this gap in immunity, may be caused by hormonal changes, as the male hormone testosterone suppresses the immune system, while the hormone estradiol protects it. It is not usually

known that testosterone is an immunosuppressive, but one study found that men who have levels The higher testosterone they have had less response than the vaccination antibody. Table 3 shows the investigation of the three types of influenza in men whose age ranges between 20-40 years. It was found that there are infections in men whose ages range from 20-30, as well as men between the ages of 30-40 years in influenza type A and no infections have appeared. The other is type B and SRV.

Table (4) ANOVA table of Screening for the three types of influenza (Flu A, Flu B, SRV) in men whose ages range from 20 to 40 years old

Tests of Between-Subjects Effects					
Dependent Variable: Number of Virus copy					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5480.711a	8	685.089	57.463	.000
Intercept	4540.089	1	4540.089	380.809	.000
age	698.978	2	349.489	29.314	.000
Flu type	3114.978	2	1557.489	130.637	.000
age * Flu type	1666.756	4	416.689	34.951	.000
Error	429.200	36	11.922		
Total	10450.000	45			
Corrected Total	5909.911	44			

a. R Squared = .927 (Adjusted R Squared = .911)

Table 4 Analysis of variance shows that there are significant differences in the incidence of influenza A type in the age group 20-30 and 30-40 years compared to other types of influenza and compared to control and there are no significant differences for type B and type SRV. Available research indicates that men suffer worse from colds and influenza than women, calling for “better

quality research” to prove this. Hospitalization rates are consistently higher for younger (before puberty) and older males (over 65 years of age). During a woman’s reproductive years, she often suffers from more severe illnesses, partly because the flu is worse for pregnant women, but also because women develop a greater inflammatory response to the flu.

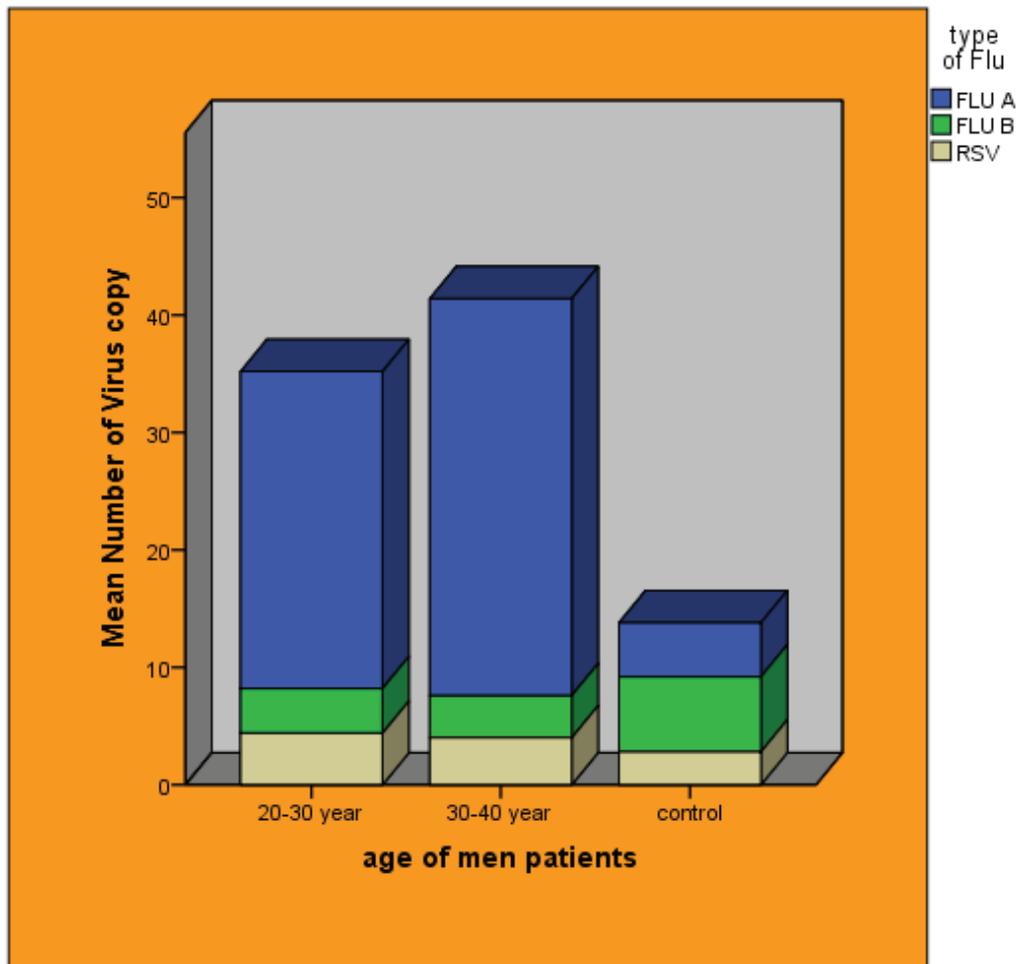


Figure 2 Screening for the three types of influenza (Flu A, Flu B, SRV) in men whose ages range from 20 to 40 years old

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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Anti-Hyperlipidemia Effects of Sijukkot Leaf Extract Ethanol (*Lactuca Indica*)

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Abstract

Lactuca indica from Indonesia (Humbang Hasundutan) is a plant used to treat as antihyperlipidemia : Cholesterol, Triglycerida (TG), High Density Lippoprotein (HDL), Low Density Lippoprotein (LDL), on 28 male rats of the wistar strain (*Rattus norvegicus*) that have been given high-fat diets were given to 3 control groups, namely rats given standard feed, groups of rats given standard and high-fat diets, and groups of rats given simvastatin with treatment for 21 days. The groups with different doses were 100 mg/kg bw, 200 mg/kg bw, 300 mg/kg bw and 400 mg/kg bw. Giving the ethanol extract of the leaves of the sijukkot plant has an effect on reducing total cholesterol levels. With the analysis obtained that the Shapiro-Wilk Normality Test with a sig value > 0.05 , the data is normally distributed. The results of the analysis in the Test of Homogeneity of Variances table obtained a Sig value of 0.510. Cholesterol Sig Value $0.510 > 0.05$; TG $0.102 > 0.05$; HDL $0.01 < 0.05$ and LDL $0.257 > 0.05$. Thus, the ethanolic extract of sijukkot leaves gave a positive effect on cholesterol levels in the serum lipid profile of High Density Lippoprotein (HDL and Low Density Lippoprotein (LDL).

Keywords: *Sijukkot (Lactuca Indica); Cholesterol; TG; LDL and HDL*

Introduction

Cholesterol is one of the lipids that can form cell membranes and the extreme layers of plasma lipoproteins. Increased levels of total cholesterol can cause various diseases such as hyperlipidemia. Cholesterol is a fat needed by the body, namely: triglycerides, low density lipoprotein (LDL) and high density lipoprotein (HDL)¹. Hyperlipidemia is a major cause of risk of coronary heart disease and atherosclerosis and can cause death. Data from

the World Health Organization² (WHO) in 2012 showed that 17.5 million people in the world died (31% of the 56.7 million deaths worldwide due to blood circulation diseases) and it is estimated that this number will continue to grow. Coronary heart disease is the third leading cause of death in the world³. Hyperlipidemia is caused by high fat food intake and is also a secondary effect of diabetes mellitus⁴. With HMG-CoA reductase inhibitor it has been used in the treatment of hyperlipidemia. Simvastatin inhibitors and one of the most prevalently used HMG-CoA reductase inhibitors, however also had side effects such as⁵. Recently, cholesterol treatment using herbal medicines is very popular because it minimizes the effects of

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using chemical drugs. The compounds contained in several types of plants that have been studied show that their secondary metabolites can be used to reduce cholesterol levels in the body. For example, secondary metabolites contained in garlic extract include: organosulfur allicin, high sulfur content including allicin, diallyl disulfide (DDS), and diallyl trisulfide (DTS), which is an essential oil and S-allyl cysteine (SAC). , water-soluble amino acids⁶, The agent of herbal have antioxidant and antidiabetic effect, also showed favorable effect to hyperlipidemia, as in *Trigonella foenum graecum*⁷ -¹¹. The *Lactuca indica* form Indonesia (Humbang Hasundutan) is called as *sijukkot*. There are founded Phytochemicals are Flavonoids, tannins, saponins, steroids and triterpenoids and have high toxicity¹². In this study, it was found that the leaves of the *sijukkot* plant (*Lactuca Indica*) from Humbang Hasundutan Indonesia had the activity of lowering cholesterol levels in rats induced with high fat. Thus the results of this study can add to the inventory of herbal medicinal plants as antihyperlipidemic activity.

Experimental

Material and Method

Plant Material

The plant of *Lactuca indica* leaves was collected from Humbang Hasundutan Indonesia, This plant was authenticated at Pharmacy Herbarium University of North Sumatra.

Extraction

Samples of *Sijukkot* (*Lactuca indica* L) leaves in the form of powder were macerated as much as 500 grams in glass jars with ethanol solvent for 3

x 24 hours. The maceration process was repeated 3 times and then filtered using Buchner to obtain the filtrate and residue. The obtained filtrate was concentrated using a vacuum rotary evaporator to obtain the extract. The extract obtained was stored in a glass bottle and covered with aluminum foil and stored in the refrigerator to avoid clumping of the extract.

Experimental Animals

The test animal used was albino male Wistar ratt (*Rattus Novergicus*) aged more than 2-3 months with a body weight of 150 - 200 grams taken from the Pharmacy lab. The test animals were kept in experimental animal cages using plastic cages with the top of the cage covered with strimin wire in such a way that the rats did not escape and a place to eat and drink was provided. The room temperature of the experimental animals was 18°C-26°C and the room was well ventilated. They were fed with standard pellet diet and water ad libitum. Adaptation is carried out for 7 days, after that, high-fat feed is given consisting of 30 grams of quail egg yolk, fat for 21 days, then the initial blood cholesterol level was measured. The test animals were divided into 7 treatment groups, each treatment consisted of 4 rats. The 7 treatment groups are:

1. Group K0(+): administered with standard pellet and water ad libitum
2. Group K0(-): administered rats fed high fat diet and standard diet.
3. Group K+: administered simvastatin 4.5 mg/kg bw. High fat and standard feed.
4. Group K1: administered fed standard, high

fat diet and given ethanol extract of the Sijukkot plant at a dose of 100 mg/Kg body weight.

5. Group K2 administered fed standard, high fat diet and given ethanol extract of Sijukkot plant at a dose of 200 mg/Kg body weight.

6. Group K3 administered fed standard, high fat diet and given ethanol extract of Sijukkot plant at a dose of 300 mg/Kg body weight.

7. Group K4 administered fed standard, high fat diet and given ethanol extract of Sijukkot plant at a dose of 400 mg/Kg body weight.

The dose of simvastatin in humans is 50 mg, then the converted dose in white rats is $50 \times 0.018 = 4.5$ mg/Kg body weight (0.018 is a conversion factor for humans to mice). The dose of simvastatin to be used is calculated based on the average body weight of the test animals. Administration of simvastatin via intraoral¹³. During 21 days of treatment, 7 groups of rats were still given food and drink. On day 21, all rats were measured for cholesterol, TG, HDL and LDL levels. Blood cholesterol levels were measured using a monotest reagent and measured using a UV-VIS spectrophotometer. The mean differences of each group were statistically analyzed using Anova One Way using SPSS For

Windows software. Test was used to analyze the effect of different doses of drugs when compared to control, with the help of Graph Pad InStat software, $P < 0.05$ is considered as significant. At the end of the experimental period, the animals were fasted overnight, blood was collected by cardiac puncture and serum was analyzed for Cholesterol, TG, HDL dan LDL.

Results

(Table 1) The results of the initial cholesterol level measurements in rats showed normal cholesterol levels in rats, namely the measurement of initial cholesterol levels by taking rat blood from the tail and then using the GCU tool. The results of cholesterol measurements of rats after being given high fat for 21 days showed that wistar rats became hyperlipidemic with levels of $81.5 \pm 1,291$ this can be seen for normal cholesterol levels of 10 – 54 mg/dl, LDL 2 – 27 mg/dl¹³. The use of simvastatin induction during treatment showed normal cholesterol levels despite being given a high-fat diet. For K1 and K2, K3, and K4 treatments, it was seen that cholesterol levels were decreasing or the administration of ethanol extract from sijukkot leaves had a positive effect.

Table 1. The effect of giving ethanol extract of sijukkot leaves to rats fed high fat.

No	Groups	Cholesterol (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
1	K0(+)	54.5±1.291	35±0.000	38.5±1.732	9±1.633
2	K0(-)	81.5±1.291	74±0.816	31.25±2.062	35.45±1.159
3	K+	53±2.160	35.5±0.577	39±2.000	6.9±3.368
4	K1	75±4.243	70.75±0.957	39.25±1.708	21.6±2.718
5	K2	62.75±1.708	65.75±0.500	40.25±0.500	9.35±1.370
6	K3	61±1.414	38±4.690	40.25±0.500	13.15±1.692
7	K4	55.25±0.957	36.25±1.258	40.5±1.000	7.5±1.793

(Figure 1)The graph shows that with the administration of high fat, the highest cholesterol level was seen, hyperlipidemia occurred in rats, in rats given high fat and simvastatin it looked normal, and in the treatment given ethanol extract from sijukkot leaves it was seen that the greater the concentration of the extract had a positive effect on cholesterol levels. With the analysis obtained that the Shapiro-Wilk Normality Test with a sig value > 0.05, the data is normally distributed. The results of the analysis in the Test of Homogeneity of Variances table obtained a Sig value of 0.510. Thus cholesterol

has a value of Sig = 0.510 > 0.05; HDL 0.01<0.05 and LDL 0.257>0.05. *Lactuca indica* originating from Sumatra Indonesia also has antioxidant activity as the species *lactuca inermis*, *Lactuca sativa* L Extract from *Lactuca indica* L from Korea which has previously been studied, it was found that the plant has good antioxidant activity¹⁴⁻¹⁷. As well as the results of this study, *lactuca indica* from Indonesia (Humbang Hasundutan) has been found to have ethanolic extract from leaves that have antihyperlipidemic activity, thereby increasing the inventory of medicinal plants.

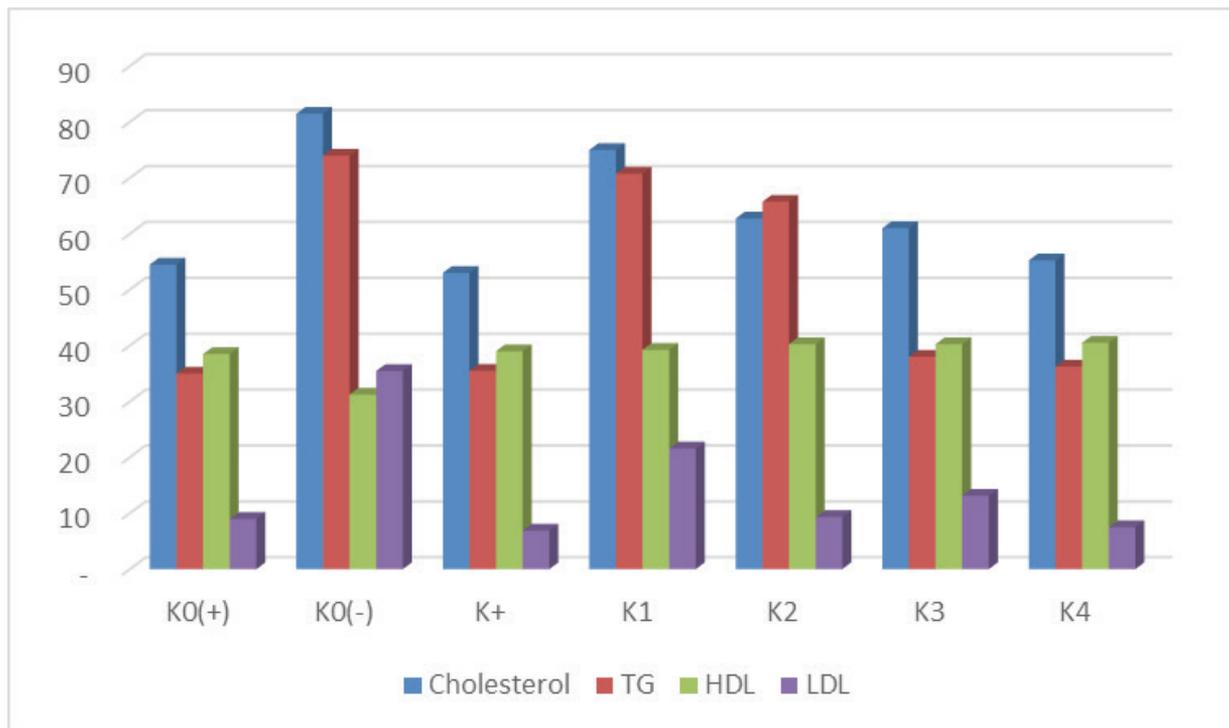


Figure 1. Chart of Cholesterol, TG, HDL and LDL cholesterol in wistar rats (*Norvegicus rattus*) given ethanol extract of sijukkot leaves (*lactuca Indica*)

Discussion

Diseases caused by an increase in cholesterol are called hyperlipidemia, the use of lowering cholesterol can be done by using drugs. Utilization of chemical drugs such as simvastatin is very

effective to lower cholesterol levels, this can be seen in the K+ group in wistar rats fed standard, high fat and simvastatin diets that have normal cholesterol levels, whereas in rats fed standard and high fat KO(-) diets, they have hyperlipidemia. However,

long-term use of drugs such as simvastatin has side effects such as neurology, tremors, dizziness, vertigo, memory loss, accelerating cataracts, and hypertension¹⁸. Therefore, the use of plants for the treatment of diseases caused by elevated cholesterol is widely preferred by utilizing herbal plants¹⁹.

Secondary effects of diabetes mellitus have been studied in the presence of cholesterol disease, therefore, the agent having some antioxidant and antidiabetic effect also showed favorable effect to hyperlipidemia. HMG-CoA reductase inhibitors²⁰. Various plants that have antioxidant and anti-diabetic activity also have cholesterol-lowering activity²¹⁻²⁴. Giving ethanol extract to 400 mg/kg BW K4 wistar rats can control cholesterol levels, with HDL being quite good, LDL and TG also normal. While feeding high fat can make cholesterol rise in rats.

Conclusion

Giving high fat to rats fed standard diet can increase cholesterol levels so that they experience hyperlipidemia, administration of ethanol extract of sijukkot leaves (*Lactuca indica*) from Sumatra (Humbang Hasundutan) has a positive effect on cholesterol, TG, HDL and LDL levels. Secondary metabolites that have antioxidant and antidiabetic activity have also been found to have cholesterol-lowering or cholesterol-neutralizing activity²⁵. *Lactuca indica* originating from Indonesia (Humbang Hasundutan) was found to have potential as an herbal medicinal ingredient as an antihyperlipidemia

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval

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