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Assessment of Reproductive Health Status and Quality of Life of Female Adolescents Living in the Slums of Dhaka, Bangladesh During COVID-19 Pandemic Situation: A Mixed-Method Study

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Abstract

Background: The COVID-19 pandemic has exacerbated the challenges of vulnerable adolescents who had reproductive health problems even before the COVID-19 pandemic. Methodology: We investigated this vulnerability through cross-sectional studies with a mixed-method approach. on female adolescents aged 15-19 years, residing in the Bauniabadh and the Ta block Jhil Par slums together with service providers. The quantitative method included a household survey of adolescents (n=144) through a semi-structured pre-tested questionnaire. The qualitative method included interviews with service providers (n=10) and with adolescents (n=9). Result: The study revealed changes in length, duration and flow of menstruation, substandard menstrual hygiene practices and impediments to the uptake of reproductive health (RH) information by adolescents during the pandemic. Married preferred delivery at home during the pandemic. There was also an increase in marriage among these adolescents and service providers had difficulty providing door-to-door RH services. Most of our female adolescents were married off during the pandemic, they lost connection with their friends and this made them feel lonely and secluded. They did not receive the support of their friends as before the pandemic. Conclusion: The study will enable the adolescent health expert to focus on the sexual and reproductive health (SRH) of vulnerable adolescents living in impoverished conditions during the COVID-19 pandemic.

Keywords: COVID-19, adolescents, slum, reproductive health, vulnerable, Bangladesh

1. Introduction

When compared to other age groups, adolescents had a lower incidence of hospitalization and death from COVID-19. However, the disease had an impact on their reproductive health, sexual health and quality of life. Menstrual health, menstrual hygiene management, family planning, and obstetric care are some of the aspects of reproductive health (RH) for adolescents. Evidence from prior large-scale disruptions, like natural catastrophes and recessions, suggests that the current pandemic would have substantial and long-term consequences for adolescents (Lindberg et al., 2020).

Adolescents make up 1.2 billion people, or nearly one-fifth of the world's population (UNICEF, 2021). Bangladesh has 36 million adolescents, accounting for 22% of the population (UNICEF, 2021). A high number of Bangladeshi migrants end up in slums in the cities. More than 5,000 slums in Dhaka city are home to an estimated four million inhabitants (UNICEF, 2021).

Adolescence is considered a vulnerable period because during this phase physical and social changes occur quickly and simultaneously (National strategy for adolescent health, 2017-18). As a result, an adolescent may experience physical and social issues such as menstrual problems, and issues in dealing with menstrual hygiene, and as for married adolescents matters of contraceptives and conception, all these situations invariably affect their Quality of life (Nasreen, Alam, and Edhborg, 2016).

Studies conducted previously in the slums of Dhaka found that the mean marriage age in slums was 13.6 years. Because the living conditions of adolescents and their families in the slums were unsafe, they were married off early. During pregnancy or if they had menstrual problems, adolescent girls did not usually visit facilities. Girls in their adolescence did not foster good menstrual hygiene (Nasreen et al.,2016). Social distancing and other parameters containing COVID made it difficult for adolescent women to get the health treatment, social services, and community support they needed to avoid child marriage and pregnancy. Adolescent girls are more likely to drop out of school and never return if schools remain closed. Job losses and rising economic uncertainty may push families to marry their daughters to alleviate financial strains (UNICEF, 2021).

During the pandemic, due to disruption in logistic services and countrywide lockdown adolescents found it difficult to avail contraceptives of their choice or plan a pregnancy. (Riley et al., 2020). There was also a rise in unwanted pregnancies and those adolescents did not get proper follow-up (Mambo et al., 2020).

Social interactions are critical in adolescents' growth to adulthood. Children and adolescents are especially sensitive to perceiving COVID-19-related restrictions on social contacts as a burden. Usual ways of communicating and seeking social support were greatly disturbed in the COVID-19 context. Face-to-face interactions were usually limited to members of the core network, such as family members. This had a significant impact on the social relationship domain of quality of life of adolescents who were no longer in school (Long et al., 2021).

Studies during the pandemic have shown that adolescents' relationships with friends deteriorated as a result of the restricted situation. The rate of children and adolescents experiencing the low health-related quality of life was notably higher during the pandemic (Deutscher, 2020).

Although there are studies worldwide on adolescent RH during the COVID-19 pandemic, RH of female adolescents in slums during the pandemic and perception of these adolescents and service providers on RH during the COVID-19 pandemic especially in low-resource settlements of Dhaka have been less studied. This paper's results expect to draw the attention of the adolescent health experts to emphasize the SRH of adolescents who live in peril

2. Methods and materials

2.1. Study Site and sample

The cross-sectional study employing a mixed-method approach was conducted in the slums of Pallabi thana of Mirpur, Dhaka. There are 107 slums under Dhaka North City Corporation (DNCC), from which Bauniabadh and 'Ta' block Jhil Par was selected purposively to match our participants and sample size. Bauniabadh slum is located in Pallabi Thana of Dhaka district, ward number 5, DNCC zone 2. It has approximately 2500 households. The slum is divided into 5 blocks A.B, C, D and E. On the other hand, 'Ta' Block Jhil par is located in Pallabi Thana of Dhaka district, ward number 6, DNCC zone 2. It has around 900 households. These two slums are approximately two kilometers apart from each other. We selected a mixed-method study design from this study, linking common themes across the survey and semi-structured interviews.

2.2. Data collection Tools

2.2.1. Quantitative survey questions

Pre-tested semi-structured Bangla questionnaire for data collection. At first, variables were identified according to the specific objectives. Then questionnaires and appropriate scales of measurement for each variable were identified.

This Questionnaire included socio-demographic questions for which questionnaires from Bangladesh Demography and Health Survey 2017 and 2018 were used. To assess the reproductive health and the access to reproductive health services, modified and adapted questionnaire derived from "Questionnaire for an interview with young people by John Cleland," A.H; Owen, J. E and MacGarvey, E.J (1995)", and Bangladesh Adolescent health and well-being survey 2019-2020 was used. To measure adolescents' social relationship domain of Quality of Life, the Bangla WHOQOL-BREF scale was used which is available on the WHO website. The WHOQOL-BREF is a 26-item instrument consisting of four domains. The social relationship domain of quality of life of the WHOQOL-BREF has 3 items, namely personal relationships, sex life and support from friends. It is scored from 1 to 5 on a response scale, which is stipulated as a five-point ordinal scale.

2.2.2. Qualitative guidelines

The qualitative portion included the in-depth interview and key informant interview guidelines. These guidelines were developed upon analysis of the quantitative part, where gaps were found as well as taking guidance from qualitative experts. The In-depth Interview guideline four code plans were there which were perception of menstrual health and menstrual hygiene management, perception of obstetric health, perception of the source of information about reproductive health and perception of social relationship domain of quality of life. The Key-Informant Interview guidelines had one code plan which was a perception of giving reproductive health services to adolescents during the COVID-19 pandemic situation

2.3. Data collection procedure

After receiving permission from IRB, and BSMMU, we took permission from the Chief Health Officer (CHO) of Dhaka North City Corporation (DNCC). The written permission of the CHO of DNCC along with the clearance of IRB, and BSMMU were submitted to the Assistant Health officer at DNCC, Mirpur Zone-2. Then a DNCC volunteer was recruited to access the slum. The DNCC volunteer introduced us to the gatekeeper of both the slums. Verbal permission from slum leader/gatekeeper of both the slums for conducting the study was taken. Along with gatekeeper of the slum and DNCC volunteer the households of the slum were reached.

Respondents comprised a simple random sampling of girls recruited from both slums after making a framework from a household listing. For the Bauniabadh slum, which is composed of 5 blocks for convenience of our study we chose blocks A, B, D, and E were chosen. Each block has several lines (roads) with families in it. Each line had about 8-10 households which were selected based on the inclusion criteria and then numbered. A framework was constructed from that household listing. A total of 160 households were listed from Baunibadh. A simple random sampling by lottery method was performed on that household list.



Figure 1: Framework of Bauniabad for simple random sampling

Unlike Baunibadh, 'Ta' block Jhil par slum was not differentiated into any lines or blocks. However, the areas were divided into three wings, which were connected by wooden bridges. There were several tin shed units in the slum. Each unit was one household and was selected based on the inclusion criteria and then numbered. A framework was constructed from that household listing. A total of 108 household listing was made from 'Ta' block Jhil Par. A simple random sampling by lottery method was performed on that household list.



Figure 2: Framework of the 'Ta' block Jhil Par slum for random sampling

From the above two frameworks, a total of 144 female adolescents both married and unmarried were enrolled in the quantitative study. The selection criteria for the respondents were age between 15 and 19 years, willingness to participate in the study, informed written consent of the parent/guardian, and consent of minors. Adolescents under 18 years of age without a parent or legal guardian who could provide informed consent were excluded from the study.

The questionnaire consisted of the following sections: the sociodemographic segment for which questions from BDHS 2017-2018 were used; the reproductive health and access to reproductive health services, modified and adapted questionnaire derived from the 'Questionnaire for an interview with young people by John Cleland' and

Bangladesh Adolescent Health and well-being survey 2019-2020 was used. To measure adolescents' social relationship domain of Quality of Life, the Bangla WHOQOL-BREF scale was used.

2.4. Statistical Analysis

Data were collected through face-to-face interviews in a private setting without parents or guardians being present. The completed questionnaires were entered into a database in SPSS version 23. Descriptive statistics and bivariate statistics were replaced. Associations were analyzed using the Chi-square and Fisher exact test. The significance p-value of the associations was analyzed.

2.5. Qualitative Analysis

Nine girls from the slum through in-depth interview guidelines and ten service providers were purposively selected for the key informant interviews that provide services to slum people. The interviews were carried out in Bangla. In-depth interviews were performed in a private environment and key informant interviews were done in the respective workplace of the service providers' workplaces. During the interview, notes were taken and interviews were recorded with voice recorders and then transcribed. Data were manually coded. All the transcripts were reviewed and themes were generated based on the analysis plan. Thematic analysis was used for this research (Barun and Clarke, 2006). Data validity was ensured by triangulation, which was again verified by comparing the data from the In-depth Interview (IDI) and Key Informant Interview (KII).

3. Results

3.1. Sociodemographic characteristics of the participants

One hundred forty-four girls participated in the study, and nine girls participated in in-depth interviews. The average age of participants was 16.9 years (SD +- 1.40). The majority of girls were unmarried (63.9 percent), and 36.1 percent were married. Around 73% of the adolescents were married in the last two years and 27% were married for more than 2 years. Approximately 52 percent of girls currently study during the pandemic and about 48 percent do not currently attend school. Approximately 26 percent of the respondent's fathers never attended school and about 28 percent of these young girls' fathers had studied till primary school. Most of the fathers (51 percent) were employed at different locations and about 33 percent were self-employed. Of the mothers of these young girls, 37 percent had never attended school and 41 percent had studied until primary school. The majority of mothers (69%) were housewives and about 12% were both garment workers and housewives.

3.2. Menstrual history of the respondents

We evaluated the menstrual history of the participants before and during the COVID-19 pandemic.

Length: Adolescents with normal menstrual cycles between 24 and 38 days were 83 percent before the pandemic, which decreased to 71 percent during the pandemic. About 5% of adolescents reported that they had a shorter cycle 23 days before the outbreak, this number grew during the pandemic, with 12 percent of adolescents complaining that they are now experiencing shorter cycles. Approximately 6 percent of girls reported having had a longer cycle than 38 days before the COVID-19 pandemic, this percentage has increased considerably, and today about 14% of girls say their menstrual cycle has been 38 days or longer since the pandemic.

Duration: Those with shorter menstrual periods of less than 5 days also increased by 23% before and 40% during the pandemic. Before the pandemic, 60% of adolescents reported menstruation lasted between 5 and 7 days, a reduction of 50% during the pandemic.

Flow: The majority of adolescents (28%) complained of a high flow during the pandemic compared to the previous pandemic (19%).

Problem: Among the teenagers surveyed, thirty-two percent said they had some form or the other of menstrual problems since the pandemic., whereas only 11% complained of having menstrual problems before the pandemic. Treatment and barriers: Of those who had menstrual problems during the pandemic, only about 35% of them sought some sort of treatment, and 38% of them faced barriers while receiving treatment.

Type of barriers.: Five out of six adolescents said the barriers were due to financial constraints and two out of six teenage girls stated fear of contracting coronavirus and difficulty in getting doctor's appointments. The menstrual history has been described in Table 2.

The quantitative findings were confirmed by individual interviews. Adolescents described their days of menstruation as a period of suffering and discomfort. Some adolescents even mentioned that their intensity of the pain had increased since the pandemic, while others said their periods had become irregular since the pandemic.

'Last year for 2-3 months I did not have periods, then I got a regular period for 2 months, then it stopped for 2 months at the beginning of this year' (Adolescent, 18 years, married).

"I have very irregular menses for 2 years, I don't even understand when I will have periods" (Adolescent, 16 years, unmarried).

Service providers who give reproductive health services to adolescents in slums said that they had noticed the influx of women and adolescents with menstrual problems since the pandemic.

"I noticed many women and also few adolescents are now coming with leukorrhea and irregular cycle in my chamber" (Doctor, 48 years)

3.3. Menstrual Hygiene Management

We compared and contrasted the menstrual hygiene practices of adolescents before and at the time of the COVID-19 pandemic. Although the majority of the girls used both sanitary napkins and cloths invariably during their menstruation, the usage of cloths increased during the pandemic. Before the pandemic, 31% of adolescent girls were using cloth which rose to 37% since the pandemic. Analysis revealed that there was no change in the location of these menstrual clothes drying, as most of them dried in the dark house, (83%) before and during the pandemic. However, around 69% of these teenage girls said to have changed their menstrual cloth or sanitary napkin less than 4 to 6 times a day before the pandemic, this number increased to 79% since the pandemic. There was a slight change in the disposal of the sanitary napkin in the garbage bin prior to and during covid (69% compared to 67%). However, the number of girls disposing of sanitary pads in toilets before the pandemic was 16%, and since the pandemic, it has increased to about 20%. Approximately 31% of individuals said they faced barriers in buying sanitary napkins during the pandemic, of which 87% were contributed due to a hike in the cost of sanitary napkins and around 35% because of the desired sanitary napkin not being available. Menstrual hygiene practices are described in Table 1

Variables		Before the pandemic	During the pandemic
		n (%)	n (%)
Sanitary products us	ed during menstruation		
Sanitary Napkin	Yes	114 (79.2)	118 (81.9)
	No	26 (18.1)	26 (18.1)
	Did not have periods	4 (2.8)	-
	before the pandemic		
Cloth	Yes	45 (31.3)	53 (36.8)
	No	95 (66.0)	91 (63.2)
	Did not have periods	4 (2.8)	-
	before the pandemic		

Table 1: Menstrual hygiene practice of adolescents (N=144)

Cotton	Yes		3 (2.1)	3 (2.1)		
	No		137 (95.1)	141 (95.1)		
	Did not have pe	riods	4 (2.8)	-		
	before the pandemi	с				
	h a					
I ne place from where the	ne sanitary product w	as brou		(110)		
Variables			(n=114)	(n=118)		
Pharmacy			85 (74.6)	93 (78.8)		
Shop			20 (17.5)	17 (14.4)		
Does not know			4 (3.5)	8 (6.8)		
NGO worker			5 (4.4)	0 (0.0)		
Place where the sanitary	y pad is disposed					
Dustbin			79 (69.3)	79 (66.9)		
Drain			17 (14.9)	16 (13.6)		
Toilet			18 (15.8)	23 (19.5)		
The menstrual cloth wa	as cleaned by		(n=45)	(n=53)		
Only water			13 (28.9)	15 (28.3)		
Water and body soap			16 (35.6)	20 (37.7)		
Water and detergent			13 (28.9)	13 (24.5)		
Throw away			3 (6.7)	5 (9.4)		
Place where menstrual cloth is dried during the			(n=44)	(n=48)		
pandemic						
Sunlight			7 (16.7)	8 (16.7)		
Inside house			35 (83.3)	40 (83.3)		
Number of times sanita	ry pad /cloth /cotton v	vas ch	anged (n=144)			
Less than 4-6 times			99 (68.8)	113 (78.5)		
More than 4-6 times			41 (28.4)	31 (21.5)		
Did not have periods before the pandemic			4 (2.8)	-		
Barriers faced in acquiring sanitary pads during pandemic (n=118)						
Yes			37 (31.4)			
No			63 (53.4)			
Does not know			18 (15.2)			
Types of barriers faced (n=37)						
The increased price the	of the pad	Yes	32 (86.5)			
		No	5 (13.5)			
Unavailability of the	desired sanitary	Yes	13 (35.1)			
napkin		No	24 (64.9)			
Fear of contracting core	onavirus	Yes	11 (29.7)			
		No	26 (70.3)			

These quantitative findings were confirmed by in-depth interviews with adolescents. The young girls complained that during the pandemic they mostly used cloth rather than sanitary napkins because most of their parents had lost their jobs. Housing and food were their primary concerns for survival in a pandemic rather than good menstrual hygiene practices.

"We did not have food at home and you are asking why did I not use the pad! I could use cloth during a pandemic and it did the same work" (17 years, unmarried).

'Since I got married in 2020, I use cloth, my husband cannot afford to buy a pad' (18 years, married).

Adolescents in the slums who were still in education disposed of the pads in the dustbin rather than individuals who were not studying at present. Also, parents who studied until primary school or more, their daughters disposed of sanitary napkins in the garbage bin rather than the daughters of parents who had never gone to school. It was

found statistically significant when associations were analyzed using the chi-square test. The results are presented in Table 2.

 Table 2: Association between the education of the adolescents and their parents with disposal of the sanitary napkin of adolescents (N=144)

Place where sanitary	Respondent studying at present					
products are disposed –	n(%)				-	
of during pandemic _	Yes	Ν	lo		-	
Dustbin	49 (62.0)	3	0 (38.0)			
Drain	6 (37.5)	1	0 (62.5)		0.02	
Toilet	63 (53.4)	5	5 (46.6)		-	
Disposal of the sanitar	y Education of	the respondent's	father *			
pads during a pandemic	n(%)					
	Never been	Studied till	Studied till	Do not		
	to school	primary	secondary	know	_	
Dustbin	17 (21.5)	24 (30.4)	21 (26.6)	17 (21.5)	0.02	
					_	
Drain	7 (43.8)	2 (12.5)	0 (0.0)	7 (43.8)		
					_	
Toilet	10 (43.5)	5 (21.7)	4 (17.4)	4 (17.4)		
Education of the responde	nt's mother*					
	n(%)				_	
Disposal of the sanitary pa	d Never been	Studied till	Studied till	Do not		
during a pandemic	to school	primary	secondary	know		
Dustbin	28 (35.4)	39 (49.4)	5 (6.3)	7 (8.9)	0.03	
					_	
Drain	11 (68.8)	3 (18.8)	2 (12.5)	0 (0.0)		
					_	
Toilet	12 (52.2)	6 (26.1)	1 (4.3)	4 (17.4)		

*Fisher Exact Test

3.4. Family Planning and Obstetric History of married adolescents

During the survey, we found that 38% of young girls had already delivered during the pandemic and around 19% had miscarried a child during the pandemic. Among the adolescents who were pregnant, the majority (64%) of them said it was their planned pregnancy and around 73% of them received ANC. However, seven out of eight of them stated that they had received this ANC at home from an NGO worker. The majority (45%) of the adolescents were delivered at home during the pandemic. Some girls (55%) who delivered during the pandemic said they faced barriers during their delivery. Financial constraints and not receiving neonatal care after delivery constituted 45% of each of these barriers and approximately 36% were contributed due to trained health care professionals not being available for delivery during the pandemic. Ten out of the 52 married adolescents who miscarried during the pandemic, around 40% of them sought treatment, and 50% of them said they faced barriers while receiving these treatments. The obstetric conditions during a pandemic are described in Table 3.

Variable (n=52)		n(%)				
	Pregnant at present	11 (21.2)				
	Delivered in pandemic	20 (38.4)				
	Miscarriage during pandemic	10 (19.2)				
Obstetric State	Did not deliver/was not pregnant/did not	11 (21.2)				
	have a miscarriage during a pandemic					
Adolescents who are pregnant (n=11)						
Planned Pregnancy	Yes	7 (63.6)				
	No	4 (36.4)				
ANC received	Yes	8 (72.7)				
	No	3 (36.4)				
Adolescents who delivered during pan	demic (n=20)					
Place of delivery of child during a	Home	9 (45.0)				
pandemic	Government Hospital	6 (30.0)				
	Private Hospital	1 (5.0)				
	NGO Clinic	4 (20.0)				
Barriers faced	Yes	11 (55.0)				
	No	9 (45.0)				
Types of barriers (n=11)						
Financial Constraints	Yes	5 (45.5)				
	No	6 (54.5)				
Fear of coronavirus	Yes	3 (27.3)				
	No	8 (72.7)				
The doctor was not available for	Yes	4 (36.4)				
delivery	No	7 (63.6)				
Was unaware of the place of delivery	Yes	2 (18.2)				
	No	9 (81.8)				
Health care provider could not come	Yes	4 (36.4)				
for home delivery	No	7 (63.6)				
Could not receive neonatal care after	Yes	5 (45.5)				
delivery	No	6(54.5)				
Adolescents who miscarried during the pandemic (n=10)						
Treatment sought after miscarriage	Yes	4 (40.0)				
-	No	6 (60.0)				
Barriers faced whilst receiving	Yes	2 (50.0)				
treatment	No	2 (50.0)				

Table 3: Obstetric History of married adolescents (N=52)

During a qualitative interview with the married girls, they said that all of them were married during the pandemic. most of these married adolescents whether they are pregnant, taking contraception or had delivered a child, none of their reproductive health decisions was taken by themselves. All married adolescents got married as early as 14 to 15 years. They remained silent when asked why they were not involved in their reproductive health decisions. The decisions were mainly taken by their mother, mother-in-law or husband. They dropped out of school after marriage and none of them did any job which would provide for them financially, all these situations made them more vulnerable. The pregnant adolescents knew that they were pregnant not by any blood test performed in the hospital, but by the signs and symptoms they developed and later when they discussed with their family members.

"My uncle is a doctor (He is not a doctor, he works in a pharmacy in the village - this information was retrieved when speaking to her after the interview), I discussed with him all my symptoms, and he gave me medicine to stop vomiting," (17 years).

None of our female adolescents who were pregnant had their antenatal check-ups done. "My mother said that I can go for checkup in the latter half of pregnancy, now since I am having no issues, I can continue like this" (16 years).

"My husband said there is no need of ANC at 5th month. If needed we can go in 7th month and ultrasound is not good for my child in the womb" (19 years).

One of our respondents delivered a child during the pandemic. The delivery was done at home under the supervision of a slum RH service provider.

"I was scared to deliver this child in hospital during a pandemic and so my mother called a health provider and she did my delivery" (18 years).

3.5. Source of Information on the reproductive health of female adolescents

More than half (51%) of the adolescents said they received RH information primarily from their mothers. Many participants (57.1%) stated they had no source of reproductive health information and around 34% did not want any kind of reproductive health information.

During qualitative in-depth interviews majority of respondents considered their mother to be their critical source of RH information. Starting from knowing about menstruation to using a menstrual cloth or sanitary pads or how and where to dispose of them- all this information was given by the mother. Female relatives such as paternal aunts and sisters played a critical role after the mother. Some said that NGOs held adolescent health meetings before the pandemic that greatly served them with reproductive health knowledge and also subjects such as *Sharirik Shikkha* (subject of pubertal growth and changes) in school books also imparted information on RH.

Adolescents who were in education spoke to a great extent about RH information than those who were not in education. Those who studied in school or college had multiple sources of RH information and had said about the necessities and importance of it, while those who were not in education, solely on their mother and did not discuss the importance of RH information.

'I learned all these from school, we have had some subjects that taught about physical changes that occur after puberty she added "Even some NGO in Bauniabad gave us lessons on what to do during menstruation, I was in class 7 at that time, I used to go with my friend to this meeting' (17 years, unmarried, studying at present).

Some adolescents were reluctant to receive any sort of RH information

"I do not know actually, I know there are meetings and seminars which say about all these in Bauniabad, but I have never been to them..... I did not want to about all this information, and that's why I did not go.... it's my body, I will know the changes" (17 years, married, out of school).

3.6. Social Relationship Domain of Quality of Life.

There are four domains of QoL. These are physical, psychological, social relationships and environmental. For our study, we chose to only explore the social relationship domain, as this domain had questions which enabled us to investigate the personal relationship, sexual life and support from friends of adolescents. Keeping in mind the social context of Bangladesh, the question relating to sexual life was restricted to married individuals. Significant associations were found between the social support that adolescents receive with them studying at present.

Variables	How satisfied	How satisfied are you with the support you get from friends?					
Studying at present	n (%)				P-value		
	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very Satisfied			
Yes	5 (6.7)	26 (34.7)	26(34.7)	18 (24.0)	0.03		
No	10 (14.5)	34 (49.3)	22(31.9)	3 (4.3)	_		

	Table 4:	Association	between ado	lescent's	support	from	friends	and th	neir stud	ying at	present
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In our in-depth interview with adolescents, we found these young girls had several RH issues like those related to menstrual health and menstrual hygiene management. Social relationships were strained because, during the pandemic, adolescents could not get the desired response to their RH problems from their parents. Most of our female adolescents were married off during the pandemic, they lost connection with their friends and this made them feel lonely and secluded. They did not receive the support of their friends as before the pandemic.

"I wanted to take treatment; but my mother said it is financially difficult for us in corona..... I just wanted relief from clotted bleeding and pain but I cannot force my mother." (17 years, unmarried, out of school).

'My friend doesn't keep in touch with me... I am married now and pregnant, they are still in school... I feel bad...they have a different circle of friends now" (18 years, married, out of school).

4. Discussion

Our findings of different patterns of menstruation pandemic are comparable to a similar study conducted in Arizona on women aged 18 to 45 years where during the pandemic 35% had an increase in cycle length (Khan et al., 2021). Another study in Turkey during the pandemic found that 10.7% of women aged 18-24 years had longer or shorter menstrual cycles (Takmaz et al., 2021). Our study revealed that around 12% of female adolescents were experiencing shorter menstrual cycles (less than 23 days) during the pandemic whereas before the pandemic only 5% encountered short cycles. Around 14% complained of a longer menstrual cycle (more than 38 days) during the pandemic, which only 6.3% said they experienced before the pandemic.

In our study, 21.5% of adolescent girls also reported a decrease in blood flow during menstruation, and 27.8 per cent of adolescents currently had a heavy flow. Similarly in their study of women of reproductive age during the Pandemic, *Phelan et al., 2020*, found that 18% of them had a heavy flow. Also comparable to our study *Li et al. in 2021 found that 25% of girls* had low blood flow during the pandemic.

The duration of menstruation of our respondents for less than 5 days was 39.6 % during the pandemic c. Similarly, a survey of women between the ages of 18 and 45 found that 50% of these women had menstruation for more than 7 days. (Ozimek et al., 2021).

During the quantitative analysis, we found that female adolescents complained they newly developed menstrual problems since the pandemic such as menorrhagia leukorrhea itching, burning in the vulval region and yellowish to greenish vaginal discharge. *Likewise, Malloy et al. 2021* in their online survey of reproductive-aged women found that 29% of women experienced severe dysmenorrhea and abnormal vaginal discharge during the pandemic. In our study, a total of 16 adolescents reported menstrual problems before COVID and during the pandemic, the number doubled (n=46). Before the Covid-19 half of the adolescents had taken services from government hospitals, but during Covid, more adolescents accessed services from pharmacy and homoeopathy treatment locally in the slum area. This reflects that adolescent could not go to the hospitals due to COVID protocols and hence sought to treatment in local areas. During the qualitative interview, we found that adolescents, especially unmarried female adolescents, rarely had treatment for menstrual problems.

In one of our IDI an unmarried adolescent S, 17 years said: *"we did not have food in the home and you are asking why did I not use a pad! I could use cloth during the pandemic"*. *Around* 31.4% of our respondents said they faced barriers in acquiring sanitary pads during the pandemic. The increased price of sanitary pads contributed to 86.5% of this barrier. These findings are consistent with an online study conducted by Plan International from 11th May

2020 to 17th May 2020 with Water, Sanitation and Hygiene (WASH) and Sexual and Reproductive Health Rights (SRHR) professionals from 24 countries including Bangladesh. This study revealed that two-thirds of WASH professionals (58%) reported that menstrual hygiene products have become more expensive since the pandemic started.

Studies have found significant associations between disposal practices and factors such as age, type of schooling, education of mother/father, social class, economic status, and occupation of parents (van Eijk et al., 2016). We have found a separate significant association (p<0.05) between the disposal of sanitary pads by adolescents and the education of both parents. Adolescents who disposed of the napkin in the dustbin, both their parents have been educated from primary to secondary levels whereas those who disposed in toilets or drain, their parents have never been to school. Equivalently a study in India showed that unsafe disposal practices, such as throwing absorbents into open spaces and burning (likely open burning, not incineration), were significantly higher in rural and slum settings (Elledge et al., 2018). Also, studies among schoolgirls in Ethiopia noted high rates of disposal in latrines at 69.3%. (Gultie, Hailu, and Workineh, 2014). In low-income communities, some adolescents disposed of their used clothes in drains and ditches, but others who were uncomfortable with disposing of menstrual clothing in the open threw them in toilets, perceiving this as a discrete disposal option (Elledge et al., 2018).

In our study, 52 out of 144 female respondents were married. The quantitative analysis showed amongst 21.2% were pregnant during the interview, 38.4% of them delivered during the pandemic and 19.2% of them had a miscarriage during the pregnancy. Obstetric services were interrupted during the pandemic. Among adolescents who had delivered during the pandemic, 72.7% received antenatal care (ANC). The majority (87.5%) of this ANC was given to them in the home by NGO workers and 12.5% of adolescents received ANC from pharmacies within the slum, of those who delivered during the pandemic (n=20), the Majority (45%) of them delivered at home. More than half of the respondents said that they faced barriers in delivering their children during the pandemic. The major problems they mentioned were lack of finance and service for newborns, among married adolescents, 10 adolescents had a miscarriage during the pandemic. Only 40% of adolescents who had a miscarriage sought treatment. This scenario is consistent with The Guttmacher Institute, New York, where they estimated a 10% decline in service coverage of essential pregnancy and newborn care in 132 low-and middle-income countries during a pandemic could lead to a huge number of additional women experiencing major obstetric complications without care, additional maternal deaths, additional newborns experiencing major complications without care and an additional newborn death. Similarly, a study amongst Ugandan youths by Mambo et al., 2020 revealed that 8 out of 44 young women (15-24 years) who were pregnant could not get maternity care during the pandemic and 5 out of 24 (15-24 years) participants who had a miscarriage could not take treatment.

For proper menstrual hygiene management and safe and healthy RH, correct and specific information is necessary for female adolescents in slums who have become more vulnerable during Covid. In the quantitative part of the study, female adolescents said that they considered the mother as their primary source of information on RH, 51.4%. In our qualitative study, we found that unmarried adolescents and those in still education had multiple sources of information regarding RH as compared to married adolescents or those who have dropped out of school. Married and those who are out of school only relied on their mother for information on RH. Around 57.1% did not have any source of RH information during the pandemic. Likewise, a study published by Rutgers in 2020 revealed that 69% of adolescents in Kenya wanted information on menstrual hygiene during the pandemic and 26% wanted information on abortion, but neither of the groups could find any.

Our study also included the social relationship domain of quality of life, taking into account the context of slum and the involvement of vulnerable adolescents in a slum – IDI guidelines on sexual life were limited to married adolescents. These girls had some RH problems, for which their social relations were strained because they could not receive the desired response to seek treatment. Most parents lost their jobs during the pandemic and their main concern was to survive rather than to address the RH of their daughters, this impacted the personal relationships aspect of the social relationship of quality of life. Additionally, most of the female adolescents were married off during the pandemic, they lost connection with their friends and which made them feel lonely and secluded, this invariably affected their support from friends' aspect of the social relationship domain. We did not find much information regarding the sexual life aspect of the social relationship domain of quality of life of adolescents, may be because the discussion of sex life in communities such as slums may not be acceptable. A study was done by

Izutsu et al., 2006 with adolescents in urban slums of Dhaka found that the social relationship domain for girls in Bangladesh is culturally limited.

4.1 Limitations of the study

During the interviews, maximum safety protocols such as social distancing and wearing a mask could not be fully assured as the slum is an overpopulated area. During the daytime, it was difficult to get consent for adolescents who were below the age of 18 as in slums both the parents go out for work and hence for this, we lost a few of our participants. Maintaining privacy and confidentiality during interviews was challenging due to the infrastructure of the slum

5.Conclusion

This study conducted on female adolescents in slums of Dhaka namely Baunibadh and 'Ta' block Jhil par, revealed changes in length, duration and flow of menstruation, substandard menstrual hygiene practices and impediments to the uptake of reproductive health (RH) information by adolescents during the pandemic. Married pregnant adolescents were inclined in taking their antenatal checkups (ANC) only during the last trimester and preferred delivery at home during the pandemic. There was also an increase in marriage among these adolescents and service providers had difficulty providing door-to-door RH services. The personal relationship aspect of the social relationship domain of QoL was strained because during the pandemic the adolescents could not utilize the health services for their RH problems as their parents prioritized spending money on food, illness, clothing and shelter rather than spending it on their daughter's RH issues. The girls who dropped out of school during the pandemic lost connection with their friends and this made them feel lonely and secluded which invariably affected their support of friends aspect of social relationship domain of quality of life. Therefore, this study concludes that in adolescents who lived in slum areas, reproductive health and quality of life were invariably affected during the COVID-19 pandemic situation.

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Ethics approval

This study was conducted according to the Declaration of Helsinki and performed after getting ethical clearance from the Institutional Review Board of Bangabandhu Sheikh Mujib Medical University (Reference No. BSMMU/2021/8654).

Competing interests

The authors declare that there are no competing interests.

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Author Contributions

All of the authors have made significant contributions to this paper and have given their approval for its submission. The concept came from Hridi and FH; statistical analysis and qualitative analysis were handled by Hridi, FH, SSI and TS. data curation was handled by Hridi, FH, and SB; Data analysis and interpretation were carried out by Hridi, FH, TS, SB, NA and SSI. The revisions were given by FH, TS, SB, UH, BB, NA, MA, ZR and SSI. During the article's drafting or editing, each author contributed essential intellectual content and acknowledges responsibility for the entire project.

References

- Adolescent wellbeing and mental health. (n.d.). Retrieved May 28, 2022, from https://www.unicef.org/laos/media/4816/file/IMPACT%20OF%20COVID-
 - 19%20ON%20ADOLESCENT%20WELLBEING%20AND%20MENTAL%20HEALTH.pdf.
- Ahmed, R., Yesmin, K., & Bangladesh, W. (2008). *Menstrual hygiene: Breaking the silence*. Retrieved March 13, 2022, from https://www.ircwash.org/sites/default/files/Ahmed-2008-Menstrual.pdf
- Ainul, S., Hossain, M., Hossain, M., Bhuiyan, M., Hossain, S., Rob, U., & Bajracharya, A. (2020). Trends in maternal health services in Bangladesh before, during and after COVID-19 lockdowns: Evidence from national routine service data. *Reproductive Health*. https://doi.org/10.31899/rh14.1037
- Braun, V., & Clarke, V. (2006). Using thematic Analysis in Psychology. *Qualitative Research in Psychology*, 3(2), 77–101. https://doi.org/10.1191/1478088706qp063oa
- Covid-19 update Flash. (2021) IEDCR. Retrieved August 13, 2021, from https://iedcr.gov.bd/covid-19/covid-19-update-flash
- Deutscher. (2020). Mental Health and Quality of Life in Children and Adolescents During the COVID-19 Pandemic (27.11.2020). Rzteblatt. https://doi.org/10.3238%2Farztebl.2020.0828
- Elledge, M., Muralidharan, A., Parker, A., Ravndal, K., Siddiqui, M., Toolaram, A., & Woodward, K. (2018). Menstrual Hygiene Management and Waste Disposal in Low- and Middle-Income Countries—A Review of the Literature. *International Journal of Environmental Research and Public Health*, 15(11), 2562. https://doi.org/10.3390/ijerph15112562
- Gultie, T., Hailu, D., & Workineh, Y. (2014). Age of Menarche and Knowledge about Menstrual Hygiene Management among Adolescent School Girls in Amhara Province, Ethiopia: Implication to Health Care Workers & School Teachers. *PLoS ONE*, 9(9), e108644. https://doi.org/10.1371/journal.pone.0108644
- Li, G., Tang, D., Song, B., Wang, C., Qunshan, S., Xu, C., Geng, H., Wu, H., He, X., & Cao, Y. (2020). Impact of the COVID-19 Pandemic on Partner Relationships and Sexual and Reproductive Health: Cross-Sectional, Online Survey Study. *Journal of Medical Internet Research*, 22(8), e20961. https://doi.org/10.2196/20961
- Lindberg, L. D., Bell, D. L., & Kantor, L. M. (2020). The Sexual and Reproductive Health of Adolescents and Young Adults During the COVID-19 Pandemic. *Perspectives on Sexual and Reproductive Health*. https://doi.org/10.1363/psrh.12151Kamruzzaman, M., & Sakib, N. (2020). *Bangladesh imposes total lockdown over COVID-19*. Www.aa.com.tr. https://www.aa.com.tr/en/asia-pacific/bangladesh-imposestotal-lockdown-over-covid-19/1778272
- Long, E., Patterson, S., Maxwell, K., Blake, C., Pérez, R. B., Lewis, R., McCann, M., Riddell, J., Skivington, K., Wilson-Lowe, R., & Mitchell, K. R. (2021). COVID-19 pandemic and its impact on social relationships and health. *J Epidemiol Community Health*, 76(2). https://doi.org/10.1136/jech-2021-216690
- Mambo, S. B., Sikakulya, F. K., Ssebuufu, R., Mulumba, Y., Wasswa, H., Thompson, K., Rusatira, J. C., Bhondoekhan, F., Kamyuka, L. K., Akib, S. O., Kirimuhuzya, C., Nakawesi, J., & Kyamanywa, P. (2020). Sexual and Reproductive Health and Rights Challenges among Ugandan Youth during COVID-19 Pandemic lockdown: An online Cross-Sectional Study. https://doi.org/10.21203/rs.3.rs-48529/v1
- Nasreen, H. E., Alam, M. A., & Edhborg, M. (2016). Prevalence and Associated Factors of Depressive Symptoms Among Disadvantaged Adolescents: Results from a Population-Based Study in Bangladesh. *Journal of Child* and Adolescent Psychiatric Nursing, 29(3), 135–144. https://doi.org/10.1111/jcap.12150
- National Institute of Population Research and Training (NIPORT), & ICF. (2020). Bangladesh Demographic and Health Survey 2017-2018.
- National Institute of Population Research and Training (NIPORT), International Centre for Diarrheal Disease Research, Bangladesh (icddr, b), & Data for Impact. (2021). *Bangladesh Adolescent Health and Wellbeing Survey 2019-20: Final Report*.
- Periods in a Pandemic. (2020). Plan International. https://plan-international.org/publications/periods-in-a-pandemic
- Riley, Sully, Ahmed, & Biddlecom. (2020). Estimates of the Potential Impact of the COVID-19 Pandemic on Sexual and Reproductive Health In Low- and Middle-Income Countries. *International Perspectives on Sexual and Reproductive Health*, 46, 73. https://doi.org/10.1363/46e9020
- van Eijk, A. M., Sivakami, M., Thakkar, M. B., Bauman, A., Laserson, K. F., Coates, S., & Phillips-Howard, P. A. (2016). Menstrual hygiene management among adolescent girls in India: a systematic review and meta-analysis. *BMJ Open*, 6(3), e010290. https://doi.org/10.1136/bmjopen-2015-010290
- World Health Organization (2012). *WHOQOL measuring the quality of life*| *the world health organization*. [online] www.who.int. Available at: https://www.who.int/tools/whoqol
- World Health Organization. (2021). Violence Against Women. Who. int; World Health Organization: WHO. https://www.who.int/news-room/fact-sheets/detail/violence-against-women



Health Shocks and Coping Mechanisms in North Central Nigeria: The Gender Perspective

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Abstract

Introduction: Reliance on out-of-pockets (OOP) payments for health services has continued to hamper access to quality healthcare across Nigeria. Socio-demographic and socio-economic characteristics of the gender of the household head as it influences and impacts health shocks and OOP payments have received very little attention globally. This study investigated the gender perspective on health shocks, health expenditures and coping mechanisms in North Central, Nigeria. Methods: This is a cross-sectional analytical study involving both quantitative and qualitative data collection methods. A total of 1,192 households were studied using multi-stage sampling technique in both rural and urban communities in North Central, Nigeria. Data was analysed with SPSS version 20, and qualitative analysis was done by thematic analysis. Results: The finding showed that 458 (38.4%) of the respondents were female-headed households (FHHs). Female-headed households were less educated, earned lower income, resided more in rural communities and were less insured than male-headed households (MHHs). Health shocks were higher among the FHHs and they also pay higher percentage of their household expenditure for healthcare through higher OOP payments. Also, more FHHs experienced Catastrophic Health Expenditure (CHE) and reported effects of health shocks on reduction in food consumption and loss of income than MHHs. Age, income, occupation and household size are all factors that influenced health shocks in this study. Conclusions: Innovative ways to financially protect women must be employed, to close up the equity gap and bring Nigeria closer to achieving UHC.

Keywords: Female, Health Shocks, Out-of-Pocket Payments, Healthcare, Coping Mechanism

1. Introduction

Generally, the Universal Health Coverage (UHC) aims at reducing the financial hardship households suffer anytime they try to use or use healthcare services. This has become a major concern globally, and thus it has

been made the target of the health sustainable development goal 3 (SDG3) (Alawode & Adewole, 2021). However, people all around the world still experience diseases and illnesses which can adversely affect their social well-being and economy. These undesirable health experiences are often referred to as "health shocks." "Health shocks" by World Health Organization (WHO) are defined as unpredictable illnesses that diminish health status (Lieve & Xu, 2008). Health shocks are also often used to refer to negative health events, which are unexpected and they impact on many other economic decisions of households especially in low-income and middle-income countries (LMICs) (Li *et al.*, 2012; Asad & Jaai, 2017; Rice *et al.*, 2018). Health shocks could be in form of death, injury or illness (Khurshid & Ajay, 2014; Dhanaraj, 2016; Mitra *et al.*, 2016).

Despite all efforts by the World Health Organization on UHC and push to achieve SDG 3, the LMICs, Nigeria inclusive, have suffered major set-backs in achieving this goal. This is because health care financing in Nigeria has been deeply characterized by declining budgetary provisions since early 80's. The health budget in Nigeria has persistently been less than 8% of the total country's budget as against the declaration made in Abuja in the year 2000 that countries should spend at least 15% of their total budget on health (Adegboye *et al.*, 2018). This gross irregularity in healthcare financing for ill health and health shocks has caused the health care system to be dominated by out-of-pockets (OOP) payments in Nigeria (Omotosho & Ichoku, 2016). World Bank states that OOP spending for healthcare in Nigeria constitutes 72% of total health expenditure and 95% of the total private health expenditure in 2015 (Adisa, 2015), this is unacceptably high despite 15 years of existence of health insurance scheme that is supposed to serve as the prepayment mechanism for all citizens (Omotosho & Ichoku, 2016; Odeyemi & Nixon, 2013).

When households are struck with health shocks, they adopt other informal payment coping mechanisms to smoothen out its social and economic effects. Payment coping mechanisms refer to ways in which households respond to health shocks and the payment mechanisms used for health services received, such as use of own money, borrowed money, sale of assets, payment by subsidy/deferment/exemptions or by community support (Odeyemi & Nixon, 2013; Onisanwa & Olaniyan, 2018). These coping mechanisms during health shocks sometimes have a negative vicious cycle effects on households, especially the poor and vulnerable (Ewelukwa *et al.*, 2013; Bonfer & Gustafsson 2017). Studies have shown that the major problem of health shocks and the negative effects it has on households in developing countries especially Nigeria is the financial burden OOP poses and the threat it exerts by pushing UHC further out of reach, with women and girls being at the receiving end than the male gender (Babatunde *et al.*, 2016; Onisanwa & Olaniyan, 2018; Urama *et al.*, 2019). The women population represents about 70% of the world's poor (Urama *et al.*, 2019), and comprises women who are female-headed households (FHHs).

The situation of poor health financing in Nigeria and the inequity in the health system is one of the major causes of the poor health indicators (Aregbeshola & Khan, 2018). These indices have been shown to be worse among women dwelling in the rural communities and among women with poor education. This is significant because the number of women who are becoming household heads is growing globally (Urama *et al.*, 2019).

The rationale of this study is to bring insight to health shocks, the pattern of health care expenditure, to identify the economic consequences of health shocks on households especially FHHs and to give recommendations on how the government can implement policies that will improve the state of health care of the country. In order to explore the gender perspective of health shocks, this paper has four objectives such as to compare the distribution of health shocks among the male and female-headed households; to compare their health expenditure pattern; coping mechanisms of household heads; as well as assessing the effects of out-of-pocket expenses on households headed by male or female. This research answered some questions. Is there any difference between the distribution of health shocks among male or female headed households in North Central Nigeria? Is there any difference in MHHs and FHHs health expenditure and what are the effects of OOP payments by the male and female headed households?

2. Methods and Materials

North Central Nigeria is one of the six geo-political zones in Nigeria. This study was carried out in Kwara and Nasarawa states which are two states that belong to the North Central political zone. Agriculture is the main stay of the economy in both states.

The study is a descriptive cross – sectional analytical study with application of quantitative and qualitative methods in Rural and Urban communities in North Central Nigeria. The study compared health shocks, household health expenditures, and coping mechanisms among the Male and Female headed households of North central Nigeria. The quantitative study was carried out through the use of the semi-structured questionnaire which was used to determine health shocks, household health expenditures and coping mechanism among Household heads in North Central Nigeria. The minimum sample size for each State for the study was 553 calculated using the formula for comparison of two proportions. However, 600 households were sampled in Kwara State and 592 households were sampled in Nasarawa State to improve the validity and reliability of the study.

Multistage sampling technique was used for the selection of respondents for the study. Two states (Kwara and Nasarawa States) were selected from states in North central Nigeria by simple random sampling technique by balloting. One Local Government was selected from each of the senatorial district in the two States by simple random sampling technique by balloting making a total of six LGAs that was selected. Two urban and two rural communities from each Local Government Area were also selected by simple random sampling. Proportionate allocation was used to allocate the questionnaires to each of the selected communities based on the population. The household questionnaire was administered to the household heads. Household out-of-pocket payments for inpatient, outpatient and routine expenses in local currency units were collected for 12-months recall period.

3. Study variables and data analysis

This research collected data to quantify and qualify the experiences of male headed and female- headed households. The questionnaire was adapted from Socio- economic questionnaire used for Impact Evaluation of Kwara State Community Health Insurance survey conducted by Amsterdam Institute for Global Health and Development (AIGHD) (SEQ, 2013). "The inequitable impact of health shocks on the uninsured in Namibia questionnaire" (Doss, 2018) and "Health shocks, coping strategies and forgone healthcare among agricultural households in Kenya" (Bonfer, 2017). It investigated households socio-economic and socio-demographic characteristics, health shocks, general household expenditure, healthcare expenditure (direct and indirect) and patterns, effects of health shocks, catastrophic health expenditure and coping mechanisms adopted by households during health shocks.

The collected quantitative data were collated and edited manually to detect omission before it was entered into the computer using the Statistical Package for Social Sciences (SPSS) 20. Associations between quantitative variables were also assessed using chi-square test. Bivariate and multivariate and regression analysis were conducted. Statistical significance level was set at p-value of < 0.05 at a confidence level of 95%.

Qualitative analysis was done by thematic analysis of the qualitative data using four domains of the conceptual framework with verbatim transcription. The transcripts were processed, coded and interpreted manually using the detailed content analysis method. The dependent variables in this study were health shocks, effects of health shocks, catastrophic health expenditure due to health shocks and the coping mechanisms used during health shocks. Coping mechanisms were grouped into six groups as: deplete assets, use savings, use insurance, borrowing, seek help and other. Catastrophic health expenditure is defined as a certain percentage of healthcare costs that endangers the household's ability to maintain its customary standard of living. Out of pocket payments: refers to the direct payments made by households to healthcare providers at the point of receiving healthcare services and it includes cash payments reported in the survey.

The independent variables in this study included socio-demographic indicators of household heads such as education level of household head which was re-categorized into no education, primary education, secondary

education, and tertiary education; employment status of household head categorized as employed and unemployed; age of household head; and sex of household head. Other independent variables include household size; area of residence (urban/rural); health insurance which is categorized as insured/uninsured; type of healthcare facility visited (recoded as public, private, alternative and other); and the household's socio-economic status (re-categorized into quartile groups based on the list of household assets owned). The survey collected a wide range of information on health status, health service utilization, health expenditures and household socioeconomic indicators.

The socio-demography was measured with age, gender, education, employment, religion, salary scale, insurance status, household size, geography location, employment status and type of employment of the household head. The Socio-Economic Status was calculated using Water/sanitation, Assets, Maternal education, and Income (WAMI) index, it was adapted from a UNICEF study (Psaki *et al.*, 2014) and the study population was classified into four quartiles (Q1, Q2, Q3 and Q4). Households were asked if they experienced any type of health shocks in the last one year respectively. Shock outcome was measured in binary, if shock occurred it is 1 and if shock did not occur it is 0.

In order to get the estimate of household expenditure, weekly food consumption, monthly non-food consumption (excluding medical/health expenditure) and yearly non-food consumption/expenditure (excluding medical/health expenditure), households were asked about their healthcare spending in the last 12 months which was further divided into direct medical costs and direct non-medical costs. Self-reported effect: the self-reported effect of health shocks was asked, if household had any effect outlined, it was scored 1 and if not 0. The effect was measured on food consumption, education, housing, income and assets. Coping strategies were evaluated in binary by asking each household head to indicate for each coping mechanism whether they used it 1 or not 0 in case of health shocks.

Focused group discussion sessions were used to obtain qualitative data from households in the North Central urban and rural communities that were not used for the quantitative data collection to prevent selection and information bias. Participants were household heads that were purposively selected based on age (18-70 years), gender, residence in the area for the last one year. The gender was used as a criterion to represent each homogeneous group which makes two homogeneous groups of male and female household heads. A total of 12 sessions were conducted. Each group consisted of about 6 - 10 persons and each session lasted about 45 to 60 minutes. The FGD was recorded on tape recorders.

Ethical approval for the study was obtained from the research and ethical committee, University of Ilorin, Kwara State, Nigeria. Written informed consent was obtained from respondents after thorough explanation of the study to them through signing for those who can write and thumb print for those who are illiterate. All information was treated with utmost confidentiality. Participation in the study was absolutely voluntary.

4. Results

4.1. Descriptive statistics

This gender perspective comparative study on household health shocks, health expenditure and coping mechanisms covered a total of 1192 households. In Kwara State 600 (50.3%) households were interviewed while 592 (49.7%) were from Nasarawa State. In all, six Local Government Areas (LGAs) were used for this study; one LGA was picked from each senatorial district in the two states (Kwara and Nasarawa). In this study, Maleheaded households (MHHs) constituted 734 (61.6%) while the Female-headed households (FHHs) constituted 458 (38.4%) of the respondents.

The differences in age, marital status, household size and area of residence for both MHHs and FHHs are statistically significant with p-value < 0.001. The MHHs were better educated than the FHHs, even though this was not statistically significant. More MHHs engage in farming while more FHHs hawks or trade, this was statistically significant with p-value < 0.001. There is statistically significant difference among the MHHs and the FHHs in terms of monthly income, social class and insurance status with p-value < 0.05. More FHHs have

lower monthly incomes than the MHHs. Also, more MHHs are insured than FHHs. More households headed by females (76.2%) experienced more health shocks than households headed by males (72.6%), even though it is not statistically significant with p-value 0.170.

The factors that influence health shocks among MHHs and FHHs are age, marital status, type of employment, income, social class, household size, area of residence and where treatment was assessed during health shocks, they were statistically significant. Education, employment and insurance status were not factors that significantly influenced health shocks among MHHs and FHHs.

More FHHs are of lower age than the MHHs. There are more FHHs that are single, widowed, divorced and separated than the MHHs. The FHHs earn lower, they are less insured and they reside more in the rural communities than the MHHs.

During the FGD sessions when asked about where treatment was sought a FHH in Rural Kwara State said: "I visit a woman up there who sells drugs for us and gives us injections" - FRE1

Additionally, a rural dweller from Nasarawa state stated that;

"We visit a private hospital nearby when ill because the primary health care that you see over there has been built for the past four years with no single equipment nor staff". -MRA2

Households who had more inpatient cases were households which were headed by the females. The difference in the prevalence of health shocks among MHHs and FHHs was statistically significant with p-value < 0.05. Also, more FHHs experienced more malaria, upper respiratory and musculoskeletal pain episodes than the MHHs. During the FGD sessions, the common types of illnesses experienced by the rural dwellers were malaria, typhoid and upper respiratory tract infections were more mentioned. However, in the urban communities, the prevalence of health shocks is high and most respondents reported malaria, diarrhoea, upper respiratory tract infections, severe body pain and surgeries.

Most expressions gotten from the FGD sessions were that illness and injuries were part of life. A middle-aged woman in rural community in Kwara stated that;

"It is not possible to be free from sickness." – FRB1

For the health care expenditure, the MHHs spent higher than the FHHs in the past one year of the study though the difference was not statistically significant at p value 0.537. The pattern of health expenditure by household heads showed that poorer households paid larger percentage of their income on health than the richer households. The FHHs even paid higher percentage of their income on health than the MHHs.

The health expenditure as a result of OOP payments because of health shocks had some negative effects on food, education and housing. Female-headed households feeding, education and income were more affected than the MHHs. During FGD sessions, a female respondent from rural Kwara reiterated that:

"Caring for sickness affects household spending especially food; it affects feeding a lot. We usually forfeit feeding for health care cost expenditures". – FRB2

Similarly, another middle age woman from rural Kwara said:

"Sometimes we even remove the money from the children's school fees so it affects children education. It also affects feeding because when we pay for health care food is reduced". - FRE2

During FGD, both MHHs and FHHs said they have had reasons to sell their products off cheaply so as to get money for health care and incurred income loss because they had to stay off work to stay with their relatives on hospital admission. A MHHs in rural Nasarawa stated during the FGD session that:

"I spent all I had to have a surgical operation done after which I had to still sell my land to cope with the drugs, follow up and pay the money I borrowed for health care, I am not yet fine but I am managing myself." - MRG2

Another middle age man in urban Nasarawa said:

"I sold my land to care for a household member that had cancer." – MUB2

In order to cope with OOP, both MHHs and FHHs spent their savings to pay for health care than other coping mechanisms because most of the households are not insured. The MHHs are more insured than households headed by the females and this difference was statistically significant with p value < 0.05. During FGD sessions, a woman from a rural community in Nasarawa stated,

"My husband was ill and was treated in the general hospital. I spent \aleph 20,000. I got money from family members. I sold crops and borrowed money from friends". -FRA3

A respondent said in rural Nasarawa,

"I have heard of health insurance before but I thought it was only for people who work with the government." -MRA3

The factors that worsened the effect of health shocks on male household heads were old age, lower years of education, low income, lower social class and higher household size and unemployment status. For FHHs, old age, lower income, lower social class and higher households' size, the worse the effects of health shocks experience.

5. Discussion

The FHHs constitute slightly more than a third of household heads, this is in line with other findings in Nigeria that show that FHHs ranges from 36% to 42% (Urama, 2019). This study showed that health shocks were more prevalent among the FHHs, this is probably because women lag behind in socio-economic developments in terms of education, income and lack of control of cultural norms like widows being dispossessed of their husband's property. This may further make them lack the purchasing power for healthcare (Holmes *et al.*, 2012; Urama *et al.*, 2019). The policy implication for this is to ensure socio-economic capacity of women becomes a priority.

Malaria was found to be the predominant illness that caused health shocks in North Central Nigeria and the highest reason for outpatient visits among FHHs, even though there is no statistically significant difference among the MHHs and FHHs. Studies have shown, however, that the number of malaria cases in Nigeria is highest in the world. Nigeria suffers the world's greatest malaria burden and it continues to be a major public health problem (Dawaki *et al.*, 2016; Oyewale *et al.*, 2018). This implies that concerted efforts must be put in place by all to reduce the morbidity and mortality caused by malaria especially among the vulnerable in which women, children and rural dwellers are mostly part of.

Consistent with the present findings, other studies found OOP spending as the main source of healthcare financing in Nigeria when households seek for healthcare during health shocks (Obansa & Orimisan, 2013). This study found that the pattern of healthcare expenditure as a result of OOP spending by households in the North Central Nigeria showed that the poorer households paid higher percentage of their income and total household expenditure on healthcare compared with richer households. This invariably means that the poorer households bear the greater burden of healthcare spending and this is even worse for households that are headed by females (Olaniyan, Chukwuedo & Obafemi, 2013; Iloka *et al.*, 2018; Azzani, Roslani & Su, 2019; Urama *et al.*, 2019). In this study, the FHHs bore greater burden of health care expenditive and sexual health needs. This was similar to what was found in a study in South-Eastern Nigeria, where FHHs incurred higher financial costs burden from seeking healthcare and also incurred more OOP payments than MHHs (Urama *et al.*, 2019). This implies that the government should broaden the free or subsidised healthcare needs for women so that they do not suffer because of her reproductive and sexual needs.

In this study based on self-reported effects of health shocks, reduction in food consumption was more reported followed by effects on income. In both the FHHs were more affected than the MHHs. This is not in line with

another study in Nigeria that found that health shocks were negatively associated with income (Onisanwa & Olaniyan, 2018).

This study showed that households used coping strategies to smooth consumption when faced with health shocks. A higher percentage of both MHHs and FHHs used more of savings to cope with health shocks. The FHHs however utilize more savings for healthcare than the MHHs. This is consistent with what was found in a study in South-eastern Nigeria (Urama *et al.*, 2019) but not in line with some other studies who found that households were more likely to borrow when faced with health shocks (Sparrow *et al.*, 2014; Onisanwa & Olaniyan, 2018). Some studies found that the choice of coping mechanism by households is based on total years of education, household size and type of shock (Onisanwa & Olaniyan, 2018). This implies that enrolment into the social health insurance schemes will reduce the use of OOP and other informal coping mechanisms during health shocks.

6. Conclusion

This study has shown that inequity exists between the MHHs and FHHs based on both their social determinants for health, that is, their socio-demographic and socio-economic characteristics. Even though it established in this study that during health shocks, most healthcare payments were made from out-of-pockets by both genders to cope with health expenditures, yet the FHHs still bears most of the consequences of the financial burden.

Therefore, strategies to enhance equity in financial protection among the poor and the rich, the rural and urban dwellers, the Male-headed households and Female-headed households must be the utmost priority for the government and policy makers. This will invariably reduce the unbearable burden of informal coping mechanisms on households during health shocks.

The government and policy makers at all levels should strengthen the financial protection mechanism by finding innovative and alternative ways of expanding health insurance coverage especially for women because of their peculiar health needs, the rural dwellers and the informal sector in general. Governments at all levels should increase efficiency in health care so as to curb wastage of scarce resources and look into public-private partnership for health.

The Federal government should increase the budgetary allocation to health care as it was stated in the Abuja 2000 declaration. It is of utmost importance that the State and Local governments promote the health insurance scheme for all.

7. Limitations

There was recall bias on the amount spent for health shocks; however this did not undermine the findings in this research. Further studies should examine other dimensions of health shocks that affect household heads such as disability of household member.

Competing interest

The authors declare that they have no competing interests.

Variables	Ger	der			
v al lables	Molo n=734 (%)	Fomolo n=158 (%)	Total n=1107 (%)	α^2/t	P
A go groups	Walt II 754 (70)	Temate II 430 (70)	10ta1 II = 1172 (70)	22 462	
< 20	104(142)	110(24.0)	214(12.0)	22.402	< 0.001
< 30	104(14.2) 260(25.4)	110(24.0) 147(22.1)	214(10.0) 407(24.1)		
30 - 39	200(33.4) 180(24.5)	147(32.1) 102(22.5)	407(34.1)		
40 - 49	180(24.5) 102(12.0)	105 (22.5)	283(23.7)		
50 - 59	102 (13.9)	58 (12.7)	160 (13.4)		
60 - 69	45 (13.9)	27 (5.9)	72 (6.0)		
$\geq 1/0$	43 (5.9)	13 (2.8)	56 (4.7)		
Mean ± SD	42.04 ± 13.32	38.84 ± 12.45	40.86 ± 13.24	4.125	0.453
Marital status				84.494	< 0.001
Single	23 (3.1)	19 (4.1)	42 (3.5)		
Married	697 (95.0)	367 (80.1)	1064 (89.3)		
Widowed	8 (1.1)	57 (12.4)	65 (5.5)		
Divorced	2 (0.3)	3 (0.7)	5 (0.4)		
Separated	4 (0.5)	12 (2.6)	16 (1.3)		
Education of HH head				1.359	0.929
No formal education	128 (17.4)	86 (18.8)	214 (18.0)		
Primary	112 (15.3)	77 (16.8)	189 (15.9)		
Junior Secondary	43 (5.9)	28 (6.1)	71 (6.0)		
Senior Secondary	207 (28.2)	126 (27.5)	333 (27.9)		
Tertiary	238 (32.4)	138 (30.1)	376 (31.5)		
Others	6 (0.8)	3 (0.7)	9 (0.8)		
Mean ± SD	11.17 ± 5.03	10.33 ± 5.44		2.593	0.010
Employed	1111/ 0100	10.00 0		0.923	0 337
Ves	713 (97 1)	449 (98.0)	1162 (97 5)	0.725	0.557
No	(13)(9)(11)	9(20)	30 (2 5)		
Type of employment	n=713	n=449	n=1162	101 505	< 0.001
Legislator	$\frac{1}{8}(11)$	7(16)	15(13)	101.505	< 0.001
Administrator	62(8.7)	7(1.0) 20(4.5)	13(1.3) 82(7.1)		
Managar/Drafagaianal	02(0.7)	20(4.3)	62(7.1)		
Tashnision / Artigan	74 (10.4) 95 (11.0)	43(10.0)	119(10.2) 100(0.4)		
Clarical worker	33(11.9)	24(3.3)	109(9.4)		
	28 (3.9)	19 (4.2)	47 (4.0)		
Sales work	37 (5.2)	26 (5.8)	63 (5.4)		
Farmer	197 (27.6)	110 (24.5)	307 (26.4)		
Hawker/Irader	65 (9.1)	135 (30.1)	200 (17.2)		
Casual labourer	30 (4.2)	15 (3.3)	45 (3.9)		
Others	127 (17.8)	48 (10.7)	175 (15.1)		
Income ('000)				27.502	< 0.001
< 50	473 (64.4)	360 (78.6)	833 (69.9)		
50 - 100	172 (23.4)	69 (15.1)	241 (20.2)		
> 100 - 150	46 (6.3)	14 (3.1)	60 (5.0)		
> 150	43 (5.9)	15 (3.3)	58 (4.9)		
Mean ± SD	97231.96±60530.23	44286.35±36385.57	71143.26±40000.00	1.868	0.062
Social class				19.230	< 0.001
Quartile 1	189 (25.7)	106 (23.1)	295 (24.7)		
Quartile 2	110 (15.0)	80 (17.5)	190 (15.9)		
Quartile 3	318 (43.3)	235 (51.3)	553 (46.4)		
Ouartile 4	117 (15.9)	37 (8.1)	154 (12.9)		
Insurance status				4.711	0.030
Yes	51 (6.9)	18 (3.9)	69 (5.8)		
No	683 (93 1)	440 (96 1)	1123 (94 2)		
Household Size				11 242	0.001
< 6	512 (69 8)	360 (78.6)	872 (73.2)	11,474	0.001
>6	222 (30.2)	98 (21 4)	320 (23.8)		
Mean + SD	5.68 ± 3.21	50(21.7) 507 ± 2.28	520(23.0) 562 + 3.03	3 /07	< 0 001
Area of residence	5.00 ± 5.24	5.07 ± 2.20	5.02 ± 5.05	10 097	< 0.001 0 001
An ca of residence	284 (28 7)	220 (48 0)	504 (42.3)	10.00/	0.001
Kulai Urban	204(30.7)	220 (40.0)	504 (42.3)		
	+30(01.3)	230 (32.0)	000 (37.7)		

Table 1: Socio-demographic and socio-economic characteristics of all household heads based on gender

p- value < 0.050 (statistically significant) χ^2 : Chi square test



Figure 1: Distribution of health shocks among household heads

Variables	Gender				
	Male n=533 (%)	Female n=349 (%)	Total n=882 (%)	χ²/t	Р
Age groups				17.456	0.004
< 30	74 (13.9)	81 (23.2)	155 (17.6)		
30 - 39	178 (33.4)	116 (33.2)	294 (33.3)		
40 - 49	138 (25.9)	78 (22.3)	216 (24.5)		
50 - 59	69 (12.9)	41 (11.7)	110 (12.5)		
60 - 69	37 (6.9)	22 (6.3)	59 (6.7)		
≥ 70	37 (6.9)	11 (3.2)	48 (5.4)		
Mean ± SD	42.76 ± 13.81	38.96 ± 12.57	41.26±13.46	4.129	< 0.001
Marital status				72.592	< 0.001
Single	12 (2.3)	16 (4.6)	28 (3.2)		
Married	514 (96.4)	279 (79.9)	793 (89.9)		
Widowed	4 (0.8)	44 (12.6)	48 (5.4)		
Divorced	0 (0.0)	2 (0.6)	2 (0.2)		
Separated	3 (0.6)	8 (2.3)	11 (1.2)		
Education of HH				0.508	0.992
head					
No formal	94 (17.6)	63 (18.1)	157 (17.8)		
education					
Primary	87 (16.3)	59 (16.9)	146 (16.6)		
Junior Secondary	30 (5.6)	22 (6.3)	52 (5.9)		
Senior Secondary	155 (29.1)	96 (27.5)	251 (28.5)		
Tertiary	163 (30.6)	107 (30.7)	270 (30.6)		
Others	6 (0.8)	2 (0.6)	6 (0.7)		
Mean ± SD	10.98 ± 5.05	10.41 ± 5.41	10.75 ± 5.21	1.535	0.125
Employed				0.824	0.364
Yes	517 (97.0)	342 (98.0)	859 (97.4)		
No	16 (3.0)	7 (2.0)	23 (2.6)		
Type of employment	n=517	n=343	n=859	99.009	<0.001
Legislator	6 (1.2)	6 (1.8)	12 (1.4)		

Table 2: Socio-demographic and socio-economic factors for households who had health shocks

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	4((0,0)	16(47)	(2(7))		
Administrator	46 (8.9)	10(4.7)	62(7.2)		
Manager/Protessio	50 (9.7)	33 (9.0)	83 (9.7)		
Ilai Taabniaian/Artican	61(12.4)	15(4 4)	70 (0.2)		
Clarical worker	04(12.4)	13(4.4) 11(2.2)	79(9.2)		
Ciefical worker	25(4.4)	11(5.2)	54 (4.0) 48 (5.6)		
Sales work	20(3.0) 150(20.0)	22 (0.4)	48 (3.0)		
Faillei Howlear/Trodor	130(29.0)	00(23.7) 102(20.1)	238(27.7) 140(16.2)		
Hawker/Irader	$\frac{37(7.2)}{10(2.7)}$	105(30.1)	140(10.3)		
Casual labourer	19(3.7)	14(4.1)	33(3.8)		
	90 (18.0)	34 (9.9)	130 (15.1)	20.227	< 0.001
Income (1000)	$2AE((A \pi))$	272 (77)		20.227	< 0.001
< 50	345 (64.7)	272 (77.9)	61 / (/0.0)		
50 - 100	121(22.7)	58 (16.6)	1/9 (20.3)		
> 100 - 150	35 (6.6)	11 (3.20	46 (5.2)		
> 150	32 (6.0)	8 (2.3)	40 (4.5)	1.000	0.070
Mean ± SD	89945.51±48663.	42428.08±	/1143.26±3/9/5.3	1.820	0.069
~	95	41398.28	1		
Social class				15.388	0.002
Quartile 1	145 (27.2)	93 (26.6)	238 (27.0)		
Quartile 2	87 (16.3)	61 (17.5)	148 (16.8)		
Quartile 3	228 (42.8)	175 (50.1)	403 (45.7)		
Quartile 4	73 (13.7)	20 (5.7)	93 (10.5)		
Insurance status				2.231	0.135
Yes	30 (5.6)	12 (3.4)	42 (4.8)		
No	503 (94.4)	337 (96.6)	840 (95.2)		
Household Size				13.661	<0.001
≤ 6	359 (67.4)	275 (78.8)	634 (71.9)		
> 6	174 (32.6)	74 (21.2)	248 (28.1)		
Mean ± SD	5.97 ± 3.42	5.09 ± 2.23	5.62 ± 3.03	4.263	< 0.001
Area of residence				3.923	0.048
Rural	225 (42.2)	171 (49.0)	396 (44.9)		
Urban	308 (57.8)	178 (51.0)	486 (55.1)		
Treatment centre				18.364	< 0.001
Public	319 (59.8)	193 (55.3)	512 (58.0)		
Private	184 (34.5)	153 (43.8)	337 (38.2)		
Alternative/Others	30 (5.6)	3 (0.9)	33 (3.7)		

Table 3: Health expenditure of households who experienced health shocks in North Central Nigeria.

Mean ± SD								
Health expenditure (₦)		Male	Female	Total	F	Р		
Health expenditure (Direct and	Mean	$28694.56 \pm$	$18380.91 \pm$	$23537.74 \pm$	0.382	0.537		
indirect)	SD	18211.99	9845.78	14476.18				
Direct health expenditure	Mean	$24452.24 \pm$	$17041.84 \pm$	$20747.04 \pm$	1.538	0.215		
	SD	13367.60	5112.09	3746.62				
Indirect health expenditure	Mean	$4242.32 \pm$	$1339.07 \pm$	$2790.70 \pm$	3.674	0.056		
_	SD	2445.23	6059.83	1927.57				



Figure 2: Household health expenditure as a percentage of the total household expenditure among the socioeconomic groups in the rural and urban communities in the last 12 months.

Effect	Gender		Total (%)	χ^2	ρ
	Male (%)	Female (%)			
Food effect				0.680	0.410
No	230 (49.1)	143 (46.1)	373 (47.9)		
Yes	238 (50.9)	167 (53.9)	405 (52.1)		
Education				0.151	0.698
No	352 (83.6)	226 (82.5)	578 (83.2)		
Yes	69 (16.4)	48 (17.5)	117 (16.8)		
Housing				0.144	0.705
No	400 (97.3)	265 (97.8)	665 (97.5)		
Yes	11 (2.7)	6 (2.2)	17 (2.5)		
Assets				3.933	0.047
No	389 (90.9)	262 (94.9)	651 (92.5)		
Yes	39 (9.1)	14 (5.1)	53 (7.5)		
Income				10.157	0.001
No	291 (64.8)	155 (53.1)	446 (60.2)		
Yes	158 (35.2)	137 (46.9)	295 (39.8)		

Table 4: Self-reported effects	s of health shoc	ks by household	heads based on Gender
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Table 5: Coping mechanisms adopted by households during health shocks.

Gender						
Coping	Male (%)	Female (%)	Total n(%)	χ^2	Р	
Depleting assets	53 (8.5)	25 (6.8)	78 (7.5)	2.295	0.130	
Borrowing	24 (3.8)	13 (3.5)	37 (3.6)	0.381	0.537	
Savings	378 (60.5)	232 (63.2)	610 (59.2)	2.800	0.094	
Seeking help	103 (16.5)	75 (20.4)	178 (17.3)	0.310	0.577	
Insurance	51 (8.2)	18 (4.9)	69 (5.8)	4.711	0.030	
Others	16 (2.6)	4 (1.1)	20 (1.9)	3.454	0.063	

References

- Adegboye, O. D., Rotimi, B. F. & Akande, T. M. (2018). Catastrophic health expenditure as a result of health shocks: challenge to universal health coverage in Nigeria. Savanna Journal of medical Research and *Practice*, 7(1), 1-6. https://doi.org/10.4314/sjmrp.v7i1.1
- Adisa, O. (2015). Investigating determinants of catastrophic health spending among poorly insured elderly International households in Urban Nigeria. journal Equity and Health, 14(79). https://doi.org/10.1186/s12939-015-0188-5
- Akinbi, J. O. & Akinbi, Y. A. (2015). Gender Disparity in Enrolment into Basic Formal Education in Nigeria: Implications for National Development. Africa Research Review. 9(3). 11-13. https://.doi.org/10.4314/afrrev.v9i3.2
- Alawode, G. O. & Adewole, D. A. (2021). Assessment of the design and implementation challenges of the National Health Insurance Scheme in Nigeria: a qualitative study among sub-national level actors, healthcare and insurance providers. BMC Public Health. 21(124). https://doi.org/10.1186/s12889-020-10133-5
- Aregbeshola, B. S. & Khan, S. M. (2018). Predictors of enrolment in the National Health Insurance Scheme among women of reproductive age in Nigeria. International Journal of Health Policy and Management, 7(11), 1015-1023. https://doi.org/10.15171/ijhpm.2018.68
- Asad, I. & Jaai, P. (2017). Heterogeneous Effects of Health Shocks in Developed Countries: Evidence from Australia. Monash Economics Working Papers 15-17, Monash University, Department of Economics. Discussion number 15/17. ISSN number 14415429. Accessed on 9 Sept 2021 from: https://ideas.repec.org/p/mos/moswps/2017-15.
- Azzani, M., Roslani, C. & Su, T.T. (2019). Determinants of Household Catastrophic Health Expenditure: A Systematic Review. Malaysia Journal of Medical Science, 26(1), 15-43 https://doi.org/10.21315/mjms2019.26.1.3
- Babatunde, R.O., Öyedeji, O., Ömoniwa, E. & Adenuga, A. (2016). Effect of community based health insurance on the livelihood of rural household in Kwara state, Nigeria. Journal of Agricultural Faculty of Gaziosmanpasa University, 33(2), 19-27. https://doi.org/10.13002/jafag914
- Bonfer, I. & Gustafsson Wright, E. (2017). Health shocks, coping strategies and forgone healthcare among Global public health journal, agricultural households in Kenya. 12(11). 1369-1390. https://doi.org/10.1080/17441692.2015.1130847
- Dawaki, S., Al-Mekhlafi, H. M., Ithoi, I. et al. (2016). Is Nigeria winning the battle against malaria? Prevalence, risk factors and KAP assessment among Hausa communities in Kano State. Malaria Journal, 15:351 https://doi.org/10.1186/s12936-016-1394-3
- Dhanaraj, S. (2016). Economic vulnerability to Health shocks and coping strategies: Evidence from Andhra Pradesh, India. Health policy plan, 31(6), pp 749 -758. https://doi.org/10.1093/heapol/czv127
- Doss, C., Oduro, A.D., Deere, C. D., Swaminathan, H., Baah-Boatena, W. & Suchitra, J.Y. (2018). Assets and shocks. A gendered analysis of Ecuador, Ghana and Karnataka. Canadian journal of development studies, 39(1), 1-8. https://doi.org/10.1080/02255189.2017
- Ewelukwa, O., Onoka, C. & Onwujekwe, O. (2013). Viewing health expenditures, payment and coping mechanisms with an equity lens in Nigeria. BMC Health Services Research, 13(87). https://doi.org/10.1186/1472-6963-13-87.
- Holmes, R., Akinrimisi, B., Morgan, J. & Buck, R. (2012). Social protection in Nigeria Mapping programmes and their effectiveness. London Overseas Development Institute.
- Ibukun, C. & Komolafe, E. (2018). Household Catastrophic Health Expenditure: Evidence from Nigeria. *Microeconomics* and macroeconomics journal. 1-8. https://doi.org/ 6(1), 10.5923/j.m2economics.20180601.01
- Iloka, C. E., Edeme, R. K., Edeh, R. A., & Ikeagu, U. F. (2018). Equity in Financing Health Care Services in Nigeria. Journal of Economics and Sustainable Development, 9(3), Accessed on 19 Jun. 2021 from: www.iiste.org ISSN 2222-1700 (Paper) ISSN 2222-2855 (Online)
- Khurshid, A. & Ajay, M. (2014). Economic impacts of health shocks on households in low and middle income countries: a review of the literature. Globalization and Health journal, 10(21), 6-18. https://doi.org/10.1186/1744-8603.
- Kwara State Government Media, Kwara State, Nigeria. (2016). Accessed on 5 May 2021 from: http://www.kwarastate.gov.ng
- Li, Y., Wu, Q., Xu, L. et al. (2012). Factors affecting catastrophic health expenditure and impoverishment from medical expenses in China: policy implications of Universal Health Insurance. Bulletin of the World Health Organization, 90(9), 664-671. https://doi.org/10.2471/BLT.12.102178.
- Lieve, A. & Xu, K. (2008). "Coping with Out-of-Pocket Health Payments: Empirical Evidence from 15 African Countries." Bulletin of the World Health Organization, 86, 849-8.
- Mitra, S., Palmer, M., Mont, D. & Groce, N. (2016). Can Households Cope with Health Shocks in Vietnam? Wiley online library, 25(7), 888-907. https://doi.org/10. 1002/hec 3196

- National Bureau of Statistics. Living Standard Measurement Study, Nigeria General Household Survey–Panel 2015/16, Wave 3. NGA_2015_GHSP-W3_v02_M Accessed on 6 May, 2022
- National Population Commission Abuja, Nigeria, (2019). Nigeria Demographic and Health Survey 2018. Accessed on 6 May, 2022 from https://dhsprogram.com
- Obansa, S. A. & Orimisan. A. (2013). Health care financing: Prospects and Challenges. *Mediterranean Journal* of Sciences. 4(1):221-236. https://doi.org/10.5901/mjss.2013.v4n1p221
- Odeyemi, I. & Nixon, J. (2013). Assessing equity in health care through the national health insurance schemes of Nigeria and Ghana: A review-based comparative analysis. *International journal of equity health*, 12(9). https://doi.org/10.1186/1475-9276-12-9
- Olaniyan, O., Chukwuedo, S. O. & Obafemi, F. N. (2013). Equity in health care expenditure in Nigeria. *International Journal of Finance and Banking Studies*, 2(3). 76-88. ISSN: 2147-4486 Accessed on 6 May, 2021 from: https://ideas.repec
- Omotosho, O. & Ichoku, H. E. (2016). Financial protection and Universal Health Coverage in Nigeria. *Iiste journal 51*. ISSN (Online) 2224-8951
- Onisanwa, D. I. & Olaniyan, O. (2019). Health shocks and consumption smoothing among rural households in Nigeria *journal of Economics and management*, *36*(2). 45-66. https://doi.org/ 10.22367/jem.2019.36.03
- Oyewale, M. M., Folusho, M. B. & Adeniyi, F. F. (2018). Housing type and risk of malaria among under-five children in Nigeria: evidence from the malaria indicator survey. Malaria Journal, open access. 17:311. https://doi.org/10.1186/s12936-018-2463-6
- Psaki, S. R., Siedman, J. C., Miller, M. et al. (2014). Measuring socioeconomic status in multi-country studies: results from the eight-country MAL-ED study. *Population Health Metrics Journal*, 12 (8). https://doi.org/10.1186/1478-7954-12-8
- Rice, T., Quentin, W., Anell, A., *et al.* (2018). Revisiting out-of-pocket requirements: trends in spending, financial access barriers and policy in ten high income countries. *BMC Health service Research*, *18*, 371. https://doi.org/10.1186/s12913-018-3185-8.
- Socio economic questionnaire. Kwara central Community health survey, AIGHD. 2013
- Sparrow, R., Van de Poel, E., Hadiwidjaja, G., Yumna, A. & Warda, N. (2014). Coping with the economic consequences of ill health. *Health economics journal*. https://doi.org/10,1002/hec.2945
- Urama, C. E., Adewoyin, Y., Ukwueze, E. R. & Ene J. C. (2019). Socioeconomic status and health shocks; analysis of coping strategies in rural households of Enugu State, Nigeria. *African Population Studies*, *33*(1), 4766-4774. Accessed 1 May, 2022 from: http://www.researchgate.net/publication/332442302
- Uzochukwu, A., & Uju, E. (2012). Implications of household's catastrophic out-of-pocket healthcare spending in Nigeria. *Journal of research in economics and international finance, 1*(5), 136-140. Accessed 5 Jun. 2022 from: **Error! Hyperlink reference not valid.**



Sex Differences in Muscle Activity During Drop-Jump

Landing Motion

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Abstract

This study investigated the effect of hip joint muscles' activity on dynamic knee-joint valgus angle during one-leg drop-jump landing motion in male and female subjects. Twenty-four healthy university students (11 males and 13 females) participated in the study. Surface electromyography was used to measure muscle activity during a one-leg landing motion. A gender difference was observed: males showed greater activity in the gluteus medius muscle compared to the adductor magnus muscle, whereas females showed greater activity in the rectus femoris muscle. A correlation between lower limb muscle activity and knee-joint valgus angle was found in male subjects but not in female subjects, suggesting that factors other than muscle activity are necessary for controlling knee joint valgus in females. Therefore, factors other than muscle activity should be investigated in the future to prevent female anterior cruciate ligament injuries.

Keywords: Anterior Cruciate Ligament Injuries, One-Leg Drop-Jump Landing, Sex Difference, Adductor Magnus Muscle, Gluteus Medius Muscle, Injury Prevention

1. Introduction

Anterior cruciate ligament (ACL) injuries are among the most common sports injuries and disorders, and the etiology of these injuries has been reported in a variety of ways (Montalvo, A. M., etc., 2019, Larwa J., etc., 2021). An ACL injury is categorized by its cause, contact or noncontact. According to many reports, contact injury is more common in male athletes and noncontact injury in females (Larwa J., etc., 2021, Yu B., etc., 2007). Notably, contact injuries are more likely to occur in female athletes than in males (Montalvo, A. M., etc., 2019). In either case, prevention of ACL injuries in the female athletes is extremely important.

Although many theories exist whether knee joint or lower leg rotation is involved in female noncontact ACL injuries (Yu B., etc., 2007), a consensus exists that valgus movement of the knee during landing and cutting on one leg is the primary mechanism of the injury (Bisciotti G. N., etc., 2019, Hewett T. E., etc., 2009, Olsen O. E., etc., 2004, Hewett T. E., etc., 2005), probably because females tend to have a larger physiological knee valgus angle and less muscle activity in the hamstrings than males and are likelier to be in a slightly flexed external knee joint position. Studied have shown that females have an increased dynamic knee valgus (DKV) angle during landing motions and are likely to be more at risk (Wilczyński B., etc., 2020, Collings T. J., etc., 2022, Nilstad A., etc., 2015, Myer G. D., et al., 2009, Myer G. D., et al., 2005). While it is questionable whether static alignment (female greater knee valgus angle than male) is a factor in this risk (Nilstad A., etc., 2015), some reports have suggested that muscle contraction or neuromuscular control is involved in stabilizing the knee (Wilczyński B., etc., 2020, Myer G. D., et al., 2009, Myer G. D., et al., 2005).

Many studies have focused on a single-leg jump landing motion (Ugalde V., etc., 2014, Nilstad A., etc., 2014, Ekegren C. L., etc., 2009), which is considered to have highest injury rate among the ACL injuries and has been validated under various names and methods, such as single-leg squat test (Ugalde V., etc., 2014), vertical drop-jump landing (Nilstad A., etc., 2014, Ekegren C. L., etc., 2009), single-leg jump-landing (Boey D., & Jc Lee M., 2020). Notably, its significance as an evaluation method is high.

From the viewpoint of muscle activity, excessive anterior subluxation of the tibia caused by the quadriceps muscle and decreased hamstring muscle activity that controls the anterior withdrawal of the tibia are the cause of ACL injury (Hewett T. E., etc., 1999). In addition, hip adduction and internal rotation are associated with increased external rotation of the knee joint, and the activity of the vastus medialis muscle, which prevents hip adduction, plays an important role in single-leg landing (Olsen O. E., etc., 2004, Myer G. D., et al., 2009). The importance of the gluteus medius (GM) during landing on one leg has been reported in males (Collings T. J., etc., 2022), but only a few reports exist for females. Although many studies have explored the relationship between the quadriceps and hamstrings, only a few have considered the coordination of the GM with the adductor magnus (AM) - the GM antagonist - and no comparative study of the characteristics of their activities between males and females exists.

In this study, we focused on a one-leg drop-jump landing (ODL) motion and aimed to calculate the muscle output and coordination of hip joint muscles and the timing of the onset of muscle activity while verifying their effects on the DKV angle in females and males. Knowing the characteristics of the above muscle activities will help elucidate the causes of noncontact ACL injuries, which are more common in females.

2. Method

2.1 Subjects

The subjects were 24 healthy university students (11 males; 20.2 ± 0.9 years old, 172.2 ± 3.6 cm, 65.8 ± 10.1 kg, and 13 females; 19.9 ± 0.7 years old, 158.5 ± 3.9 cm, 51.4 ± 5.8 kg). Subjects with current pain or a history of orthopedic disease in the lower limbs were excluded. The dominant leg was examined, the leg was used to kick the ball.

2.2 Measurement Items

2.2.1 Static knee joint valgus (SJV) angle (Shultz S. J., etc., 2006)

The angle between the superior anterior iliac spine, the midpoint connecting the center of the medial and lateral knee joint spaces, and the midpoint connecting the medial and lateral malleolus were measured using an SJV angle on a goniometer. The measurement was performed in the one-leg standing posture, with the dominant leg supporting the subject not to lose balance.

2.2.2 Joint Angles and Muscle Activities during One-leg Drop-jump Landing (ODL) Motion

The subjects were instructed to jump down from a 30-cm-high platform to a 20-cm-forward fall, land on one leg, and maintain a static posture. They were also instructed to jump forward and were careful not to jump up during

the motion. The upper limbs were fixed in front of the chest, with the elbows flexed. The subjects practiced twice before, and the number of measurements was one time.

2.2.2.1 DKV and Dynamic Knee Flexion (DKF) Angles

The angle between the superior anterior iliac spine, the midpoint connecting the center of the medial and lateral knee joint clefts (just on the patella), and the midpoint connecting the medial malleolus and lateral malleolus were designed as a DKV angle in the frontal plane. In addition, the angle between the greater trochanter, lateral knee joint cleft, and lateral malleolus was designed as a DKF angle in the sagittal plane. Two digital video cameras (Casio EX-FC100) were used to capture images at 210 frames per second. The height of the cameras was 1 m from the floor and the distance was set to capture whole-body movements. Each joint angle was photographed and calculated using Image J (1.53j, National Institutes of Health, USA). Motion was initiated by a light cue, and the two cameras were synchronized based on that timing.

2.2.2.2 Muscle Activity during Motion

Ultium electromyography (EMG, Noraxon EM-U810MM) was used to measure muscle activity. The target muscles were the rectus femoris, biceps femoris, GM, and AM muscles. The electrodes were applied to the rectus femoris muscle at the center of a line drawn from the superior anterior iliac spine to the center of the patella, the biceps femoris at the distal one-third of a line drawn from the sciatic tubercle to the fibular head, the GM at the center of a line drawn from the public sub-tubercle to the tuberosity of adductor pollicis brevis. Maximum voluntary contraction (MVC) was measured in advance in a fixed position on the BIODEX (Biodex Medical Systems) using the amount of muscle activity during the maximum voluntary isometric contraction of each muscle. Measurements were taken once for 5 s. After rectification and smoothing, the MVC was calculated as the average of the values obtained during the 3 s before and after the 5 s, excluding the 1 s before and after the 5 s.

EMG measurements during the ODL motion were performed, initiated by the light cue described above, and synchronized with motion capture. The sampling frequency was set at 2000 Hz, band-passed at low (50 Hz) and high frequency (500 Hz), and then filtered. The EMG waveform was converted to the root mean square every 10 ms. Muscle activity during ODL was analyzed in three intervals: pre-landing, post-landing, and during maximal valgus of the knee joint (hereinafter defined as "maximal valgus"). "Pre-landing" was the mean value calculated from 100 ms before toe-ground to the timing of toe-ground, "post-landing" was from toe-ground to the timing of maximal valgus of the knee joint after landing, and "maximal valgus" was from 50 ms before the timing of maximal valgus of the knee joint. Each muscle activity value of the three intervals for each subject was calculated as "MVC, devided by the value of the MVC. The ratio of rectus femoris to biceps femoris (hamstrings/quadriceps: H/Q ratio) and the ratio of AM muscle to GM muscle (AM/GM ratio) were calculated from the mean values for each interval.

2.3. Analysis Method

Each data was compared between the sexes; SJV, DKF, and DKV angles, and each muscle %MVC, H/Q ratio, and AM/GM ratio in the three intervals (pre-landing, post-landing, and maximal valgus). IBM SPSS Statistics Version 24 was used for statistical analysis. All data were checked for normality using the Shapiro-Wilk test, and when normality was present, a two-sample unpaired *t*-test was used, and when not normal, a Mann-Whitney test was used. Correlations between the maximum knee valgus angle and the other items were calculated separately for each gender and analyzed using Pearson's correlation coefficient for normality and Spearman's rank correlation coefficient for non-normality. The significance level was set at 5%.

2.4. Ethical Considerations

This study was designed and conducted following the Declaration of Helsinki. This study's aim and the measurement details were explained to the subjects orally and in writing in advance, and measurement was conducted after their written consent was obtained. The measurement was started after obtaining the consent of

the faculty member in charge of the ethical review at the Department of Physical Therapy, Faculty of Health Care, Takasaki University of Health and Welfare.

3. Results

3.1. Gender Differences in Each Joint Angle and Muscle Activity (Table 1)

The SKV angle was significantly bigger in females than in males, but no gender difference in the DKV angle was found during the ODL motion. For muscle activity, only the rectus femoris muscle at a maximum valgus angle was significantly greater in females. The AM/GM ratio was significantly lower in males across all periods, but the H/Q ratio did not differ between sexes.

		Male (n=11)	Female (n=13)	p value
Static knee valgus angle (deg)		1.55±1.44	6.08±2.56	< 0.01*
Dynamic knee valg	gus angle (deg)	10.7±7.7	11.6±9.36	0.807
Maximal knee flex	ion angle (deg)	60.5(57.9,68.3)	60.2(57.9,69.6)	0.191
	RF	39.9(27.7,63.9)	63.2(51.0,78.4)	0.063
D 1 1	BF	40.7(27.3,53.4)	36.7(24.3,71.0)	0.910
Pre-landing	GM	46.8(30.9,94.3)	73.4(54.9,153.9)	0.106
%MVC (%)	AM	37.5(19.4,49.8)	74.7(37.0,115.2)	0.119
	H/Q ratio	130.6(104.9,231.8)	106.1(91.4,126.4)	0.106
	AM/GM ratio	29.1(21.2,32.4)	88.4(47.6,121.4)	0.026^{*}
	RF	56.8±61.8	95.4±50.8	0.054
	BF	40.1(26.1,64.4)	55.0(26.4,71.7)	0.649
Post-landing	GM	55.0(42.2,74.3)	93.7(42.2,18.1)	0.106
%MVC (%)	AM	61.4(37.9,92.6)	91.6(59.0,94.6)	0.228
	H/Q ratio	49.6(42.1,105.2)	58.8(50.8,76.8)	0.865
	AM/GM ratio	42.6(31.4,77.4)	107.8(97.0,141.6)	0.009^{*}
	RF	36.0(18.6,47.8)	71.1(40.1,90.9)	0.035*
	BF	39.5(26.8,64.2)	43.2(29.5,53.1)	0.820
Maximal valgus	GM	50.8(25.5,66.7)	86.2(51.6,97.6)	0.106
%MVC (%)	AM	41.9(29.2,85.7)	83.8(46.1,132.6)	0.865
	H/Q ratio	91.0(71.6,208.3)	57.2(42.9,141.5)	0.167
	AM/GM ratio	67.4±45.6	138.0±81.4	0.018^{*}

unpaired t-test or Mann-Whitney's U-test

RF : rectus femoris BF : biceps femoris

GM : gluteus medius muscle AM : adductor magnus muscle H/Q: hamstrings/quadriceps

3.2. Correlations to DKV Angle by Gender (Table 2)

The following results were observed in males: although no correlation existed between the DKV and SKV angles (r = -0.229) or DKF angle (r = -0.100), a negative correlation was found between the DKV angle and %MVC of the biceps femoris, GM, and AM muscles in the correlation of muscle activity before landing. In addition, a negative correlation was found between the DKV angle and %MVC of the biceps femoris muscle in muscle activity during post-landing and maximal valgus. Negative correlations were also found in the H/Q ratio after landing and during maximal valgus, and a negative correlation was found only after landing in the AM/GM ratio. In female subjects, no correlation was found in all data.
Table 2: Correlation with dynamic knee joint valgus angle in male subjects							
		RF	BF	GM	AM	H/Q	AM/GM
		%MVC	%MVC	%MVC	%MVC	ratio	ratio
	Pre-landing	r=-0.451	r=-0.629*	$r = -0.673^*$	r=-0.691*	r=0.181	r=-0.509
male	Post-landing	r=-0.082	r=-0.745*	r=-0.364	r=0.518	r=-0.709*	r=-0.609*
	Maximal valgus	r=-0.188	r=-0.791*	r=-0.418	r=-0.327	r=-0.7*	r=-0.422
	Pre-landing	r=0.148	r=-0.368	r=-0.374	r=0.093	r=-0.342	r=0.475
females	Post-landing	r=0.454	r=-0.066	r=-0.305	r=0.255	r=-0.462	r=0.453
	Maximal valgus	r=0.563	r=-0.001	r=-0.028	r=0.379	r=-0.412	r=0.285

Pearson's correlation coefficient or Spearman's rank correlation coefficient * : p<0.05

RF : rectus femoris BF : biceps femoris

GM : gluteus medius muscle AM : adductor magnus muscle H/Q: hamstrings/quadriceps

4. Discussion

4.1. SKV angle by gender

The SKV angle was significantly larger in females than in males, similar to the results of a previous study (Nilstad A., etc., 2015). This suggests that this study's physiological knee valgus angle is greater in females than in males. However, because no relationship was found between the SKV and DKV angles, the SKV angle does not necessarily affect the DKV angle, and it cannot be said that an increase in the SKV angle causes ACL injury, which is similar to the results of Nilstad et al. (Nilstad A., etc., 2015). Although the incidence of ACL injury is lower in males than in females, suggesting that males may have a larger DKV angle that does not directly lead to ACL injury, it is also necessary to examine how the actual occurrence of trauma is related to an increase in the DKV angle.

4.2. Gender Differences in Muscle Coordination

The correlation results in male subjects showed that the DKV angle decreased with increased muscle activity of the biceps femoris, GM, and AM muscles before landing. In other words, the coordinated contraction of these three muscles before landing prevented excessive DKV, suggesting that male subjects might have had greater predictive postural control by muscle activity than females. Notably, predictive postural control ability to avoid the injured limb position before landing is necessary to prevent ACL injury (Hewett T. E., etc., 2013), and males can manage predictive postural control with cooperative lower limb muscle activity. The lack of correlation between muscle activities in females suggested that they might use physical functions other than muscle activity for postural control.

In this study, the AM/GM ratio was calculated to examine the coordination between the GM and AM muscles. The AM/GM ratio was significantly smaller in males than females in all the periods, suggesting that the GM muscle activity might be greater or the AM muscle activity smaller during the ODL motion. Notably, the GM muscle activity tends to increase during landing motion in males (Hewett T. E., etc., 1999), and Padua et al. reported that medial knee displacement appeared to be associated with increased hip-adductor activation. These reports suggested that GM muscle activity is important during the jump landing motion for the male subjects, and it may be used to control the hip joint's adduction and the abduction of the knee joint (Padua D. A., et al., 2012). However, the AM/GM ratio was higher in the female subjects, and the values increased after landing, suggesting that the greater activity of the AM muscle has the action in the female subjects might exert a hip adduction and relatively increase the knee valgus angle. Russel et al. reported that females tended to land in more knee valgus than males, but GM muscle activation did not differ between the sexes (Russell K. A., etc., 2006). Thus, GM did not appear to be responsible for the sex differences in knee valgus, suggesting that the adductor muscles, not the GM, might affect DKV. In addition, the AM muscle has the action of hip internal rotation, and when the hip internal rotation occurs, the lower leg anatomically tends to be in an externally rotated position. Notably, females

tend to have hip adduction and internal rotation due to the excessive contraction of the AM muscle during the ODL motion, resulting in external rotation of the lower leg and knee joint. The increased muscle activity of the AM muscle, a characteristic of the female muscle activity, might result in external rotation of the lower leg - a phenomenon that should be noted from ACL injury viewpoint.

Notably, the dominance of the quadriceps muscle acts on ACL shearing forces, and the hamstring activity inhibits ACL tension (Myer G. D., et al., 2009, Withrow T. J., et al., 2006, Draganich L. F., & Vahey J. W., 1990). From this study, the female subjects had greater rectus femoris muscle activity than males, and in males, an increase in biceps femoris muscle activity was associated with a decrease in the DKV angle, whereas this relationship was not observed in females. Kernozek et al. reported that the female athletes who suffered an ACL injury had a combination of decreased hamstring strength but not quadriceps strength compared to males, suggesting that males can control external knee movement using the hamstring muscle activity, whereas females could not control forward movement of the tibia sufficiently and might thus be at risk for an ACL injury (Kernozek T. W., etc., 2005). However, the maximum knee flexion angle in this study was about 60 degrees, which might not have met the generally reported risk factors of mild flexion and valgus of the knee joint for an ACL injury. A previous report stated that "there is no problem at flexion angles greater than 60 degrees, but the risk is greater at flexion angles between 0 and 45 degrees" (Arms S. W., etc., 1984). Therefore, the flexion position in the present study was deep flexion as a risk factor for ACL injury.

As no significant relationship was found between the DKV angle and muscle activity in the female subjects, the muscle activity may not be the only cause of prevention for taking a risky position of ACL injury. Other factors besides the muscle activity, such as neuromuscular activation strategies (Padua D. A., et al., 2012), trunk movements (Hewett T. E., etc., 2009), and poor core stability (Larwa J., etc., 2021, Zazulak B. T., etc., 2007), may be related to taking such a jump landing position.

4.3. Limitations and Future Prospects

This study could not capture the motion of internal/external rotation of the hip joint and lower leg because the motion was analyzed in two dimensions using a digital video camera. It is expected to analyze the motion in the horizontal plane using a 3D motion analyzer and to examine the relationship between the hip and ankle joints and the dynamic knee external rotation angle in the future.

The study's results suggested that males could control the knee joint external rotation by the lower limb muscle activity, but females had difficulty controlling the external rotation by the lower limb muscle activity, and it might be difficult to prevent ACL injury by strength training alone. In addition, the external rotation of the knee joint was considered a passive change in angle caused by a sudden increase in load. In the future, it will be necessary to prevent ACL injury in females by focusing on the muscle activity, kinematics, and balance ability.

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References

- Arms S. W., Pope M. H., Johnson R. J., Fischer R. A., Arvidsson I., & Eriksson E. (1984). The biomechanics of anterior cruciate ligament rehabilitation and reconstruction. *Am J Sports Med*, 12(1), 8-18. doi: 10.1177/036354658401200102.
- Bisciotti G. N., Chamari K., Cena E., Bisciotti A., Corsini A., & Volpi P. (2019). Anterior cruciate ligament injury risk factors in football. *J Sports Med Phys Fitness*, 59(10), 1724-1738. doi: 10.23736/S0022-4707.19.09563-X.
- Boey D., & Jc Lee M. (2020). The relationship between Y-balance test scores and knee moments during singleleg jump-landing in netball. *Int J Sports Phys Ther*, 15(5), 722-731. doi: 10.26603/ijspt20200722.

Collings T. J., Diamond L. E., Barrett R. S., Timmins R. G., Hickey J. T., du Moulin W. S., Williams M. D.,

Beerworth K. A., & Bourne M. N. (2022). Strength and biomechanical risk factors for non-contact ACL injury in elite female footballers: a prospective study. *Med Sci Sports Exerc*, online ahead of print. doi: 10.1249/MSS.00000000002908.

- Draganich L. F., & Vahey J. W. (1990). An in vitro study of anterior cruciate ligament strain induced by quadriceps and hamstrings forces. *J Orthop Res*, 8(1), 57-63. doi: 10.1002/jor.1100080107.
- Ekegren C. L., Miller W. C., Celebrini R. G., Eng J. J., & Macintyre D. L. (2009). Reliability and validity of observational risk screening in evaluating dynamic knee valgus. J Orthop Sports Phys Ther, 39(9), 665-674. doi: 10.2519/jospt.2009.3004.
- Hewett T. E., Lindenfeld T. N., Riccobene J. V., & Noyes F. R. (1999). The effect of neuromuscular training on the incidence of knee injury in female athletes. A prospective study. *Am J Sports Med*, 27(6), 699-706. doi: 10.1177/03635465990270060301.
- Hewett T. E., Myer G. D., Ford K. R., Heidt R. S. Jr., Colosimo A. J., McLean S. G., van den Bogert A. J., Paterno M. V., & Succop P. (2005). Biomechanical measures of neuromuscular control and valgus loading of the knee predict anterior cruciate ligament injury risk in female athletes: a prospective study. *Am J Sports Med*, 33(4), 492-501. doi: 10.1177/0363546504269591.
- Hewett T. E., Torg J. S., & Boden B. P. (2009). Video analysis of trunk and knee motion during non-contact anterior cruciate ligament injury in female athletes: lateral trunk and knee abduction motion are combined components of the injury mechanism. *Br J Sports Med*, 43(6), 417-422. doi: 10.1136/bjsm.2009.059162.
- Hewett T. E., Di Stasi S. L., & Myer G. D. (2013). Current concepts for injury prevention in athletes after anterior cruciate ligament reconstruction. *Am J Sports Med*, 41(1), 216-224. doi: 10.1177/0363546512459638.
- Kernozek T. W., Torry M. R., VAN Hoof H., Cowley H., & Tanner S. (2005). Gender differences in frontal and sagittal plane biomechanics during drop landings. *Med Sci Sports Exerc*, 37(6), 1003-1012.
- Larwa J., Stoy C., Chafetz R. S., Boniello M., & Franklin C. (2021). Stiff Landings, Core Stability, and Dynamic Knee Valgus: A Systematic Review on Documented Anterior Cruciate Ligament Ruptures in Male and Female Athletes. *Int J Environ Res Public Health*, 18(7), 3826. doi: 10.3390/ijerph18073826.
- Montalvo, A. M., Schneider D. K., Webster K. E., Yut L., Galloway M. T., Heidt R. S. Jr., Kaeding C. C., Kremcheck T. E., Magnussen R. A., Parikh S. N., Stanfield D. T., Wall E. J., & Myer G. D. (2019). Anterior Cruciate Ligament Injury Risk in Sport: A Systematic Review and Meta-Analysis of Injury Incidence by Sex and Sport Classification. J Athl Train, 54(5), 472-482. doi: 10.4085/1062-6050-407-16.
- Myer G. D., Ford K. R., & Hewett T. E. (2005). The effects of gender on quadriceps muscle activation strategies during a maneuver that mimics a high ACL injury risk position. *J Electromyogr Kinesiol*, 15(2), 181-189. doi: 10.1016/j.jelekin.2004.08.006.
- Myer G. D., Ford K. R., Barber Foss K. D., Liu C., Nick T. G., & Hewett T. E. (2009). The relationship of hamstrings and quadriceps strength to anterior cruciate ligament injury in female athletes. *Clin J Sport Med*, 19(1), 3-8. doi: 10.1097/JSM.0b013e318190bddb.
- Nilstad A., Andersen T. E., Kristianslund E., Bahr R., Myklebust G., Steffen K., & Krosshaug T. (2014). Physiotherapists can identify female football players with high knee valgus angles during vertical drop jumps using real-time observational screening. J Orthop Sports Phys Ther, 44(5), 358-365. doi: 10.2519/jospt.2014.4969.
- Nilstad A., Krosshaug T., Mok K. M., Bahr R., & Andersen T. E. (2015). Association between anatomical characteristics, knee laxity, muscle strength, and peak knee valgus during vertical drop-jump landings. *J Orthop Sports Phys Ther*, 45(12), 998-1005. doi: 10.2519/jospt.2015.5612.
- Olsen O. E., Myklebust G., Engebretsen L., & Bahr R. (2004). Injury mechanisms for anterior cruciate ligament injuries in team handball: a systematic video analysis. *Am J Sports Med*, 32(4), 1002-1012. doi: 10.1177/0363546503261724.
- Padua D. A., Bell D. R., & Clark M. A. (2012). Neuromuscular characteristics of individuals displaying excessive medial knee displacement. *J Athl Train*, 47(5), 525-536. doi: 10.4085/1062-6050-47.5.10.
- Russell K. A., Palmieri R. M., Zinder S. M., & Ingersoll C. D. (2006). Sex differences in valgus knee angle during a single-leg drop jump. *J Athl Train*, 41(2), 166-171.
- Shultz S. J., Nguyen A. D., Windley T. C., Kulas A. S., Botic T. L., & Beynnon B. D. (2006). Intratester and intertester reliability of clinical measures of lower extremity anatomic characteristics: implications for multicenter studies. *Clin J Sport Med*, 16(2), 155-161. doi: 10.1097/00042752-200603000-00012.
- Ugalde V., Brockman C., Bailowitz Z., & Pollard C. D. (2014). Single leg squat test and its relationship to dynamic knee valgus and injury risk screening. *PM R*, 7(3), 229-235. doi: 10.1016/j.pmrj.2014.08.361.
- Wilczyński B., Zorena K., & Ślęzak D. (2020). Dynamic knee valgus in single-leg movement tasks. Potentially modifiable factors and exercise training options. A literature review. *Int J Environ Res Public Health*, 17(21), 8208. doi: 10.3390/ijerph17218208.
- Withrow T. J., Huston L. J., Wojtys E. M., & Ashton-Miller J. A. (2006). The relationship between quadriceps muscle force, knee flexion, and anterior cruciate ligament strain in an in vitro simulated jump landing. Am J Sports Med, 34(2), 269-274. doi: 10.1177/0363546505280906.
- Yu B., & Garrett W. E. (2007). Mechanisms of non-contact ACL injuries. *Br J Sports Med*, 41 (Suppl 1), i47-51. doi: 10.1136/bjsm.2007.037192.

Zazulak B. T., Hewett T. E., Reeves N. P., Goldberg B., & Cholewicki J. (2007). Deficits in neuromuscular control of the trunk predict knee injury risk: a prospective biomechanical-epidemiologic study. *Am J Sports Med*, 35(7), 1123-30. doi: 10.1177/0363546507301585



Radiation Dose to Breast During Head Computed Tomography Scan Among Nigerian Population: A Cross-Sectional Study

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Abstract

Background: The extent of the dose received by the breast during head CT, needs quantification to effectively predict the risk of carcinoma and gene mutation. Objective: The study aimed to evaluate the radiation dose to the breast during a head CT scan in Enugu State. Methods: This cross-sectional study which involved 52 adults women selected purposively, described the radiation doses received by shielded and unshielded breasts during head CT examinations. The relationship between the anthropo-technical parameters Age, height, weight, BMI, breast circumferences, and distance from the External Auditory Meateus (EAM) to the TLD, kVp, mA, and the DLP was determined using Pearsons' Correlation. Results: The mean of CTDIvol, DLP, and breast circumferences (left shielded and right unshielded) are 29.43±40.0mGy, 540.8±107.03mGy, and left shielded breast (4.71±2.45mGy) and right unshielded breast (5.0 ± 2.32 mGy). There were positive correlations but not statistically significance between breast absorbed dose and BMI (r = 0.152, p = 0.291), linear distance from EAM to TLD (r = 0.032, p = 0.828) and mAs (r = 0.100, p = 0.492), while there was negative correlation but not statistically significance exist with subjects' age (r = -0.112, p = 0.439). Conclusion: There was a significant difference in the amount of radiation doses received by shielded and unshielded breast greatly reduces the radiation dose received by the breast during head CT. Therefore, we recommend that shielding of the breasts during head CT should form a standard protocol in our setting.

Keywords: Computed Tomography, Dose, Radiation

1. Introduction

The last two decades have witnessed a surge in the use of computed tomography (CT) in radiological diagnosis and this method has become the major non-natural source of ionizing radiation to the population. CT investigations deliver to the patients more radiation than all other radiological imaging modalities and contribute disproportionately to the overall radiation dose. Radiation received by organs in the primary beam can be clinically

justified. However, the dose received by organs outside the primary beam cannot be justified when the organs are radiosensitive. Breast, thyroid, eyes and gonads receive a greater proportion of radiation due to increased scatter and lack of other overlying tissues to partially absorb some of the dose (Abuzaid et al., 2017).

Breast dose is high in CT examination with breast in scanning planes. Radiation dose to the breast is also significant when breast is exposed only to scatter radiation. Breast doses received through scatter radiation during CT scan of the head may account for up to one-fifth of an average mammographic dose per one view (Beaconsfield et al., 1998). Since, it is not possible to reduce radiation load to organs in CT scanning plane (Brnic et al., 2003), the tissues outside should be protected against scatter, whenever, it does not sacrifice image quality (Brnic et al., 2003). The breast is a gland in close proximity to the head which is considered the most common examination in CT (Adejoh & Nzotta, 2016). It is therefore at risk of scattered radiation during head CT procedures. The extent of dose received by the breast during head CT, needs quantification to effectively predict risk of carcinoma and gene mutation. To the best of the researcher's knowledge and based on literature search, there is paucity of data on radiation dose to the breast during head CT scan and the impact of the anthropometric parameters on the radiation received in this setting. The study aimed to evaluate radiation dose to the breast during head CT scan in Enugu State using TLD and to find out if any relationship exists between CT breast dose and anthropometric parameters as well as determine the extent to which lead sheet shielding reduces dose to the breast during head CT scan.

2. Materials and Methods

2.1. Study Design and Setting

This was a cross-sectional study carried out at the radiology unit of a private diagnostic center in Enugu, Enugu State, Nigeria.

2.2. Population and Sample Size Determination

This study population comprised of all patients referred for head CT scans at the selected study center within the study period (October 2018 to February 2019)

The sample size of 52 was determined using Yamani (1967) formula for known population.

n =	$\frac{N}{1+N(e)^2}$	
Where n	=	desired sample size
Ν	=	population of study
e	=	accepted error limit (0.05)

From the head CT reports' archives of the study centre, a total population of 60 female patients underwent Brain CT from March 2018 to December 2018.

This gave			
n=	60	60	
1+60	0(0.025)	1.15	= 52

2.3. Sampling Technique and Ethical Consideration

A purposive sampling method was adopted for selecting a sample size of 52 from the target population, since only female subjects with developed breasts without any history of mastectomy were selected and included in this study. Ethical approval (ERC/FHST/NAU/2018/2078) for the study was obtained from the Human Research and Ethical Committee of Faculty of Health Sciences and Technology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Nnewi Anambra State. A thorough explanation of the study procedures was made to the participants by the researchers and the entire participants signed a written consent form.

2.4. Inclusion and exclusion criteria:

Only female subjects from 18 years of age and above, subjects with developed breasts and who have not undergone mastectomy in any of the breast were included in this study. All subjects with any history of mastectomy and those below the age of 18 years were excluded from this study.

2.5. Equipment and accessories:

The equipment and accessories used in this study include; 1) General electric (GE) Bright speed CT scanner manufactured in July, 2007 and installed in 2015, with 8-slice per rotation capacity. The scanner was also self – calibating and its software was activated daily. 2) One hundred and four LI-TLD (TLD-100) chips annealed before use were obtained from the radiation safety Adviser (RSA) Nigeria Nuclear Regulatory Authority (NNRA) Abuja and were used for the study. 3) ZT WHO standard scale and height meter with error level of 0.05 were used. It was manufactured by Halogic Incorporation United States of America in the year 2008. 4) Radalert 100 survey meter was used to record the background radiation. It was manufactured by Toshiba in the year 2009 in Germany.5) Measuring tape was used to measure both breast circumferences.6) Transparent cello tape. 7) Data capture sheet and 8) Lead sheet of 0.35mm lead equivalent.

2.6. Examination procedures and method of data collection

The subjects who consented to this study were asked to stand erect in an anatomical position and without shoes, and were made to empty pockets of any objects like mobile phones or bunch of keys that could add a gram or more to the weight. The subjects stood erect on the beam balance without resting hands or bodies on the table or wall. The weight, in (kg) was read to the nearest 0.5kg, while still standing erect and without motion, the heels, gluteal muscles and occipital protuberance touching the vertical bar of the height scale, the short, horizontal bar of the scale was adjusted to make firm contact with the vertex of the head. The height was then read off to the nearest 0.1 centimeter. The body mass index was obtained by dividing weight (in kg) by square of height (m²)

BMI = weight (kg)Height (m²)

The following information was collected; patients age, weight (Kg), Height (meters) Body mass index (BMI) kgm-1), Distance from the External auditory meatus to TLD (cm), both breast circumferences (cm) and technical parameters. Data were entered by the researchers assisted by a radiographer in the facility.

The background radiation in the CT suit of the study centre was recorded by the researchers daily using Rad alert 100 survey meters prior to the patient's positioning for the CT scan throughout the period of the study. Before the commencement of CT examinations, the subjects were asked to change to the departmental gown made up of radiolucent materials to avoid image artifacts.

The subjects were properly positioned for the investigations and this was achieved by ensuring that the laser light crosses the outer cantus of the eye, which should be equidistant to the external auditory meatus. The subjects were immobilized using straps. The similar procedure used by Brinicz et al. (2003) was adopted for evaluating the radiation doses to the breast during head CT. For each subject, two TLD chips were used and each enclosed in cellophane and labeled "shielded" and "unshielded." With the subject in supine position, the TLD holder was attached by cello tape to the skin of the unshielded right breast, 2 cm above and 2 cm lateral to the nipple. The second TLD labeled "Shielded" was fixed underneath the lead sheet on the left breast at the same position as in the right breast. A lead sheet was also used to cover the area of the TLD and clavicle, extending to the mid axillary line. This was done so that each patient will serve as her own control. The band straps were ensured not to be on the chest region to ensure radiation was not attenuated. Then, standard non-contrast conventional head CT protocol consisting of initial scout view, axial scans was carried out at each study with exposure parameters of kVp of 120, mAs of 150, slice thickness of 5mm and gantry rotation time of I and scan duration 14 seconds. Finally, the two sachets of TLD chip labeled (shielded and unshielded) were removed from the patient after the exposure and put

together into another sachet labeled with the patient's hospital and CT identification number, date of investigation, patient age and exposure parameter used.

2.6.1 Reading/Processing of the TLD

Each batch of the exposed TLD chips was taken to the center for Energy, Research and Training (CERT), Zaria for reading. The reading was done using Harshaw 4500 dual channel TLD reader at the physics and protection section of the center for Energy, Research and Training (CERT) of the Ahmadu Bello University, Zaria, and Kaduna State Nigeria. The reading involved heating the chips to high temperature for them to give out luminescence which is proportional to the amount of radiation exposure received and stored by the TLD chips. The Harshaw 4500 TLD Reader is interfaced with windows Win REMSTM and software resident on a personal computer (PC), which is connected to the reader via a serial communication port.

2.7. Data Analysis

The Data were categorized into Anthropometric (measurements on subjects), technical data of the CT scanner and dose data. The anthropometric data included weight, height, body mass index both breast circumference and distance from the external auditory meatus to the TLD. The technical data however are exposure parameters, while dose data were CTDi vol, DLP and dose data from read TLDs. These data were analyzed with the aid of Statistical Package for the Social sciences (SPSS) version 20.0 (SPSS incorporated, Chicago, Iliniois, USA) Mean, mode and range were used to summarize all anthropo-technical data. Results were given as mean ± standard deviations. The relationship between the anthropo-technical parameters Age, height, weight, BMI, Both breast circumference and distance from the EAM to the TLD, kVp, mA, gantry tilt angle and the DLP was determined using a univiariate Pearson correlation tests. All tests were carried out at an alpha level of 5% (0.05). The results are described into descriptive statistics displayed in tables.

3. Results:

3.1. Demographic characteristics of the subjects

The age range of the subjects was 18-50 years with mean age of 36 ± 8 .oyears. The weight range was 60-80kg with BMI of 29.79 ± 3.05 kg/m², and a mean breast size of 51.6 ± 9.5 cm (Table 1).

Table 1: Demographic characteristics of subjects							
Parameters	n = 50						
i ai ainctci ș	Range	Mean ± Standard Standard ange Deviation Error					
Age (years)	18 - 50	36 ± 8.0	1.13	- 0.204			
Height (cm)	1.52 - 1.74	161 ± 4.0	0.01	0.900			
Weight (kg)	55 - 89	77.6 ± 7.2	1.02	1.000			
Body Mass Index (BMI)	21.8 - 34.6	29.72 ± 3.05	0.40	- 0.713			
Right breast circumference (cm)	24 - 67	52.1 ± 8.7	1.23	- 0.810			
Left breast circumference	25 - 67	51.9 ± 9.7	1.40	- 0.636			

3.2. Dose characteristics of the subjects

The mean and standard deviations for the CTDIvol, DLP and breast circumferences (left shielded and right unshielded) are 29.43 ± 40.0 mGy, 540.8 ± 107.03 mGy, and left shielded breast (4.71 ± 2.45 mGy) and right unshielded breast (5.0 ± 2.32 mGy) (Table 2). The result of the paired-sample T-test between the left and right breast revealed that there were no significance differences in the mean circumferences of the breasts (p=0.747) and the breast absorbed dose (p=0.612). Also, the correlation analysis done revealed that there were positive correlations but not statistically significance between breast absorbed dose and BMI (r=0.152, p=0.291), Linear distance from EAM to TLD (r=0.032, p=0.828) and mAs (r=0.100, p=0.492), while there was negative correlation but not statistically significance exist with subjects' age (r=-0.112, p=0.439) (Table 3).

Table 2: Dose characteristics of subjects							
Parameters		n = 50					
	Range	Mean ± Standard Deviation	Standard Error	Skewness			
Distance of TLD from EAM (Cm)	21 - 35	30.9 ± 6.62	0.37	-1.028			
CTDIvol (head)	Fixed	29.43 ± 0.0					
DLP (head)	451 - 1230	540.8 ± 107.03	15.15	5.6			
Breast dose LB; shielded)	1.8 - 15.4	4.71 ± 2.45	0.35	2.41			
Breast dose (RB; unshielded)	1.6 – 12.2	5.0 ± 2.32	0.33	1.1			
Breast dose,	1.60 - 15.40	4.84 ± 2.40	0.24	1.64			
mGy (combined)	0.09 - 0.80	0.24 ± 0.12	0.2	2.21			
	0.08 - 0.60	0.25 ± 0.12	0.2	1.40			
		0.24 ± 0.12	0.01	1.66			

Key: LB= left breast, RB= Right breast

Table 3: Inferential statistics of the variables

Parameters	Paired-sample T-Tests of means					
1	Mean ± SD (Difference)	t-statistic	p-value	Remarks		
RBC& LBC (cm)	0.22 ± 4.8	0.325	0.747	Not statistically significant		
SLB&URB (mGy)	0.26 ± 3.6	0.511	0.612	Not statistically significant		
SLB&URB (mSv)	0.10 ± 0.2	- 0.360	0.720	Not statistically significant		
		Corr	Correlations			
		Correlation				
	n	coefficient (r)	p-value	Remarks		
Age with breast circumference	50	-0.205	0.154	Not statistically significant		
Age with mGy	50	-0.112	0.439	Not statistically significant		
BMI with breast circumference	50	0.141	0.329	Not statistically significant		
BMI with mGy	50	0.152	0.291	Not statistically significant		
Distance from EAM to TLD& Breasts (mGy)	50	0.032	0.828	Not statistically significant		
mAs& Breasts (mGy)	50	0.100	0.492	Not statistically significant		

Key: RBC= Right Breast Circumference, LBC= Left Breast Circumference, SLB= Shielded Left Breast, URB= Unshielded Right Breast

4. Discussion

The result of this study showed that the mean absorbed dose for the shielded breast was slightly lower than that of the unshielded breast with reduction of 29%. This finding is in agreement with to the findings of the previous studies by Brinic et al. (2003), Hohl et al. (2018), Parker et al. (2008) and Gunn et al. (2009), which also reported reductions in the absorbed dose between the unshielded and shielded organs evaluated. They all reported that the absorbed dose of the shielded structures is usually lower than that of the unshielded structures. This implies that shielding of an organ during radiation investigations significantly reduces the amount of radiation absorbed dose received by the organ. In Hohl et al. (2008) study, which was conducted to evaluate the radiation dose of breast and thyroid gland, reported a mean dose without shield of 17.2 ± 0.5 mSy and 11.4 ± 0.6 mSy resulting in dose reduction of 33.7%. According to Hohl et al. (2008), plain Bismuth breast shield greatly decreases radiation dose in MDCT without altering the image quality. Similarly, Brinic et al. (2003) in their study, which evaluated the efficacy of breast shielding during CT head, reported mean doses of 0.28mGy and 0.13mGy for unshielded and shielded breasts respectively, with dose reduction of 57%. Parker et al. (2008) study, also documented reduction in absorbed dose between shielded and unshielded breast of 56-61% with the left and right unshielded breasts having mean absorbed doses range of 14.20-4.7mGy and 13.83-19.36mGy respectively, while the shielded right and left breasts absorbed doses were 6.64-8.12mGy and 6.7-8.03mGy respectively. In Gunn et al. (2009) study, they also found that the mean absorbed dose was higher with an unshielded breast than the shielded one. According to Gunn et al. (2009), great dose reduction was observed to the thyroid gland and breast with shield.

We found also that there was no significance difference in the mean of the right and left breast circumferences. There were positive correlations but not statistically significance between breast absorbed dose and BMI, Linear distance from EAM to TLD and mAs, while there was negative correlation but not statistically significance exist with subjects' age. This implies that increased in body mass index, linear distance from EAM to TLD and mAs also resulted in increase in the radiation dose received during CT head while as the age increases, the radiation dose received decreases.

4.1. Limitation of the study

The majority limitation of this study is that the findings cannot be generalized due to the small sample of this study.

5. Conclusion

There was significant difference in the amount of radiation doses received by shielded and unshielded breasts during head CT, with low radiation doses received by shielded breasts. Shielding of the breast greatly reduces the radiation dose received by the breast during head CT. Increased in body mass index, linear distance from EAM to TLD and mAs resulted in increase in the radiation dose received during CT head while radiation dose receives decreases with increased age of the patients. Therefore, we recommend that shielding of the breasts during head CT should form a standard protocol in our setting and the anthropometric parameters should be considered when selecting CT exposure factors.

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References

Abuzaid, M.M., Elshani, W., Hancef, C., Alyafei, S. (2017). Thyroid shield during brain CT scan: dose reduction and image quality evaluation. Imaging in medicine. 9(3)

Beaconsfeld, T., Nicholson, R., Thornton, A., & Al-Kutoubi, A. (1998). Would thyroid and breast shielding be beneficial in CT of the head? Eur Radiol. 8: 664-7

- Brnic, Z., Vekic, B., Hebrang, A., & Anic, P. (2003) Efficacy of breast shielding during CT of the head. EurRadiol.13(11),2436 - 2440
- AdeJoh, T., & Nzotta, C. C. (2016). Head Computed Tomography: Dose output and relationship with anthropotechnical parameters. West African journal of Radiology. 23(2), 113-117.

- Yamane, T. (1967). *Statistics: An Introductory Analysis*. 2nd Ed. Harper and Rao, New York. p.886. Hohl,C., Muhlenbruch, G., Wildberger, J.E., Leidecker, C., Suss, C., Schmidt, T., Gunther, R.W., & Mahaken AH.(2006). Estimation of radiation exposure in low-dose multislice computed tomography of the heart and comparison with a calculation program. Eur Radiol. 16:1841-1846 DOI: 10.1007/s00330-005-0124-y
- Parker, M.S., Kelleher, N.M., Hoots, J.A., Chung, J.K., Fatouros, P.P., Benedict, S.H. (2008). Absorbed radiation dose of the female breast during diagnostic multidetector chest CT and dose reduction with a tungstenantimony composite breast: preliminary results. ClinRadiol. 63(3),278-288.
- Gunn, M.L., Kanal, K.M., Kolokythas, O., & Anzal, Y. (2009). Radiation dose to the thyroid gland and breast from mutidetector computed tomography of the cervical spine: does bismuth shielding with and without a cervical collar reduce dose? J Comput Assist Tomogr. 33(6),987 -990



Leptomeningeal Schistosomiasis: A Case Report of an Atypical Location of Neuroschistosomiasis Presenting as Adult-Onset Seizure

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Abstract

We report a case of a 19-year-old male, single, right-handed, student, Filipino currently living in Novaliches, Quezon City who consulted for the first time at our institution due to stiffening of extremities with a pertinent travel history from the Island of Samar. Evaluation and diagnostics showed a leptomeningeal enhancement and thereafter, a biopsy was made revealing deposition of schistosoma ova at the leptomenineal area. Schistosomiaisis (also known as Bilharzia or Blood Fluke Disease) is widely distributed in the Philippines affecting 24 provinces in Luzon, Visayas, and Mindanao, with 5 million people at risk and approximately 1 million affected in the year 2003. Cerebral schistomiasis is a severe and neglected complication which occurs in less than 5% of infected individuals. Symptoms are non-specific such as headache, vomiting, confusional states, and focal seizures. Typical neuroimaging findings are expected at the spinal cord, cerebellum, and the subcortical area, however leptomeningeal involvement is rarely reported. We report this case to the medical community to give light on the different presentations of the said disease

Keywords: Schistosomiasis, Neuroschistosomiasis, Leptomeninges, Focal Seizure

1. Introduction

Schistosomiaisis (also known as Bilharzia or Blood Fluke Disease) is widely distributed in the Philippines affecting 24 provinces in Luzon, Visayas, and Mindanao affecting 5 million people at risk, with approximately 1 million affected in the year 2003 (Olveda *et al.*, 2014). Infection is transmitted by snails living in fresh water such as lakes, rivers, streams and ponds. Initial symptoms usually appear within days or weeks after cercarial penetration which migrates to the lungs then to the target organs via the vascular system. Symptoms include a skin rash, fever, headache, muscle ache, bloody diarrhea, cough, malaise, and abdominal pain in the early stages of the disease. If untreated, schistosomiasis can become a chronic illness as the flatworm eggs damage the lining or the main parenchyma of the target organ (Imai *et al.*, 2011).



Figure 1: shows the distribution of Schistosomes in the Philippines

This type of parasitic infection is composed of several species with 5 of them causing significant disease entities. According to Olveda *et al.* (2014), there are three species are present in the Philippines namely *S. japonicum, S. haematobium, and S. mansoni to which each specie has a predilection to affect an organ system particularly the integumentary, urogenital, gastrointestinal, and hepatobiliary system. Although there are some cases in which the parasite may involve an "atypical location" so called ectopic schistosomiasis which affects the lung, heart, and in some cases the central nervous system.*

Neuroschistosomiasis is a relatively an uncommon disease with the risk of the infection depending on the level of immunity and the magnitude of the schistosomal invasion. About <5% of patients will develop neuroschistosomiasis where spinal and cerebellar complications are prevalent. This was further elaborated by Amaral and Andrade (2015) as S. japonicum infection has a predilection for cortex, subcortex, basal ganglia or internal capsule. The reason for this predilection is explained two different mechanisms. The first is by egg embolism and second is by worm migration through valveless veins as cited by the study of Wu et al. (2011) to which the study involved 11 patients from the Rennin Hospital, Wuhan University in China. Eggs from the portal system could embolize to the brain along the vertebral venous or Batsons' plexus, via atrial/septal defects or patent foramen ovale, or via pulmonary venous shunts, as a result of hepatic and pulmonary hypertension. The size and shape of the eggs make it favorable in their migration to the brain especially in S. japonicum explaining it's cerebral predilection. In contrast with S. mansoni and S. haematobium which have larger eggs and prominent spines. In relation to the second mechanism, adult flukes could enter the Batson plexus and then migrate upwards to the brain and deposit their eggs directly. Both theories explain the most common pathologies such as transverse myelitis and a cerebellar mass (George et al., 2009). In transverse myelitis, the most prevalent clinical manifestations as studied by Amaral and Andrade (2015) were weakness in the lower limbs (94%), back pain (84%), bladder dysfunction (75%) and impotence (80%). The most common site of injury was the thoracolumbar junction (65%) and 73% of parasitological stool examinations were negative.

Several neuroimaging techniques such as computed tomography scan (CT Scan) and magnetic resonance imaging (MRI) reveal certain patters in the diagnosis of neuroschistosomiasis. CT scans showed irregular low-dense and isodense lesions that were mixed in the focus area, as mixed boundary was not very clear. There are also reports of having multiple calcified areas at the subcortical area associated with edema and manifestations of the "glove sign" with obvious mass effect. Typical MRI findings showed a scattered gyrus-like, nodular, or irregular-shaped spots of strengthening (Wu *et al.*, 2011). However due to the variation of clinical manifestations, a high index of suspicion is still warranted.

In our case, we describe a case of cerebral schistosomiasis presenting as a new onset seizure and a solitary mass like lesion affecting the meninges of the brain

2. Case Report

A case of a 19-year-old Filipino male, single, right-handed, unemployed, from Novaliches, Quezon City who sought consult for the first time at our institution on August 3, 2020 due to stiffening of extremities. He is a grade9 student with a total of 8 formal years of schooling The patient was born and raised in Catbalogan City, Samar Island for 13 years where they lived near rice-fields. He had no comorbidities with no history of trauma and surgery. He is a nonsmoker, non-alcoholic beverage drinker and denies illicit drug use. Review of the family history showed only hypertension on father side.

The patient was apparently well until the 2nd week of October 2019, when he started to experience headache, located at the right hemicranium, which was dull in character, graded 3-4/10 on PAS, non-radiating, occurring at no particular time of the day. After 1 month the patient was seen having seizures described as stiffening of the left extremities associated with head veering to the left, and loss of consciousness which lasted for 2-3 minutes which occurred for three times. The patient also had auditory and visual hallucinations prior to the seizure episode. He was initially brought a nearby hospital which requested for neuroimaging and was initially given Levetiracetam 500mg tablet twice a day to which the patient was compliant. He was eventually sent home. In the interim the patient had recurrence of the seizures with the same semiology for about 2-3 times per month, from which he tolerated his condition. However, on the 1st week of August 2020 the patient had 6 episodes of seizure associated with vomiting hence consulted at our institution.

Upon consultation, the vital signs, general physical examination, and review of systems were unremarkable. Neurologic examination revealed that the patient was awake, oriented to time, place and person. He could follow commands. Cranial nerve examination noted no anosmia, 3mm equally briskly reactive to light pupillary response, no visual field cuts, primary gaze at the midline, full extraocular muscle movement, funduscopic exam was unremarkable, intact gross hearing, no facial asymmetry, uvula and tongue at midline. On manual motor testing, patient scored 5/5 on all extremities, with no sensory deficit and normoreflexive on all extremities. No Babinski was noted, no nuchal rigidity, no apraxia, no aphasia, no frontal release signs, no parietal signs.

Basic laboratory examinations (Appendix A) such as complete blood count, electrolytes and coagulation studies were normal. Other ancillaries such as Random blood sugar, Creatinine were normal. Chest Xray and 12-Lead ECG were normal (Appendix A).



Figure 1: The non-contrast CT scan on axial view showing finger-like hypodensities at the right temporal lobe with no midline shift nor hydrocephalus. The cranial vault was intact.



Figure 2: The cranial MRI done 6 months after the 1st imaging with gadolinium contrast shows areas of irregular hyperintensities likely corresponding to vasogenic edema on T2 segment (A – Axial, B – Coronal)



Figure 3: The cranial MRI with gadolinium contrast shows a serpentine-like enhancement at the right temporal lobe with the corresponding MRS ratio

The patient was admitted and was initially managed as a case of adult-onset seizure probably secondary to an intracranial mass. The patient was started on Levetiracetam 500mg tab, 1 tablet twice a day for the control of seizures. Dexamethosone 4mg tablet, 1 tablet twice a day was also started for the vasogenic edema of the patient. Patient was admitted for 21 days with no neurologic nor behavioral changes. Patient was then subjected to excision of tumor and histopathologic studies were done which revealed a Cerebral Schistosomiasis at the subarachoid space and Virchow spaces overlying the right temporal lobe. He was given Praziquantel 60mg per kilogram as loading dose, a total of 2350 mg was loaded. Patient was observed for adverse drug reactions and was eventually discharged. Follow-up done after 1 month post-surgery with no recurrence of seizures and no focal neurologic deficits. Patient was able to go back to school.

3. Discussion

Focal epilepsy due to neuroschistosomiasis in the Philippines has been estimated to be from 2% to 5% among infected individuals. Diagnosing cerebral schistosomiasis can be difficult, since neurological symptoms and radiological findings are nonspecific. In some reported cases of neuroschistosomiasis, brain tumors, such as meningioma and glioma, had been suspected initially. Moreover, as in the present case, patients with neuroschistosomiasis may have no clinical evidence of systemic disease such as loose stools nor blood tinged urine. Stool/Urine examinations only reveal up to 40-50% of neuroschistosomiasis especially if the patient had the exposure a few years prior.

Our case demonstrates one of the neglected diseases in the Philippines. The patient had no history of 'cercarial dermatitis' nor the common prodromes of the said disease, possibly due poor health seeking behavior or perhaps

because it went unrecognized. Unremarkable examination and laboratory findings (on blood and stool exam) added to the diagnostic difficulty. With the benefit of histological diagnosis, the study suggests that the serpiginous enhancements seen on MRI represent focal zones of intense tissue reaction to eggs, leading to chronic pathology involving the vasculature. The treatment of cerebral schistosomiasis is highly effective and safe. Praziquantel eradicates the adult worms and concomitant corticosteroids reduce the granulomatous inflammation and are used for all schistosomal subtypes. However, the effect on chronic granulomatous disease and ova remains unknown. For this case, a high index of suspicion and a good clinical history revealing the pertinent travel history to endemic areas may help with the diagnosis and proper treatment for the patient.

4. Conclusion

This is a case of a 19-year-old male who presented with adult onset seizure with a history of travel to the province of Samar, with no known co-morbidities, no family history of seizure. Further laboratory work-up was inconclusive. Neuroimaging with MRS guidance revealed non-specific findings. We report this case as one of the neglected diseases in our country. Making the diagnosis is challenging, as manifestations may be protean and presentation with neurological complications can occur several years after the initial infection. Therefore, we recommend a high index of suspicion and an aggressive diagnostic approach in comparable cases.

Ethical Consideration

Patient form was secured before submission of manuscript

Financial Support and Sponsorship

None

Conflicts of Interest

There are no conflicts of interest.

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References

- Olveda, D.U., Li, Y., Olveda, R.M., Lam, A.K., McManus, D.P., Chau, T.N.P., Harn, D.A., Williams, G.M., Gray, D.J. and Ross, A.G.P. (2014). Bilharzia in the Philippines: past, present, and future. *International Journal of Infectious Diseases*, [online] 18, pp.52–56. doi:10.1016/j.ijid.2013.09.011.
- Imai, K., Koibuchi, T., Kumagai, T., Maeda, T., Osada, Y., Ohta, N., Koga, M., Nakamura, H., Miura, T., Iwamoto, A. and Fujii, T. (2011). Cerebral Schistosomiasis Due to Schistosoma haematobium Confirmed by PCR Analysis of Brain Specimen. *Journal of Clinical Microbiology*, 49(10), pp.3703–3706. doi:10.1128/jcm.01073-11.
- El Beltagi, A., Salem, K. and Hanoun, M. (2021). Neuro-schistosomiasis with palm tree contrast enhancement pattern, a report of three cases, and review of literature. *BJR*|*case reports*, [online] 7(1), p.20200053. doi:10.1259/bjrcr.20200053.
- Wu, L., Wu, M., Tian, D., Chen, S., Liu, B., Chen, Q., Wang, J., Cai, Q., Ji, B., Wang, L., Zhang, S., Ruan, D., Zhu, X. and Guo, Z. (2011). Clinical and Imaging Characteristics of Cerebral Schistosomiasis. *Cell Biochemistry and Biophysics*, 62(2), pp.289–295. doi:10.1007/s12013-011-9294-1.
- George, J., Rose, D., Hazrati, L.-N., Majl, L. and Hodaie, M. (2009). Cerebral schistosomiasis--an unusual presentation of an intracranial mass lesion. *The Canadian Journal of Neurological Sciences*. *Le Journal Canadien Des Sciences Neurologiques*, [online] 36(2), pp.244–247. doi:10.1017/s0317167100006624.
- Amaral, R. and Andrade Filho, A. (2015). The clinical and epidemiological profile of neuroschistosomiasis. *Journal of the Neurological Sciences*, 357, pp.e109–e110. doi:10.1016/j.jns.2015.08.350.

Appendix A

CXR		Clear Lung-field								
ECG initial		Normal Sinus	Normal Sinus rhythm							
СВС	Hgb	Het	WBC	Ν	L	E	Plt			
8/6	16.2	45	10.0	53	27	3	224			
8/13	16.3	46	11.5	70.5	16.2	1.2	256			
8/17	16.4	36	16.9	71	16	4.14	305			
8/20	16.4	48	11.8	53.1	30.6	1.5	250			
8/23	14.9	43	19.8	80.4	7.7	4.1	197			
8/26	16.9	49	17.1	81.3	5.5	3.9	184			
8/29	14.2	43	8.0	76	19	0.2	221			
COAG	РТ	% A	INR	CON	РТТ	CON				
8/6	11.2	103.6	0.93	11.7	23.4	26.6				
8/15	9.0	136.6	0.75	11.6	21.1	27.1				
Chem	Crea	Na	К							
8/7	83	145	3.3							
8/13	97.24	143	3.35							
8/17	97.24	140.0	4.1							
8/20	88.4	139	3.8							
UA	Color	Trans	рН	SG	СНО	CHON	RBC			
8/17	Yellow	Clear	5.5	1.030	Neg	neg	0			

Appendix B – BIOPSY SLIDES



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Outcomes of COVID-19 in Children After Wide Distribution of COVID Vaccine, Albaha, Saudi Arabia

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Abstract

Background: The WHO announced COVID-19 as a pandemic on 12 March 2020, and has become a major public health around the world. Objectives: The study aimed to evaluate clinical presentation and outcomes of COVID-19 cases after wide distribution of COVID vaccine. Methods: Cross sectional study, retrospective file review of the children aged less than 14 years, and confirmed by positive swab test to have COVID 19, from May 2021 to May 2022. Results: A total of 125 patients with positive tests were included in this study. Males made up 52.8% (66/125) of the population, while females made up 47.2% (59/125). The age group of 5–10 years was the most affected, with 46.2%. The common symptoms noted were fever 90.4%, followed by abdominal pain 45.6%, vomiting 44%, diarrhea 41.6%, running nose 39.2%, cough 36%, and headache 25%. Conjunctivitis (4%) and skin rash (1.6%) were the least common symptoms. Only four patients needed PICU admission. No mortality was reported in this study. Conclusions: The study concluded that the COVID severity became less with a short hospital course after wide distribution of the COVID vaccine. Male patients were more affected than female patients in the age group 5-10 years. Severe cases were associated with comorbidities.

Keywords: COVID-19, Children, Vaccine, Saudi Arabia

1. Introduction

The World Health Organization (WHO) declared COVID-19 as a pandemic disease in March 2020 [WHO report on COVID-19, 2020]. The disease prevalence among countries is different [R. Shekerdemian LS et al]. Children have the same risk of infection as adults but may present with mild clinical manifestations. [Tsabouri, MD, PhD. Ekaterini Siomou, Jun 2020]. The common presentation signs are upper respiratory tract infection, fever, cough, and rhinorrhea, nausea, vomiting, and diarrhea (Riccardo Castagnoli, Apr 2020). Loss of taste and smell were reported in children, and increasing with age in children [Nallasamy K, 2021]. No significant differences have been reported between male and female children. Children can have COVID-19 through close contact with positive family members and with other students in the school [Zimmermann P, Curtis N, 2020]. Laboratory changes reported in patients include: lymphocytosis, lymphopenia, neutropenia, high liver and muscle enzymes, increased lactate dehydrogenase, elevated C-reactive protein (CRP) level, elevated erythrocyte sedimentation rate, [Chen W et al. 2020]. The presenting symptoms, severity, and outcome of COVID-19 are variable in each country. The care for the pediatric age group depends on local data, and guidelines. In children with hypoxia, the primary therapies were supportive care and oxygen administration. COVID Vaccine effectiveness over time was noted and the protection against severe disease was reported (ACS, NACI, April 2022). The hospitalization rates and the severity of the illness in vaccinated patients were lower in the pediatric age group (Oliver J. Watson et al., June 2022).

2. Objectives

The study aimed to evaluate the clinical presentation and outcomes of positive cases after the wide distribution of the COVID vaccine and after the local health authorities in Saudi Arabia held the strict measures applied to control COVID-19 in March 2020.

3. Methods

This was a hospital-based cross-sectional study carried out at King Fahad Hospital in Albaha, Saudi Arabia. Retrospective file review of the children aged less than 14 years was confirmed with a positive COVID-19 test from May 2021 to May 2022. The COVID protocols of the Saudi health authorities were applied [Abdullah A Algaissi et al. May 2020; Shen KL et al. Jun 2020]. The Declaration of Helsinki, ethical principles for medical research involving human subjects, were applied in our study [Helsinki Declaration 2013]. The diagnosis was confirmed by a positive nasopharyngeal swab PCR. All the cases admitted to COVID ward were enrolled in a structural protocol which included symptoms, signs, relevant investigation, duration of stay, and treatment. Information regarding demographic and clinical details, including age, sex, and history of contact, comorbidities, clinical features, and lab investigations, was recorded. The study was approved by the hospital's ethical research committee. There are no sources of funding. No conflict of interest. Children in an age group from birth to 14 years old, admitted with positive COVID-combined nasal and oropharyngeal swabs were included. Data was entered on a computer and analysed by Microsoft Excel 2020.

4. Results

A total of 125 positive cases were admitted to the Pediatric department, King Fahad Hospital, Albaha, Saudi Arabia, from May 2021 to May 2022 and were statistically analyzed. Based on the age distribution, the majority were in the age group of 6–10 years, 46.4% (58/125), followed by the 5-year age group at 33.6% (42/125), with the least being in the age group of 10–14 years, 20% (25/125), (table1). Among genders, males were more common at 52.2% (66/125), and females at 47.5% (59/125). Based on symptoms, the most common symptoms noticed were fever 90.5% (113/125), followed by abdominal pain 45.6% (57/125), vomiting 44% (55/125), diarrhea 41.6% (52/125), running nose 39.2% (49/125), cough 36% (45/125), headache 20% (25/125), and the least common symptoms noticed were conjunctival conjunction 4% (5/125) and skin rash 1.6% (2/125), (table2). Only 3.2% (4/125) of the children admitted to PICU were, one child had a known case of nephrotic syndrome, one had SCD, one had CHD, and one had cerebral palsy with spastic quadriplegia and was on ventilator support. Most of the children had a history of house and school contact. Lymphopenia was noted in the majority of admitted children with positive CRP.

5. Discussion

The data for this study were collected after the local health authorities in Saudi Arabia removed the strict measures applied to control COVID-19 in March 2020, to compare the spread and outcomes of the disease in children during and after the pandemic. In comparison with our previous study conducted at King Fahad Hospital before the vaccine was widespread, we found that the symptoms became milder, and the hospital course decreased

[Almawazini et al. 2022]. Symptomatic treatment is applied for positive cases only. This study found that after the vaccination was implemented in Saudi Arabia, the number and severity of COVID-19 in children were significantly reduced [Oliver J Watson Jun 2022]. Since COVID-19 was a major health issue all over the world, this study reported the clinic status of the children admitted to our tertiary centre. The most common symptoms observed were fever (90.5%), GI symptoms (50.8%), and respiratory symptoms (43.2%). This study revealed that gastrointestinal symptoms were predominant and severity of COVID 19 was less when compared to other studies [Meena et al., Jun 2020]. Only four patients, or 3.2% of the children, were severely ill and needed intensive care admission. Limited sample sizes and confounding factors may have affected this outcome in our study. Only two patients needed mechanical ventilation. One child was a known case of congenital heart disease and the second patient had cerebral palsy with spastic quadriplegia, and this outcome was less when compared to other studies [Nallasamy et al. 2021]. In our study, comorbidities were seen more in children aged 6–10 years (17.2% (10/58) followed by 11.9% (5/42) in children 5 years, while it was 8% (2/25) in patients older than 10 years. Compared to other studies, it was 10.6% for -5-year age groups and 3.7% for children aged 6–10 years. Comorbidities in children increase the risk of intensive care admission and the risk of severe disease [Ma X et al. 2021].

6. Conclusion

This study revealed that the number and severity of positive COVID cases were significantly decreased after the vaccination was applied in Albaha, Saudi Arabia. School and household contacts were the main risks. Comorbidities had higher risk of severe disease. Mild symptoms, short course of disease and had good outcome was reported in all admitted patients.

Age group	number	Male	Female
1-5 yr.	42 (33.6%)	20	22
6-10 yr.	58 (46.4%)	34	24
11-14 yr.	25 (20%)	12	13
Total	125	66	59

Findings	N (%)	male	female	P value
fever	113 (90.4%)	57	56	0.74
Abdominal Pain	57 (45.6%)	37	20	0.06
Vomiting	55 (44%)	25	30	0.21
Diarrhea	52 (41.6%)	28	24	0.24
Runny nose	49 (39.2%)	23	26	0.65
Cough	45 (36%)	23	22	0.67
Headache	25 (20%)	13	12	0.66
conjunctivitis	5 (4%)	3	2	0.71
Skin rash	2 (1.6%)	1	1	0.89

References

- World Health Organization (WHO). WHO characterizes COVID 19 as a pandemic [EB/OL], Geneva, Switzerland: World Health Organization, 2020. Available at: https://www.who.int/emergencies/diseases/novel ttps://www. who.int/emergencies/ diseases/novel, coronavirus-2019/events as they happen, accessed on 6 Jan, 2021.
- R Shekerdemian LS, Mahmood NR, Wolfe KK, Riggs BJ, Ross CE, McKiernan CA, et al. Characteristics and Outcomes of Children with Coronavirus Disease 2019 (COVID-19) Infection Admitted to US and Canadian Pediatric Intensive Care Units. JAM Pediatr, doi:10.1001/jamapediatrics.2020.1948. [PubMed].
- Sophia Tsabouri, MD, PhD. Ekaterini Siomou, MD, PhD. Risk Factors for Severity in Children with Coronavirus Disease 2019, A Comprehensive Literature Review. Published: July 30, 2020: https:// doi: 10.1016/j.pcl.2020.07.014. Review article, P321-338, Feb 01, 2021.
- Riccardo Castagnoli, MD; Martina Votto, MD; Amelia Licari, MD; Ilaria Brambilla, MD, PhD; Raffaele Bruno, MD; Stefano Perlini, MD; Francesca Rovida, PhD; Fausto Baldanti, MD; Gian Luigi Marseglia, MD. Severe

Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection in Children and Adolescents A Systematic Review.JAMA Pediatr. 2020; 174(9):882-889. Doi: 10.1001/jama pediatrics.2020.1467 published online April 22, 2020].

- Nallasamy K, Angurana SK, Jayashree M, Mathew JL, Bansal A, Singh MP et al. Clinical profile, hospital course and outcome of children with COVID-19. Indian J Pediatr. 2021;88(10):979-84. doi: 10.1007/s12098-020-03572.
- Zimmermann P, Curtis N. Coronavirus Infections in Children Including COVID-19. An Overview of the Epidemiology, Clinical Features, Diagnosis, Treatment and Prevention Options in Children. Pediatr Infect DisJ. 2020 May;39(5):355-368. doi: 10.1097/INF.00000000002660.
- Chen W, Zheng KI, Liu S, Yan Z, Xu C, Qiao Z. Plasma CRP level is positively associated with the severity of COVID-19. Ann Clin Microbiol Antimicrob, 2020 May 15;19(1):18. doi: 10.1186/s12941-020-00362-2.
- An Advisory Committee Statement (ACS), National Advisory Committee on Immunization (NACI) Updated guidance on a first booster dose of COVID-19 vaccines in Canada Published: April 12, 2022.
- Oliver J Watson, PhD, Gregory Barnsley, MSc, Jaspreet Toor, PhD, Alexandra B Hogan, PhD. Peter Winskill, PhD. Prof Azra C Ghani, PhD. Show footnotes. Global impact of the first year of COVID-19 vaccination: a mathematical modelling study. Open Access Published: June 23, 2022. DOI:https://doi.org/10.1016/S1473-3099(22)00320-6.
- Abdullah A Algaissi, Naif Khalaf Alharbi, Mazen Hassanain, Anwar M.Hashem. Preparedness and response to COVID-19 in Saudi Arabia: Building on MERS experience. J Infect Public Health, 2020 Jun;13(6):834-838. doi: 10.1016/j. jiph. 2020.04.016. Epub 2020 May 11.
- Shen KL, Yang YH, Jiang RM, Wang TY, Zhao DC, Jiang Y, et al. Updated diagnosis, treatment and prevention of COVID-19 in children: experts' consensus statement (condensed version of the second edition). World J Pediatr, 2020 Jun;16(3):232-239. doi: 10.1007/s12519-020-00362-4.
- WMA Declaration of Helsinki, ethical principles for medical research involving human subjects. Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964 and amended by the: 29th WMA General Assembly, Tokyo, Japan, October 1975. 35th WMA General Assembly, Venice, Italy, October 1983. 41st WMA General Assembly, Hong Kong, September 1989. 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996. 52nd WMA General Assembly, Edinburgh, Scotland,October2000, 53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added). 55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added). 59th WMA General Assembly, Seoul, Republic of Korea, October, 2008 64th WMA General
- Assembly, Fortaleza, Brazil, October 2013. https://www.wma.net/policies-post/wma-declaration-ofhelsinkiethical-principles-for-medical-research-involving-human-subjects/.
- Almawazini, A., Alghamdi, A. O., Alnashi, S., Ahmed, M. S. A. S., Alsharkawy, A., Hassan, C. I., Alghamdi, M. A., Alshumrani, K. M., Yahiya, A., Jaman, M., Nakhali, A., Alghamdi, O., Alghamdi, E., Zahrani, M. Almawazini, H., Almawazini, M., Alshumrani, M., Ata, T. Madco, M., PayonGa, A., & Kundoly, T.Review of COVID-19 in Children Admitted to King Fahad Hospital, Albaha, Saudi Arabia in 2020, Journal of Health and Medical Sciences, Vol.5, No.1, 2022: 18-23. ISSN 2622-7258. DOI: 10.31014/aior.1994.05.01.203.
- Meena J, Yadav J, Saini L, Yadav A, Kumar J. Clinical Features and Outcome of SARS-CoV-2 Infection in Children: A Systematic Review and Meta-analysis. Indian Pediatr. 2020 Sep 15;57(9):820-826. doi: 10.1007/s13312-020-1961-0. Epub 2020 Jun 24. PMID: 32583808; PMCID: PMC7498550.
- Ma X, Liu S, Chen L, Zhuang L, Zhang J, Xin Y. The clinical characteristics of pediatric inpatients with SARS-CoV-2 infection: a meta-analysis and systematic review. J Med Virol. J Med Virol. 2021;93(1):234-40. DOI: 10.1002/jmv.26208.



Are Medical Technologists still needed in Medical Laboratories in a Technologically Advanced Future?

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Abstract

An emerging trend in modern medical laboratories is automation, and it is having a positive impact on the quality of service to patients and on the safety of medical laboratory staff. The use of automation in medical laboratories enable many tests by analytical instruments with minimal use of an analyst. These automated instruments result in increasing the capabilities of a laboratory to process more workload with minimum involvement of manpower. Total Laboratory Automation (TLA) has many advantages including workload reduction, less time spent per sample, increased number of tests done in less time, use of a smaller sample amount, decreased risks for human errors, and higher reproducibility and accuracy. With the future practice of medical technologists in a technologically advanced future in peril. What edge do medical technologists have over Artificial Intelligence and Robotics that would still make them essential in medical laboratories in a future that is technologically advanced?

Keywords: Laboratory Automation, Artificial Intelligence, Robotics, Medical Technologists, Medical Laboratory Scientists

1. Introduction

There has been rapid evolution of clinical laboratories since 1990s that is mainly driven by technological advances whose main focus is on automation(Burtis, 1995; Panteghini, 2004; Bajwa, 2014). Today, there is a consensus in the bioanalytical industry that automation in bioanalytical laboratories improves its function and productivity (Armbruster, Overcash and Reyes, 2014; Archetti *et al.*, 2017; Ellison *et al.*, 2018; Yeo and Ng, 2018; Lippi and Da Rin, 2019). Services of medical laboratories are an essential component of high-quality healthcare delivery and require adequate space and equipment so that high-quality work and safety of patients, visitors, customers, and staff are not compromised (Mosadeghrad, 2014; Carey *et al.*, 2018).

Medical laboratories are potentially dangerous places due to biological hazards. Introducing automation results in a reduction of manipulation of biological sample by the staff, in particular transport of samples, subsampling, analytical operations, and waste management (Aita *et al.*, 2017). In addition to reducing occupational hazards, automation reduces tedious labor, employee turnover, permits the reallocation of staff for expansion and growth, and generally improves productivity (Lippi & Da Rin, 2019).

There is already a technological revolution happening in medical laboratories wherein machines have made tasks and procedures safer and more efficient (Thimbleby, 2013; Shah *et al.*, 2019). With total automation of medical laboratories a distinct possibility in the near future and more and more tasks of medical technologists are being delegated to artificial intelligence and machines, the main question of this discussion paper is "would we still need medical technologists in medical laboratories in a technologically advanced future?"

2. The Medical Technologist

A medical technologist, also known as a clinical laboratory scientist or medical laboratory scientist, analyzes a variety of biological specimens (Tan and Ong, 2002; Ortiz and Hsiang, 2018). They are the third largest medical profession after doctors and nurses (Resources and Administration, 2016), their responsibility lies in the performance of scientific testing on samples and reporting the results to physicians. Medical laboratory scientists perform complex tests on samples from patients using sophisticated equipment like microscopes. The work of medical technologists play an important role in the identification and treatment of medical conditions (Hosogaya, 2015; Ortiz and Hsiang, 2018). This type of analysis requires extensive knowledge of abnormal and normal physiology, ability to correlate laboratory data to specific diseases as well as extensive knowledge of instrumentation and individual test principles. It has been estimated that 70-80% of all decisions with regard to a patient's diagnosis, hospital admission, treatment, and discharge are based on the results of the tests that are performed by the medical technologists (Ortiz and Hsiang, 2018).

Medical Technologists work closely with other healthcare professionals in the diagnosis, monitoring of disease processes, as well as the monitoring of the effectiveness of therapy. Areas of medical laboratory training include chemistry, immunology, microbiology, hematology, toxicology, transfusion medicine, and molecular diagnostics (Bayot and Naidoo, 2020). Medical technologists have a wide variety of duties and responsibilities that includes: Examining and analyzing body fluids, blood, cells, and tissues; identify microorganisms capable of infection; analyze the chemical constituents of body fluids; measuring the efficacy of specific treatments by testing; evaluating test results for accuracy and aid in the interpretation to help physicians; cross matching blood for blood transfusions; monitoring patient outcomes; and establishing quality assurance programs that monitor and ensure the accuracy of test results (Wood, 2002; Hosogaya, 2015).

3. Automation in Medical Laboratories

Automation has a strong contribution to revolutionizing a host of human activities that results in the provision of unquestionable benefits on system performance (Archetti et al., 2017; Yeo and Ng, 2018). The multifaceted and abundant advancements of automation technologies have also resulted in a profound impact on the organization of medical laboratories, where many manual tasks have now been partially or completely replaced by labor-saving and automated instrumentation. Laboratory automation is commonly classified according to the sophistication of instruments integration (Da Rin, Zoppelletto and Lippi, 2016; Ellison et al., 2018; McAdam, 2018), which ranges from "no automation" which means all analyzers exist as stand-alone machines, "partial laboratory automation" which means there is the development of "automation islets" where laboratory analyzers are interconnected and are partially integrated with pre-analytical workstations such as in the serum work area which integrates clinical chemistry and immunochemistry testing, and "total laboratory automation (TLA)" which means that most analyzers performing different types of tests (such as immunochemistry, clinical chemistry, hemostasis, hematology, and others) on different sample matrices (serum, whole blood, citrated or heparinized plasma) are integrated physically as modular systems or connected by assembly lines (e.g. conveyer belts) (Lippi & Da Rin, 2019). In TLA, various preanalytical and postanalytical steps (e.g. sample input, check-in, sorting, decapping, centrifugation, separation, aliquoting, sealing, and storage) are performed automatically in workstations that are physically connected with the analyzers and are managed efficiently by software programs.

4. Potential Advantages of Total Laboratory Automation

4.1. Lower costs in the long term

There is evidence that automation can successfully lower the costs of laboratory diagnostics (Archetti *et al.*, 2017). The return on investment (ROI) is more appreciated more in the long term after reaching the "break-even point" when the high initial cost will be offset. The major economic revenue of automation comes from the merging of many diagnostic platforms within a consolidated system resulting in reduction of manual workforce needed for the management of high-volume testing (Ellison *et al.*, 2018).

4.2. Decreased congestion in the laboratory

The decrease of personnel essential for the performance of tests would also result in lower staff congestion within the laboratory (Lippi & Da Rin, 2019). An optimized layout of integrated workstations would prevent the unnecessary movement from one workstation to another by staff resulting in minimizing the distance covered by personnel for the performance of multiple analyses on different instrumentation.

4.3. Improved efficiency

Other than benefits in cost-containment, automation also provides other advantages within the laboratory environment that is commonly attributable to the use of customizable assembly lines that can be organized to meet specific requirements and layouts of laboratories (Armbruster, Overcash and Reyes, 2014). Studies demonstrate that an efficiently designed automated laboratory may be variably effective in reducing turnaround time (TAT) and concomitantly improving laboratory productivity (Angeletti *et al.*, 2015; Chung *et al.*, 2018; Bhatt, Shrestha and Risal, 2019). These goals are achieved through workflow optimization.

4.4. Improved sample management

Information technology has drastically contributed to the improvement of medical laboratory organization and work (Carey *et al.*, 2018; Sutton *et al.*, 2020). Modern generation of laboratory instrumentation come equipped with advanced software programs (i.e. all samples can be stored within online stockyards that can be automatically retrieved and re-analyzed hours or days after initial testing) that allow better sample management (Lippi & Da Rin, 2019). Setting of decision rules that are based on a predefined criteria now permits auto verification of data, automatic re-analysis of samples with suspect or highly abnormal results, as well as a triggering reflex (reflective) and add-on testing that ultimately contributes to an improvement in the quality and safety of diagnostic testing (Rimac *et al.*, 2018; Wu *et al.*, 2018).

4.5. Enhanced standardization for certification/accreditation

The consolidation of different diagnostic areas within the same workspace would result in increased accuracy and repeatability throughout the entire testing process that has been enabled by automated operations. This in turn will grant paramount benefits in terms of standardization, thus resulting in the simplification of accreditation and certification procedures (Zima, 2017; Lippi and Da Rin, 2019).

4.6. Improved quality of testing

Automation allows delegating the bulk of manual ordinary activities such as specimen sorting, loading, centrifugation, decapping, aliquoting, and sealing from humans to machines (Zima, 2017; Yeo and Ng, 2018; Lippi and Da Rin, 2019). Improved process standardization will result in tangible benefits to the quality of the total testing process and also lowering the risk of diagnostic errors, especially errors that emerge from manually-intensive activities (Yeo and Ng, 2018).

4.7. Lower sample volume

One of the benefits of an automated medical laboratory is a reduction in the number of blood tubes needed for testing (David Hopper, 2016). The same serum tube can be used for multiple immunochemistry and clinical chemistry tests which allows to consistently reduce the total volume of blood required for testing. More importantly, a reduced sample volume will also result in a lower impact on biological waste disposal which results in additional economic savings (National Research Council, 2011).

4.8. More efficient integration of tests results

Consolidation of different diagnostic areas within the same space results in an improved ability to navigate and manage the flow of data from delivery, analytical, and archival systems (Tozzoli *et al.*, 2015; Lippi and Da Rin, 2019). Automation of medical laboratories enables the integration of a vast array of test results produced by different analyzers. This will not only enable the definition of a larger, more complex, and accurate auto-validation criteria, but would also enable laboratory personnel to gain a broader picture of the patient's results, thus resulting in an improved ability to detect potential errors or identify critical situations that need immediate communication to clinicians (Lippi & Da Rin, 2019).

4.9. Lower biological risk for operators

Worker safety is one of the most significant advantages of automation. Automated systems not only remove human operators from the workplace, but also defends them against the risks of performing biologically hazardous operations and handling of materials that are biohazardous (Armbruster et al., 2014; Lippi & Da Rin, 2019).

4.10. Staff Requalification and Job Satisfaction

One of the major advantages of automation in medical laboratories is the minimization of manually-intensive labor that results in net saving of staff needed for the management of the laboratory workflow (Lippi & Da Rin, 2019) This will enable the requalification of personnel, elimination of manpower, and the redefinition of job roles towards value-added tasks such as implementation of new tests like genomics, proteomics, theranostics, or quality assessment that would ultimately lead towards personalized (laboratory) medicine.

5. Potential Disadvantages of Total Laboratory Automation

5.1. Higher costs in the short-term

Association with an initial escalation of costs is inevitably related to the implementation of TLA because accommodating the project requires environmental modifications, heavy-duty air conditioners, and soundproofing for installation of the system and for the new hardware (Lippi & Da Rin, 2019)

5.2. Increased costs for supplies (i.e. maintenance, energy, and supplies)

The new hardware implementation that consists mainly of pre-analytical workstations, assembly lines, and sample storage units, carries subsidiary costs for system functioning (i.e. energy and water) and for supplies (e.g. caps for sealers and tips for aliquotters). TLA would require a higher level of maintenance compared with manually-operated instrumentation (Lippi and Da Rin, 2019).

5.3. Space requirement and infrastructure constraints

The accommodation of multiple analyzers and new hardware into a pre-existing environment may be difficult, especially when the environment is not purpose-built for this reason. But an advantage of TLA are flexible models that may be used when the environment does not allow developing an ideal solution, when renewing possibilities are limited, the configuration of the TLA system can be designed to fit in the local environment, so that the

orientation of analyzers and access for repair or maintenance would be possible (Archetti *et al.*, 2017; Lippi and Da Rin, 2019).

5.4. Overcrowding of personnel

An unquestionable benefit of the implementation of TLA is a reduction in the need for staff to move many times from one analyzer to another. A drawback of this is the consolidation of many different analyzers within the same area may increase the risk of the generation of overcrowded work environments, where many technicians occupy the same space at the same time (Lippi and Da Rin, 2019). To avoid this from happening, there is a need to develop an efficient plan that is aimed at identifying a lean laboratory layout concept.

5.5. Increased generation of heat, noise, and vibrations

The consolidation of numerous analyzers in an area will concentrate heat, noise, and vibrations in a narrow environment. This may result in the perception of excessive warming and increased exposure to acoustical or electrical noise in the work environment (Lippi and Da Rin, 2019).

5.6. Increased risk of downtime

The higher is the sophistication of the system, the greater the risk of a system failure that would generate serious consequences on laboratory functioning (Archetti *et al.*, 2017; Lippi and Da Rin, 2019). Critical system failures that involve assembly lines would require restoring manual procedures for the management of samples (i.e. manual sorting, centrifugation, decapping, aliquoting, loading, and unloading). As a result, there is production of variably protracted downtimes, delaying analysis of specimens and prolonging the TAT (turnaround time).

5.7. Psychological dependence on automation

Replacement of manual activities with automation results in loss of locus-of-control in the staff, rapid deterioration of skills, and inefficient resumption of manual functioning when automation fails (Lippi and Da Rin, 2019).

5.8. Differential requirements for sample management

Different types of samples with different biological matrices (i.e. heparinized or citrated plasma, whole blood serum, etc.) can be introduced simultaneously into the system. This means that all samples may be managed the same way along their path to the analyzers but different biospecimens may need different preparations before being tested like EDTA-anticoagulated samples for hematologic testing that do not need centrifugation (Armbruster, Overcash and Reyes, 2014; Lippi and Da Rin, 2019).

5.9. Generation of potential bottlenecks

The optimal management of urgent testing is a critical issue in TLA. The larger the volume of routine testing, the higher the risk of the creation of bottlenecks that may reduce system productivity and TAT (Lippi and Da Rin, 2019).

5.10. Disruption of staff trained in specific technologies

The development of the "core lab," where a majority of laboratory staff will be committed, is a consequence of the consolidation of many different diagnostic areas. The larger the volume of knowledge requirement, the lower the competency of staff on specific technologies (Armbruster, Overcash and Reyes, 2014; Lippi and Da Rin, 2019). The enhancement of workforce flexibility may contribute to a decrease in competency and skills in specific tasks, most especially in laboratory services that are shifting towards TLA, where there is incorporation of several diagnostic lines (e.g. immunochemistry, clinical chemistry, coagulation, hematology, microbiology, and virology). Although automation and technology have made the performance of tasks much easier, some specific and practical skills may be lost over time. Overall, there is a limitation on the experience that laboratory specialists harvest on

a daily basis due to automation and this would potentially lead to decreased skills and expertise in analytical procedures (Armbruster, Overcash and Reyes, 2014; Archetti *et al.*, 2017; Lippi and Da Rin, 2019).

5.11. Risk of transition toward a manufacturer's-driven laboratory

The establishment of a strategic relationship with manufacturers and suppliers is essential for the achievement of an efficient TLA. This implies that the technology should be more accurately defined according to the expected laboratory layout. Full commitment to a single supplier or manufacturer paves a way to manufacturer's-driven laboratory and this would substantially limit or even prevent laboratory professionals from organizing or managing their own laboratories (Lippi and Da Rin, 2019).

Automation is considered as one of the most significant breakthroughs that have taken place in laboratory diagnostics over the past decades (Archetti *et al.*, 2017; Yeo and Ng, 2018). The ability to connect multiple diagnostic specialties to one single track has been proven to enhance efficiency, organization, standardization, quality, and safety of laboratory testing, and also providing a substantial return of investment in the long term and enable the requalification of staff (Lippi and Da Rin, 2019). Technological breakthroughs happen at a growing rate and as a result, technology is revolutionizing human wellness and care. The structure and organization of the healthcare industry have been drastically changed by technological advancements (Thimbleby, 2013). But the ultimate question remains, are medical technologists still needed in medical laboratories in a technologically advanced future? Medical technologists should learn more about emerging technologies in the healthcare industry like what are the capabilities of these technologies, especially intelligent machines, and search for ways where they can use these technologies to complement the tasks they are performing daily.

There are ways medical technologists are irreplaceable in the healthcare industry

Healthcare will need humans in the future

Artificial Intelligence (AI) will become routine in healthcare practice during the next 10-15 years (Jiang *et al.*, 2017; Buch, Ahmed and Maruthappu, 2018; Davenport and Kalakota, 2019). AI will transform what it means to be a healthcare provider: some tasks will disappear, while there will be additional tasks to the work routine. AI systems use deep and machine learning to analyze enormous amounts of data to generate predictions and recommend interventions. The advances in computing power have enabled the creation and cost-effective analysis of enormous datasets (Dunjko and Briegel, 2018; Kersting, 2018; Vollmer *et al.*, 2020). Digital microscopy analysis influences machine-learning algorithms trained with the use of tens of thousands of specimen images. These algorithms can quickly, consistently, and accurately identify and classify cellular and particulate objects in serum, urine, or tissue samples and are able to handle high volumes of samples for analysis (Delahunt *et al.*, 2015; Xing *et al.*, 2018; Rai Dastidar and Ethirajan, 2020).

AI is wholly dependent on data in its computation. A problem with this dependence is that AI is unable to verify the accuracy of the underlying data they are given (Cresswell, Cunningham-Burley and Sheikh, 2018; Kim *et al.*, 2019; Yang *et al.*, 2020). AI assumes that the data they are given are perfectly accurate, reflect high quality, and are representative of excellent care and outcomes. An advantage of clinicians against AI is the capability of clinicians to make assumptions and care choices based on unstructured data (Dash *et al.*, 2019; Hong *et al.*, 2019; Palanisamy and Thirunavukarasu, 2019). Experienced medical technologists develop intuition that enables them to identify an abnormal finding even though the sample provided might look identical to normal findings. This gives experienced medical technologists an edge over AI because clinical judgment may not be well represented by data (Walton, 2018; Patel *et al.*, 2019).

Medical technologists also have a non-linear working method. Although data, measurements, and quantitative analytics are also important factors of a medical technologist's work, the development of a diagnosis and developing a proper treatment regimen for patients are not linear processes and would require creativity and problem solving skills that robots and algorithms may never have (Kaartemo and Helkkula, 2018; Kelly *et al.*, 2019). The lifestyles of people vary to the degree that people differ. This feature also applies to diseases, which means no case is the same.

Another reason why medical laboratories will still need medical technologists in the future is the fact that complex digital technologies will require competent professionals (Groenier, Pieters and Miedema, 2017). Take for example, IBM's Watson. It's a unique AI for oncologists that is capable of providing clinicians evidence-based options. But a limitation of this technology is it is not capable of providing the best treatment option available for the patient and can only come up with potential suggestions (Lee *et al.*, 2018; Liu *et al.*, 2018). No robot or algorithm may be capable of incontrovertibly interpreting the complex, multi-layered analysis needed in determining the best treatment intervention for patients. While these technologies may be able to provide the data, the interpretation will always remain a territory for humans (Bader and Kaiser, 2019; Dear, 2019; Araujo *et al.*, 2020).

And the final reason why medical technologists would still be needed in laboratories in the future is the fact that there will always be tasks that algorithms and robots can never complete (Ozawa *et al.*, 1992; Royakkers and van Est, 2015; Kaartemo and Helkkula, 2018). There are responsibilities and duties which technologies cannot perform and would need the procedural expertise of humans to complete. Clinical laboratory testing plays a crucial role in the detection, diagnosis, and treatment of diseases. The Medical Technologists who perform most of these laboratory tests, are involved in the examination and analysis of body fluids, tissues, and cells. They usually look for the presence of bacteria, parasites and other microorganisms in the body. They analyze the chemical contents of fluids, match blood transfusions, and test for drug levels in the blood to show a patient's response to a specific treatment. They also prepare specimens for examination, to count cells, and look for abnormal cells in the blood and other body fluids. Clinical laboratory scientists as they are called from other countries use microscopes, cell counters and other sophisticated laboratory equipment to confirm the findings of the machine. After testing and examining a specimen, laboratory scientists analyze the results and relay them to the attending physicians. In these instances, humans will always be faster, more reliable, or cheaper than technologies in these tasks.

Collaborating with technologies

It should not be humans vs. technologies since technological innovations will always serve to help people (Pohoryles and Tommasi, 2017; Edwards-Schachter, 2018; von Schomberg and Blok, 2019). It doesn't matter if it's AI or robotics, healthcare professionals must accept that these technologies have a massive influence on the way the healthcare system operates, and healthcare professionals must start utilizing their power. Collaboration between technology and humans should be the most suitable response (Thimbleby, 2013; Simoes, 2015; Mitchell and Kan, 2019).

Medical technology is designed to improve the detection, diagnosis, and treatment, and monitoring of diseases. As such, it has linkages with many other disciplines for specific diagnostic or therapeutic purposes. The use of sophisticated technology in medical laboratories isn't a question of replacing but it's a question of complementing. The role of AI in healthcare is to complement and not to replace people by delivering actionable intelligence to human experts (Kaartemo and Helkkula, 2018; Laï, Brian and Mamzer, 2020). AI could pave a way for the creation of a world where human abilities are amplified as technology processes, analyzes, and evaluates the abundance of data in the world today. This allows humans to spend more time engaged in creativity, high-level thinking, and decision-making (Loh, 2018; Reddy, 2018; Ahuja, 2019). The challenge now falls on how laboratories can successfully use sophisticated technologies in ways that enable their human workforce, aiding them to become faster, more efficient, and more productive.

6. Conclusion

Medical Technologists would still continue to be needed by growing as professionals, the more basic and routine tasks of laboratory testing will be delegated to machines and AI, while medical technologists attend to more complex issues that require creativity, high-level thinking, decision-making and tasks that require expert level procedural expertise. Essentially, as sophisticated technologies will not be able to replace medical technology practice, there is no need for medical technologists to feel anxious about the security of their employment. With the challenge of the increasing sophistication of technology, as long as medical technologists are willing to develop professionally and transform into more exceptional versions of the outstanding healthcare providers that they are today, human medical technologists will still prevail in a technologically advanced medical future.

References

- Ahuja, A. S. (2019) 'The impact of artificial intelligence in medicine on the future role of the physician', PeerJ. PeerJ Inc., 2019(10), doi: 10.7717/peeri.7702.
- Aita, A. et al. (2017) 'Patient safety and risk management in medical laboratories: theory and practical application', Journal of Laboratory and Precision Medicine. AME Publishing Company, 2, pp. 75–75. doi: 10.21037/jlpm.2017.08.14.
- Angeletti, S. et al. (2015) 'Laboratory Automation and Intra-Laboratory Turnaround Time: Experience at the University Hospital Campus Bio-Medico of Rome', Journal of Laboratory Automation. SAGE Publications Inc., 20(6), pp. 652–658. doi: 10.1177/2211068214566458.
- Araujo, T. et al. (2020) 'In AI we trust? Perceptions about automated decision-making by artificial intelligence', AI and Society. Springer, pp. 1-13. doi: 10.1007/s00146-019-00931-w.
- Archetti, C. et al. (2017) 'Clinical laboratory automation: A case study', Journal of Public Health Research. Page Press Publications, 6(1), pp. 31–36. doi: 10.4081/jphr.2017.881.
- Armbruster, D. A., Overcash, D. R. and Reyes, J. (2014) 'Clinical Chemistry Laboratory Automation in the 21st Century - Amat Victoria curam (Victory loves careful preparation).', The Clinical biochemist. Reviews. The Australian Association of Clinical Biochemists, 35(3), 143-53. pp. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25336760 (Accessed: 29 June 2020).
- Bader, V. and Kaiser, S. (2019) 'Algorithmic decision-making? The user interface and its role for human involvement in decisions supported by artificial intelligence', Organization, SAGE Publications Ltd, 26(5), pp. 655-672. doi: 10.1177/1350508419855714.
- Bajwa, M. (2014) 'Emerging 21st century medical technologies', Pakistan Journal of Medical Sciences. Professional Medical Publications, 30(3), p. 649. doi: 10.12669/pims.303.5211.
- L. Naidoo. P. (2020)Clinical Laboratory, StatPearls. Available Bayot, M. and at: http://www.ncbi.nlm.nih.gov/pubmed/30570979 (Accessed: 29 June 2020).
- Bhatt, R. D., Shrestha, C. and Risal, P. (2019) 'Factors Affecting Turnaround Time in the Clinical Laboratory of the Kathmandu University Hospital, Nepal.', EJIFCC. International Federation of Clinical Chemistry and Laboratory Medicine, 30(1), pp. 14-24. Available at: http://www.ncbi.nlm.nih.gov/pubmed/30881271 (Accessed: 29 June 2020).
- Buch, V. H., Ahmed, I. and Maruthappu, M. (2018) 'Artificial intelligence in medicine: Current trends and future possibilities', British Journal of General Practice. Royal College of General Practitioners, pp. 143-144. doi: 10.3399/bjgp18X695213.
- Burtis, C. A. (1995) 'Technological trends in clinical laboratory science', Clinical Biochemistry. Elsevier, pp. 213-219. doi: 10.1016/0009-9120(94)00075-7.
- Carey, R. B. et al. (2018) 'Implementing a quality management system in the medical microbiology laboratory', Clinical Microbiology Reviews. American Society for Microbiology, 31(3). doi: 10.1128/CMR.00062-17.
- Chung, H. J. et al. (2018) 'Experimental fusion of different versions of the total laboratory automation system and improvement of laboratory turnaround time', Journal of Clinical Laboratory Analysis. John Wiley and Sons Inc., 32(5). doi: 10.1002/jcla.22400.
- Cresswell, K., Cunningham-Burley, S. and Sheikh, A. (2018) 'Health care robotics: Qualitative exploration of key challenges and future directions', Journal of Medical Internet Research. Journal of Medical Internet Research, 20(7). doi: 10.2196/10410.
- Dash, S. et al. (2019) 'Big data in healthcare: management, analysis and future prospects', Journal of Big Data. SpringerOpen, 6(1), p. 54. doi: 10.1186/s40537-019-0217-0.
- Davenport, T. and Kalakota, R. (2019) 'The potential for artificial intelligence in healthcare', Future Healthcare Journal. Royal College of Physicians, 6(2), pp. 94-98. doi: 10.7861/futurehosp.6-2-94.
- David Hopper, L. (2016) 'Automated Microsampling Technologies and Enhancements in the 3Rs', ILAR Journal. Oxford Academic, 57(2), pp. 166-177. doi: 10.1093/ILAR/ILW020.
- Dear, K. (2019) 'Artificial Intelligence and Decision-Making', RUSI Journal. Routledge, 164(5-6), pp. 18-25. doi: 10.1080/03071847.2019.1693801.
- Delahunt, C. B. et al. (2015) 'Automated microscopy and machine learning for expert-level malaria field diagnosis', in Proceedings of the 5th IEEE Global Humanitarian Technology Conference, GHTC 2015. Institute of Electrical and Electronics Engineers Inc., pp. 393–399. doi: 10.1109/GHTC.2015.7344002.
- Dunjko, V. and Briegel, H. J. (2018) 'Machine learning & artificial intelligence in the quantum domain: a review of recent progress', Reports on Progress in Physics. IOP Publishing, 81(7), p. 074001. doi: 10.1088/1361-6633/AAB406.
- Edwards-Schachter, M. (2018) 'The nature and variety of innovation', International Journal of Innovation Studies. Elsevier BV, 2(2), pp. 65–79. doi: 10.1016/j.ijis.2018.08.004.
- Ellison, T. L. et al. (2018) 'Implementation of total laboratory automation at a tertiary care hospital in Saudi Arabia: Effect on turnaround time and cost efficiency', Annals of Saudi Medicine. King Faisal Specialist Hospital and Research Centre, 38(5), pp. 352–357. doi: 10.5144/0256-4947.2018.352.

- Groenier, M., Pieters, J. M. and Miedema, H. A. T. (2017) 'Technical Medicine: Designing Medical Technological Solutions for Improved Health Care', *Medical Science Educator*. Springer, 27(4), pp. 621–631. doi: 10.1007/s40670-017-0443-z.
- Hong, L. et al. (2019) 'Big Data in Health Care: Applications and Challenges', Data and Information Management. Walter de Gruyter GmbH, 2(3), pp. 175–197. doi: 10.2478/dim-2018-0014.
- Hosogaya, S. (2015) 'Role of Medical Technologists' Training in the Future', *Rinsho byori. The Japanese journal of clinical pathology*, 63(1), pp. 137–140. Available at: http://europepmc.org/article/med/26524891 (Accessed: 29 June 2020).
- Jiang, F. et al. (2017) 'Artificial intelligence in healthcare: Past, present and future', Stroke and Vascular Neurology. BMJ Publishing Group, pp. 230–243. doi: 10.1136/svn-2017-000101.
- Kaartemo, V. and Helkkula, A. (2018) 'A Systematic Review of Artificial Intelligence and Robots in Value Cocreation: Current Status and Future Research Avenues', *Journal of Creating Value*. SAGE Publications, 4(2), pp. 211–228. doi: 10.1177/2394964318805625.
- Kelly, C. J. *et al.* (2019) 'Key challenges for delivering clinical impact with artificial intelligence', *BMC Medicine*. BioMed Central Ltd., p. 195. doi: 10.1186/s12916-019-1426-2.
- Kersting, K. (2018) 'Machine Learning and Artificial Intelligence: Two Fellow Travelers on the Quest for Intelligent Behavior in Machines', *Frontiers in Big Data*. Frontiers Media SA, 1, p. 6. doi: 10.3389/fdata.2018.00006.
- Kim, D. W. et al. (2019) 'Design characteristics of studies reporting the performance of artificial intelligence algorithms for diagnostic analysis of medical images: Results from recently published papers', Korean Journal of Radiology. Korean Radiological Society, 20(3), pp. 405–410. doi: 10.3348/kjr.2019.0025.
- Laï, M. C., Brian, M. and Mamzer, M. F. (2020) 'Perceptions of artificial intelligence in healthcare: Findings from a qualitative survey study among actors in France', *Journal of Translational Medicine*. BioMed Central Ltd., 18(1), p. 14. doi: 10.1186/s12967-019-02204-y.
- Lee, W.-S. et al. (2018) 'Assessing Concordance With Watson for Oncology, a Cognitive Computing Decision Support System for Colon Cancer Treatment in Korea', JCO Clinical Cancer Informatics. American Society of Clinical Oncology (ASCO), (2), pp. 1–8. doi: 10.1200/cci.17.00109.
- Lippi, G. and Da Rin, G. (2019) 'Advantages and limitations of total laboratory automation: A personal overview', *Clinical Chemistry and Laboratory Medicine*. De Gruyter, pp. 802–811. doi: 10.1515/cclm-2018-1323.
- Liu, C. *et al.* (2018) 'Using artificial intelligence (watson for oncology) for treatment recommendations amongst Chinese patients with lung cancer: Feasibility study', *Journal of Medical Internet Research*. Journal of Medical Internet Research, 20(9). doi: 10.2196/11087.
- Loh, E. (2018) 'Medicine and the rise of the robots: A qualitative review of recent advances of artificial intelligence in health', *BMJ Leader*. BMJ Publishing Group, pp. 59–63. doi: 10.1136/leader-2018-000071.
- McAdam, A. J. (2018) 'Total laboratory automation in clinical microbiology: A micro-comic strip', *Journal of Clinical Microbiology*. American Society for Microbiology. doi: 10.1128/JCM.00176-18.
- Mitchell, M. and Kan, L. (2019) 'Digital Technology and the Future of Health Systems', *Health Systems and Reform*. Taylor and Francis Inc., pp. 113–120. doi: 10.1080/23288604.2019.1583040.
- Mosadeghrad, A. M. (2014) 'Factors Affecting Medical Service Quality.', *Iranian journal of public health*. Tehran University of Medical Sciences, 43(2), pp. 210–20. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26060745 (Accessed: 29 June 2020).
- National Research Council (2011) Prudent Practices in the Laboratory, Prudent Practices in the Laboratory. National Academies Press. doi: 10.17226/12654.
- Ortiz, G. B. and Hsiang, W. (2018) 'Focus: Medical Technology: Medical Technology', *The Yale Journal of Biology and Medicine*. Yale Journal of Biology and Medicine, 91(3), p. 203. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6153631/ (Accessed: 29 June 2020).
- Ozawa, K. *et al.* (1992) 'The expanding role of robotics in the clinical laboratory', *Journal of Automatic Chemistry*, 14(1), pp. 9–15. doi: 10.1155/S146392469200038.
- Palanisamy, V. and Thirunavukarasu, R. (2019) 'Implications of big data analytics in developing healthcare frameworks – A review', *Journal of King Saud University - Computer and Information Sciences*. King Saud bin Abdulaziz University, pp. 415–425. doi: 10.1016/j.jksuci.2017.12.007.
- Panteghini, M. (2004) 'The future of laboratory medicine: understanding the new pressures.', *The Clinical biochemist. Reviews*. The Australian Association of Clinical Biochemists, 25(4), pp. 207–15. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18458714 (Accessed: 29 June 2020).
- Patel, B. N. et al. (2019) 'Human-machine partnership with artificial intelligence for chest radiograph diagnosis', npj Digital Medicine. Springer Science and Business Media LLC, 2(1), pp. 1–10. doi: 10.1038/s41746-019-0189-7.
- Pohoryles, R. J. and Tommasi, D. (2017) 'Innovation: society, research & technology', *Innovation*. Routledge, pp. 385–387. doi: 10.1080/13511610.2017.1383730.
- Rai Dastidar, T. and Ethirajan, R. (2020) 'Whole slide imaging system using deep learning-based automated focusing', *Biomedical Optics Express*. The Optical Society, 11(1), p. 480. doi: 10.1364/boe.379780.
- Reddy, S. (2018) 'Use of Artificial Intelligence in Healthcare Delivery', in eHealth Making Health Care Smarter.

InTech. doi: 10.5772/intechopen.74714.

- Resources, H. and Administration, S. (2016) Allied Health Workforce Projections, 2016-2030: Medical and Clinical Laboratory Technologists. Available at: http://www.ascls.org/advocacy-issues/licensure. (Accessed: 29 June 2020).
- Rimac, V. et al. (2018) 'Implementation of the Autovalidation Algorithm for Clinical Chemistry Testing in the Laboratory Information System', *Laboratory Medicine*. Oxford Academic, 49(3), pp. 284–291. doi: 10.1093/LABMED/LMX089.
- Da Rin, G., Zoppelletto, M. and Lippi, G. (2016) 'Integration of Diagnostic Microbiology in a Model of Total Laboratory Automation', *Laboratory Medicine*. Oxford Academic, 47(1), pp. 73–82. doi: 10.1093/LABMED/LMV007.
- Royakkers, L. and van Est, R. (2015) 'A Literature Review on New Robotics: Automation from Love to War', *International Journal of Social Robotics*. Springer Netherlands, 7(5), pp. 549–570. doi: 10.1007/s12369-015-0295-x.
- von Schomberg, L. and Blok, V. (2019) 'Technology in the Age of Innovation: Responsible Innovation as a New Subdomain Within the Philosophy of Technology', *Philosophy and Technology*. Springer, pp. 1–15. doi: 10.1007/s13347-019-00386-3.
- Shah, P. et al. (2019) 'Artificial intelligence and machine learning in clinical development: a translational perspective', *npj Digital Medicine*. Springer Science and Business Media LLC, 2(1), pp. 1–5. doi: 10.1038/s41746-019-0148-3.
- Simoes, E. (2015) 'Health information technology advances health care delivery and enhances research', *Missouri medicine*. Missouri State Medical Association, 112(1), pp. 37–40. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6170093/ (Accessed: 29 June 2020).
- Sutton, R. T. *et al.* (2020) 'An overview of clinical decision support systems: benefits, risks, and strategies for success', *npj Digital Medicine*. Springer Science and Business Media LLC, 3(1), pp. 1–10. doi: 10.1038/s41746-020-0221-y.
- Tan, L. and Ong, K. (2002) 'The Impact of Medical Technology on Healthcare Today', Hong Kong Journal of Emergency Medicine. SAGE Publications, 9(4), pp. 231–236. doi: 10.1177/102490790200900410.
- Thimbleby, H. (2013) 'Technology and the future of healthcare.', *Journal of public health research*. PAGEPress, 2(3), p. e28. doi: 10.4081/jphr.2013.e28.
- Tozzoli, R. *et al.* (2015) 'Automation, consolidation, and integration in autoimmune diagnostics', *Autoimmunity Highlights*. Springer-Verlag Italia s.r.l. doi: 10.1007/s13317-015-0067-5.
- Vollmer, S. et al. (2020) 'Machine learning and artificial intelligence research for patient benefit: 20 critical questions on transparency, replicability, ethics, and effectiveness', *The BMJ*. BMJ Publishing Group, 368. doi: 10.1136/bmj.l6927.
- Walton, P. (2018) 'Artificial Intelligence and the Limitations of Information', *Information*. MDPI AG, 9(12), p. 332. doi: 10.3390/info9120332.
- Wood, J. (2002) 'The role, duties and responsibilities of technologists in the clinical laboratory', *Clinica Chimica Acta*. Elsevier, 319(2), pp. 127–132. doi: 10.1016/S0009-8981(02)00033-5.
- Wu, J. et al. (2018) 'Establishing and Evaluating Autoverification Rules with Intelligent Guidelines for Arterial Blood Gas Analysis in a Clinical Laboratory', SLAS TECHNOLOGY: Translating Life Sciences Innovation. SAGE Publications Inc., 23(6), pp. 631–640. doi: 10.1177/2472630318775311.
- Xing, F. et al. (2018) 'Deep Learning in Microscopy Image Analysis: A Survey', IEEE Transactions on Neural Networks and Learning Systems. Institute of Electrical and Electronics Engineers Inc., 29(10), pp. 4550– 4568. doi: 10.1109/TNNLS.2017.2766168.
- Yang, Y. *et al.* (2020) 'The diagnostic accuracy of artificial intelligence in thoracic diseases', *Medicine*. Lippincott Williams and Wilkins, 99(7), p. e19114. doi: 10.1097/MD.000000000019114.
- Yeo, C. P. and Ng, W. Y. (2018) 'Automation and productivity in the clinical laboratory: Experience of a tertiary healthcare facility', *Singapore Medical Journal*. Singapore Medical Association, pp. 597–601. doi: 10.11622/smedj.2018136.
- Zima, T. (2017) 'Accreditation of Medical Laboratories-System, Process, Benefits for Labs', Journal of Medical Biochemistry. Society of Medical Biochemists of Serbia and Montenegro, 36(3), pp. 231–237. doi: 10.1515/jomb-2017-0025.



Evaluation of Toxicity of the Bioactive Components from the Stem of Buyo (*Piper betle L.*) Extracts

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Abstract

Extracts of Buyo (Piper betle L) were subjected to a bioscreening study to detect cytotoxicity activity by the brine shrimp lethality bioassay. Specifically, the researchers opted to use the stem part of the plant since there has been no study about its cytotoxic activity. The result obtained for the ethanol extract was promising. The researchers concluded that ethanol extract is the most active in cytotoxicity activity, with a value of 205.3525, against the other extracts. This extract can be regarded as a promising candidate for a plant-derived antitumor or anticancer compound. Also, it is suggested that the extracts should be subjected to other tests and further experimentations to elaborate on its essential biological properties.

Keywords: Brine Shrimp Lethality Test, Cytotoxicity, Piper betle L., Medicinal Properties, Green Heart

1. Introduction

The Philippines has a diverse species of plants (Sakilan JM et al., 2019). These plants have unique components that give the plants a unique chemical composition. Buyo, for example, is one of the most valuable plants that inhabits not only in the Philippines but also in other nearby countries. This plant was named as the Golden Heart of Nature since it has many important biological activities and significant phytoconstituents that bring about outstanding contributions to people using the plant. Buyo has written its history to the world of herbal medicine with its promising power to alleviate some of the people's illnesses. The leaves of this plant contributed a lot of biological activities such as antibacterial activity, gastroprotective activity, antioxidant activity, antidiabetic activity, radioprotective activity, the effect on the cardiovascular system and platelet inhibition activity, antifertility activity, immunomodulatory activity, cholinomimetic effect, hepatoprotective activity, as an oral care agent, and neuropharmacological profile.

Medicinal plants are natural resources yielding valuable phytochemical products, which are often used in the treatment of various diseases. A substantial part of the population in developing countries, uses folk medicines for their daily healthcare. In the Philippines, most of the people use folk medicines as part of their everyday healthcare.

Some of the traditional medicine involves the use of crude plant extracts, which may contain an extensive diversity of molecules, often with indefinite biological effects (Olowa and Nuñeza, 2013).

Anthropologists have found traces of betel in the spirit caves in Northwest Thailand dating back as to 5500-7000BC, which is even before systematic and organized agriculture came to be practiced. There have been similar findings in Timor in Indonesia going back to 3000 BC and in the blacked teeth of a human skeleton in Palawan in (Pradhan et al., 2013) the Philippines, going back to 2600 BC. It had found a place in the most ancient Sri Lanka Historical Book "Mahawamsa" written in *palli*. Even today, some hardened betel chewers in Thailand, Myanmar, and Indonesia with black teeth as (Pradhan et al., 2013) a result of long years of chewing (Chaveerach et al., 2006). There is archaeological evidence that the betel leaves have been chewed along with the Arica nut since very ancient times it is not known when these two different (Pradhan at al., 2013) stimulant substances were first put together. It may be difficult to ascertain the period when the tradition of chewing was started. However, it was mentioned in the Vatsyayana's Kamsutra, and Kalidas' Raghuvamsa reflects the antiquity of this practice. The social status of betel can also be appreciated from the fact that it was a great honor to receive betel from kings and nobles. Such was the status of betel in ancient India. During this period (Circa 600 AD), words like Tambuladhikara, Tambuladyaka, Tambuladayini, and Tambulika were used in different texts. Some of the common usages are mentioned in Kadamberi. Betel has been referred to in Sakta-tantra as one of the means of achieving siddhi. It was believed that without betel chewing and offering betel to Guru, no siddhi could be gained. Tambool has also been referred to as facilitating the sadhak in chewing dharma, yasha aisvarya, Srivairagya, and mukti. Tambool finds frequent mention in writings from the fifth century onwards especially, Reetikaaleen Hindi poetry (Wenke et al., 1983; Sharma, 2003; Prokopczyk et al., 1991).

Piper betle Linn. (Local name 'Buyo') Piperaceae, a dioecious, annual creeper, climbing by many small adventitious rootless, grows to a height of about one (10) meter, generally grown in hotter and damper parts of the country (Wenke et al., 1983; Sharma, 2003). It is extensively found in damp forests and is propagated in the Philippines and other countries in South-East Asia, such as India, Vietnam, and China. In India, it is found in Uttar Pradesh, Bihar, Bengal, Orissa, Tamilnadu, Andhra Pradesh, and Karnataka. In Tamilnadu, three varieties of *Piper betle* leaves, Sirugamani, Karpoori, and Vellaikodi, are accessible mostly (Prokopczyk et al., 1991).

The study of the bioactive compounds from plant sources and extracts in the chemical laboratory is often hampered by the lack of a suitable, simple, and rapid screening procedure. There are many procedures for bioassay that are employed using whole animals, isolated tissues, or biochemical systems. A practical method for general toxicity screening is, therefore, essential as a preliminary stage in the study of bioactive plants. A model animal that has been used for this purpose is the brine shrimp, *Artemia salina* (Kumar, 1993; Wenke et al., 1983; Sharma, 2003; Prokopczyk et al., 1991; Imran and Amin, 2011).

Availability of the eggs, the ease of hatching them into larvae, the rapid growth of the nauplii, and the relative ease of maintaining a population under laboratory conditions (Svoboda and Hampson, 1999) have made the brine shrimp a simple and effective animal test in biological sciences and toxicology. Combined with a reference standard, the brine shrimp test offers a bioassay that can be rapid, simple, bench-top, and, more importantly, inexpensive, and reproducible (Imran and Amin, 2011).

The physiological or biological effect to be observed in the screening is critical. One of the most superficial biological responses is to monitor the lethality since there is only one criterion: either dead or alive. In that case, the statistical analysis is relatively easy. The lethal concentration for 50% mortality after 24 hours of exposure (the chronic LC_{50}) is determined as the measure of the toxicity of the extract or compound. The choice of time, governed by the solubility of the extractor substance, is mostly one of convenience since the test is to be rapid and kept simple (Wenke et al., 1983; Sharma, 2003; Prokopczyk et al., 1991; Imran and Amin, 2011).

The brine shrimp (*Artemia salina*) is a simple zoologic organism (an arthropod). The use of brine shrimp test (BST) as a tool to measure general bioactivity in plant extracts was initiated in 1982 and then modified in 1991 as a simple, rapid, in-house, bench-top, and low-cost prescreen for cytotoxicity and 9KB (human epidermoid carcinoma of the nasopharynx) cytotoxicity was observed in an initial study. The usefulness of brine shrimp as a prescreen for antitumor activity was confirmed in a blind comparison with *in vitro* cytotoxicity and 3PS activity.
The brine shrimp bioassay has been implemented as a test for the last 20 years and has led to the discovery of the cytotoxic effects of a wide range of plants and bioactive compounds so diverse in their chemical structure. This method is now widely used all over the world with great success (Kumar, 1993; Wenke et al., 1983; Sharma, 2003; Prokopczyk et al., 1991; Imran and Amin, 2011).

The primary purpose of the study is to evaluate the toxicity of the bioactive components of Buyo (*Piper betle*) in the different stem extracts. The brine shrimp lethality test was used for the evaluation of cytotoxicity of the plant extracts.

2. Materials and Methods

2.1. Plant Materials

The plant samples (Figure 1) were collected from Barangay Hinaplanon, Iligan City, Lanao del Norte, Philippines. The Taxonomic identity of the plant was confirmed by Prof. Edgardo C. Aranico, plant taxonomist of the Department of Biological Sciences of the College of Science and Mathematics, Mindanao State University-Iligan Institute of Technology.



Figure 1: Piper betle

The researchers opted to use the stem part of the plant because there has been no study about its biological activities (e.g., cytotoxicity). The stems were washed with running tap water to remove any dirt before the drying process. The stems were cut into small pieces and dried at 40°C for two weeks.

2.2. Extract Preparation



Figure 2: Schematic Diagram of the Procedures and Protocol of the Experiment

2.3. Brine Shrimp Lethality Test

Test samples of the plant extracts, in triplicate, were prepared as follows: One hundred grams (100 g) each of the crude extract was initially dissolved in 10 mL of dimethyl sulfoxide (DMSO) and further diluted with seawater to produce the required concentrations. Appropriate amounts (5000-, 500-, 50- and 5- μ L for 10,000-, 1,000-, 100- and 10-ppm, respectively) were transferred to vials containing small filter paper discs, air-dried overnight to evaporate the solvent, and further dried under nitrogen gas. Ten brine shrimps were transferred to each sample vial, and boiled, filtered seawater was added to make 5mL. Tests for each concentration were done in triplicate. Control experiments using DMSO was also performed for the four concentrations in triplicates. All the vials were maintained under illumination. The number of dead and alive nauplii was counted after 6 and 24 hours. The results were evaluated, and the acute and chronic LC₅₀ values were determined using the Reed-Muench method.

3. Results and Discussion

The extracts studied in this work showed significant lethality against brine shrimp, which has been successfully used as a simple biological test to guide the experiment.

F 4 0.4-5	6 hours of Exposure			24 hours of Exposure				
Extracts	1000ppm	500ppm	100ppm	10ppm	1000ppm	500ppm	100ppm	10ppm
PbE	100%	21.43%	0%	0%	100%	93.75%	21.88%	8.33%
PbEW	11.76%	6.35%	3.30%	1.69%	73.68%	54.72%	14.93%	6.02%
PbD	15.15%	5.00%	2.25%	1.71%	43.59%	18.64%	11.25%	4.04%

The mortality rate of the brine shrimp after 6 hours and 24 hours of exposure to various doses of plant stem extracts of *Piper betle* is shown in Table 1. For 6 hours of exposure, at 100ppm and 10ppm, almost the PbEW (*Piper betle*

ethanol: water, 50:50) and PbD (*Piper betle* decoction extract) showed no toxicity against the brine shrimps. Also, for 24 hours of exposure, at 10ppm, almost the three extracts showed no toxicity against the brine shrimps. Meanwhile, for the 6 hours of exposure, at 100ppm and 10ppm, the PbE (*Piper betle* ethanol extract) showed no toxicity against brine shrimps. Thus, the plant stem extracts of *P. betle* are not toxic or harmful when prepared at lower concentrations (100ppm and 10ppm). The results suggested that a higher concentration of plant stem extracts of *P. betle* is needed to kill 50% of the brine shrimps.

Extracts	Acute LC ₅₀	Chronic LC ₅₀
PbE	668.3439 ppm	205.3525 ppm
PbEW	>1000 ppm	434.010 ppm
PbD	>1000 ppm	>1000 ppm

Table 2: LC50 Results of the Various Extracts

The interpretation of results was based on the concept of the rate of LC_{50} and its corresponding range of concentration. If the value of LC_{50} is lower than 50 ppm, it signifies high mortality rates; if the value of LC_{50} is up to 500 ppm, it signifies promising results; and if the value of LC_{50} is up to 800 ppm, it signifies moderate toxicities.

The LC₅₀ results of the three extracts evaluated in this screening are listed in Table 2. From the assay, it was found that acute LC₅₀ in PbE (*Piper betle* ethanol extract) showed moderate toxicity with 668.3439. The chronic LC₅₀ of PbE showed promising results having the value of 205.3525. The acute LC₅₀ of PbEW (*Piper betle* ethanol: water, 50:50) showed >1000ppm. The chronic LC₅₀ of PbEW showed promising results with a value of 434.010. The acute LC₅₀ and chronic LC₅₀ of PbD (*Piper betle* decoction extract) showed >1000ppm. Based on the results, the brine shrimp lethality of *Piper betle* extracts was found concentration dependent. The crude extract is toxic if it has an LC₅₀ value of less than 1000 µg/mL, while non-toxic if it has greater than 1000 µg/mL (Meyer et al., as cited by Cabrido, 2015).



Figure 3: Estimated LC50 of the Active Extracts with Different Times of Exposure

4. Conclusions

Crude stem extracts of *Piper betle* does not exhibits toxicity. Among the three extracts, only PbE and PbEW showed moderate toxicity against *Piper betle* with a value of 205.3525 and 434.010, respectively. These extracts

can be regarded as a promising candidate for a plant-derived antitumor or anticancer compound. PbD showed no significant cytotoxicity activity since the value of LC_{50} is greater than 1000 µg/mL. The assay is limited only to the evaluation of the cytotoxicity activity of the plant stem extracts; hence this study insufficiently determines the properties of the potential and essential bioactive compounds. It is suggested that the extracts should be subjected to other tests and further experimentations to elaborate on its essential biological properties.

References

Chaveerach A., Mokkamul P., Sudmoon R., Tanee T., "Ethnobotany of the Genus Piper (Piperaceae) in Thailand," *Ethnobotany Research and Applications*, 2006, 4, pp. 223-231.

- Imran Chowdhury I., Amin R. Binzaid S., "Optimal control on environments for improving the *Piper betle* (paan growth)," *Life Sciences Leaflets*, 2011, 60 (17), pp. 5-615.
- Kumar N., "Betalvine (*Piper betle* L.) Cultivation: A unique case of plant establishment under anthropogenically regulated microclimatic conditions," *Indian Journal of History of Science*, 1993, 34(1), pp.19-32.
- Olowa, L., & Nuñeza, O. (2013). Brine Shrimp Lethality Assay of the Ethanolic Extracts of Three Selected Species of Medicinal Plants from Iligan City, Philippines. Isca. in. Retrieved 30 August 2020, from http://www.isca.in/IJBS/Archive/v2/i11/12.ISCA-IRJBS-2013-177.pdf.
- Pradhan, D., Suri, K., Pradhan, D., & Biswasroy, P. (2013). Golden Heart of the Nature: Piper betle L.. Phytojournal.com. Retrieved 30 August 2020, from http://www.phytojournal.com/voll1ssue6/Issue march 2013/19.pdf.
- Prokopczyk B., Rivenson A., Hoffmann D., "A Study of Betel Quid carcinogenesis IX. Comparative carcinogenicity of 3-(methylnitrosamino) propionitrile and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone upon local application to mouse skin and rat oral mucosa", *Cancer Letters*," 1991, 60 (2), pp. 153-157.
- Sakilan JM, Demayo CG and Opanasopit P: Phytochemical analysis and determination of antimicrobial, antioxidant, and anticancer activity of the leaf ethanolic extracts of *Piper sarmentosum* roxb. in lapuyan zamboanga del sur, Philippines. Int J Pharm Sci & Res 2019; 10(12): 5715-22. doi: 10.13040/IJPSR.0975-8232.10(12).5715-22.
- Sharma D. C., "Betel Quid and Areca Nut are Carcinogenic without Tobacco," *The Lancet Oncology*, 2003, 4(10), pp. 587.
- Svoboda, K., & Hampson, J. (1999). Bioactivity of essential oils of selected temperate aromatic plants: pharmacological antibacterial, antioxidant, antinflammatory and other related activities.. Restorationisnow.com. Retrieved 30 August 2020 from https://www.restorationisnow.com/uploads/3/7/8/6/37869251/bioactivity of essential oils- antibacterial. antioxidant. anti-inflammatory.pdf.
- Tripathi, S. (2014). *Review study on potential activity of Piper betle*. Phytojournal.com. Retrieved 30 August 2020, from http://www.phytojournal.com/vol3Issue4/Issue nov 2014/17.1.pdf.
- Wenke, Gottfriend, Hoffman, Dietrich, "A Study of Betel Quid Carcinogeneses: On the in vitro N-nitrosation of arecoline," *Carcinogenesis*, 1983, 4(2), pp. 169-72



Calvarial Metastasis with Dural Sinus Invasion: A Case Report

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Abstract

Metastatic disease to the calvaria with invasive dural venous sinus extension presents unique diagnostic and management challenges in cancer patients. Treatment options are not standardized. We report a case of a 62-year-old female who presented with breast cancer calvarial metastasis and upper sagittal sinus extension, and in whom a complete resection of the lesion was performed. Surgery may be helpful in selected cancer patients for symptomatic relief as well as survival benefits.

Keywords: Calvarial Metastasis, Dural Sinus Invasion, Surgical Treatment

1. Introduction

Metastases are the most common malignancies of the central nervous system. Rising incidence is due to the availability of more advanced neuroimaging techniques and raising survival rate (Newton, 2008; Takei et al., 2016). Unfavourable prognosis, high morbidity and mortality are associated with central nervous system metastases (Nayak et al., 2009). Skull metastases remain a neglected complication and are of less clinical importance than brain metastases (Harrison et al., 2018; Mitsuya et al., 2011). Their precise incidence is underreported in the literature. Few studies have been published on skull metastases in the literature (Harrison et al., 2018; Nasi-Kordhishti et al., 2021; Ozgiray et al., 2016; Stark et al., 2003). Multiple therapeutic modalities are used in the treatment of cranial metastases: radiotherapy, surgery, and chemotherapy. However, no treatment standard has been developed (Harrison et al., 2018).

2. Case presentation

A 62 years old female patient has operated two years ago for invasive ductal carcinoma. She has had a tumorectomy followed by radiotherapy and hormonotherapy. Our patient came to the clinic complaining of a headache, and calvaria mass started to appear for six months. Clinical examination revealed a painful frontal calvarial mass. Computed tomography (CT) scan revealed an osteolytic lesion in the frontal bone with endocranial

extension. Magnetic resonance imaging (MRI) showed (figure 1) a midline process, with exo and endocranial extension, measuring 53 * 45 * 30mm. The lesion is intermediate signal T1 and T2 and is enhanced after Gadolinium injection, with dura mater contrast enhancement and thrombosis of the upper sagittal sinus.



Figure 1: Preoperative cerebral MRI. **a** Sagittal T1-weighted image shows a bone lesion with exo and endocranial extension. **b** Coronal T2-weighted image shows superior sagittal sinus infiltration. **c** MRI-angiogram shows obstruction of the anterior third of the superior sagittal sinus.

The whole-body CT scan investigation did not show other metastases. The patient underwent a complete tumor resection by piecemeal technique (figure 2) with superior sagittal sinus resection and cranioplasty.



Figure 2: Peroperative image. **a** Extracranial calvarial metastasis. **b** and **c** Dural metastasis with sous dural invasion. **d** and **e** Resection of metastasis after ligation of the upper sagittal sinus. **f** Macroscopic aspect of the tumor after resection by piecemeal technique.

Postoperative MRI showed complete resection (figure 3). The histological examination showed poorly differentiated breast carcinoma. The patient had 30 sessions of skull radiation. Our patient is in good condition without any complications after one year of treatment.



Figure 3: Postoperative cerebral MRI. **a** Sagittal T1-weighted image, **b** Coronal T2-weighted image, and **c** Coronal T1-weighted with contrast image shows complete resection of the tumor.

3. Discussion

Breast, prostate, and lung cancer are the most common primary tumors associated with cranial metastases (Harrison et al., 2018; Newton, 2008). 24% of breast cancer patients have metastatic skull lesions (Heo et al., 2017). Metastatic involvement of the dura mater is a rare event and usually arises by direct extension from skull metastases (Heo et al., 2017; Laigle-Donadey et al., 2005). Calvarial metastases are most commonly asymptomatic. When symptomatic, they most often manifest as a mass under the scalp with local pain. In a series reported by Michael et al. (Michael et al., 2001), 70% of patients present a local mass as the initial symptom. Invasive metastases can manifest as headache, vomiting, or even neurological deficits (Harrison et al., 2018; Nasi-Kordhishti et al., 2021; Newton, 2008; Ozgiray et al., 2016).

MRI remains the test of choice to explore skull metastases. MRI can be used to assess soft tissue involvement and intracranial extension. Calvarial metastases often present as osteolytic lesions (Harrison et al., 2018; Mitsuya et al., 2011). Mitsuya proposed an MRI classification based on three questions: concerning the location (either in the calvarium or in the cranial base), concerning distribution within the plan of the cranial bone (circumscribed or diffuse), and concerning invasion (intraosseous or invasive) (Mitsuya et al., 2011).

Four therapeutic modalities should be considered: irradiation, chemotherapy, hormonal therapy, and surgical resection. The treatment of skull metastases is most often palliative. In cases of solitary metastasis, the treatment should be considered for curative purposes. Radiotherapy is the first option. In diffuse cranial lesions, whole brain radiation therapy is often necessary. In oligo metastatic forms, it is reasonable to consider focused radiotherapy such as radiosurgery. Radiosurgery provides better tumor control with side effects comparable with those of standard radiation therapy (Mitsuya et al., 2011). Surgical treatment is poorly described in the literature. Only a minority of patients with skull metastases are candidates for surgical resection. Complete gross total resection is associated with a prolonged survival rate (Harrison et al., 2018; Nasi-Kordhishti et al., 2021). The tumor can be removed en bloc or using the piecemeal technique. The en bloc resection technique is based on the method described by Kinjo to ensure complete resection of meningiomas that invade the skull or the soft tissues. Michael et al. have extended this technique to include the dural sinus. En bloc resection is more effective in limiting operative blood loss (Michael et al., 2001). Dural sinus invasion increases the complexity of the surgery, however, more aggressive resection surgery is recommended and is associated with low morbidity (Harrison et al., 2018; Newton, 2008; Ozgiray et al., 2016). Surgery should be indicated in case of (Harrison et al., 2018; Ozgiray et al., 2016; Sawaya, 1997; Stark et al., 2003): - the presence of a neurological deficit - massive bone destruction, infiltration of the dura - painful mass - single metastases - diagnostic confirmation. The involvement of the venous sinus does not seem to affect the indications for surgical intervention. It is recommended for sinus resection selection patients that the tumor is localized in the anterior third of the sagittal sinus or that the sinus is occluded on imaging. Michael et al. concluded that surgery was a relatively safe and effective treatment option (Michael et al., 2001). Reconstruction of bone defects is usually performed simultaneously as resection surgery but can be delayed from a few weeks to a few months (Harrison et al., 2018; Ozgiray et al., 2016). Depending on the type of tumor, the use of hormone therapy, targeted therapy, or conventional chemotherapy may be appropriate. Recent advances in chemotherapy have contributed to the treatment of metastatic skull tumors and systemic bone metastases (Harrison et al., 2018).

5. Conclusion

Metastatic disease to the calvaria with intracranial dural sinus invasion presents unique diagnosis and management challenges in cancer patients. The management should be multidisciplinary. A complete resection must be sought after a proper investigation and appropriate neurosurgical plan. Surgical resection undoubtedly cannot influence the underlying disease but enables the patient to avoid local pain, and it might improve neurologic symptoms with low morbidity and mortality.

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References

- Harrison, R. A., Nam, J. Y., Weathers, S. P., & DeMonte, F. (2018). Intracranial dural, calvarial, and skull base metastases. In Handbook of Clinical Neurology (1st ed., Vol. 149). https://doi.org/10.1016/B978-0-12-811161-1.00014-1
- Heo, M. H., Cho, Y. J., Kim, H. K., Kim, J. Y., & Park, Y. H. (2017). Isolated pachymeningeal metastasis from breast cancer: Clinical features and prognostic factors. Breast, 35. 109-114. https://doi.org/10.1016/j.breast.2017.07.006
- Laigle-Donadey, F., Taillibert, S., Mokhtari, K., Hildebrand, J., & Delattre, J. Y. (2005). Dural metastases. Journal of Neuro-Oncology, 75(1), 57-61. https://doi.org/10.1007/s11060-004-8098-1
- Michael, C. B., Gokaslan, Z. L., DeMonte, F., McCutcheon, I. E., Sawaya, R., & Lang, F. F. (2001). Surgical resection of calvarial metastases overlying dural sinuses. Neurosurgery, 48(4), 745-755. https://doi.org/10.1227/00006123-200104000-00009
- Mitsuya, K., Nakasu, Y., Horiguchi, S., Harada, H., Nishimura, T., Yuen, S., ... Endo, M. (2011). Metastatic skull tumors: MRI features and a new conventional classification. Journal of Neuro-Oncology, 104(1), 239-245. https://doi.org/10.1007/s11060-010-0465-5
- Nasi-Kordhishti, I., Hempel, J. M., Ebner, F. H., & Tatagiba, M. (2021). Calvarial lesions: overview of imaging features and neurosurgical management. Neurosurgical Review. https://doi.org/10.1007/s10143-021-01521-
- Navak, L., Abrey, L. E., & Iwamoto, F. M. (2009). Intracranial dural metastases. Cancer, 115(9), 1947–1953. https://doi.org/10.1002/cncr.24203
- Newton, H. B. (2008). Skull and dural metastases. Cancer Neurology In Clinical Practice: Neurologic Complications of Cancer and Its Treatment: Second Edition, 145–161. https://doi.org/10.1007/978-1-59745-412-4 10
- Ozgiray, E., Perumal, K., Cinar, C., Caliskan, K. E., Ertan, Y., Yurtseven, T., ... Oner, K. (2016). Management of Calvarial Tumors: A Retrospective Analysis and Literature Review. Turkish Neurosurgery, 26(5), 690-698. https://doi.org/10.5137/1019-5149.JTN.12537-15.0
- Sawaya, R. (1997). Lesions of the calvaria: Surgical experience with 42 patients. Annals of Surgical Oncology, 4(1), 28–36. https://doi.org/10.1007/bf02316808
- Stark, A. M., Eichmann, T., & Mehdorn, H. M. (2003). Skull metastases: Clinical features, differential diagnosis, and review of the literature. Surgical Neurology, 60(3), 219-225. https://doi.org/10.1016/S0090-3019(03)00269-6
- Takei, H., Rouah, E., & Ishida, Y. (2016). Brain metastasis: clinical characteristics, pathological findings and molecular subtyping for therapeutic implications. Brain Tumor Pathology, 33(1), 1–12. https://doi.org/10.1007/s10014-015-0235-3



Prevalence and Risk Factors of Malaria among Pregnant Women receiving Antenatal Care in a Health Facility in Delta State, Southern Nigeria

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Abstract

Background: Malaria is one of the major causes of morbidity and mortality in developing countries including Nigeria. Malaria in pregnancy is estimated to cause about 15% of maternal deaths globally, accounting for over 10,000 maternal and 200,000 neonatal deaths annually. This study determined the prevalence and risk factors of malaria infection among pregnant women receiving antenatal care in a health facility in Delta State. Methods: This was a facility-based cross-sectional study carried out among 418 pregnant women who were selected by systematic sampling technique. Clinical malaria was confirmed using microscopy method (Giemsa staining technique) while a pre-tested, structured, interviewer administered questionnaire was used to collect information on soscio-demographic characteristics and obstetrics history of the respondents. Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 20.0 and the level of statistical significance was set at p< 0.05. Results: The mean age of the participants was 29.9 ± 5.7 years. Two hundred and sixty three (62.9%) pregnant women tested positive for malaria. Respondents who were pregnant for the second time (AOR = 0.521, 95% CI: 0.28 - 0.99, p = 0.045) and those from a family size of 1-6 (AOR = 2.123, 95% CI: 1.12 - 4.04, p = 0.022) were more likely to test positive to malaria parasite. Conclusion: In this study, the prevalence of malaria in pregnant women was high and the significant predictors were; family size of 1-6, skill level 1, first trimester and multigravidae. The Delta State Ministry of Health should ensure increasing access to IPT, ITNs/LLIN and health education on malaria in all hospitals rendering ANC services.

Keywords: Prevalence, Risk Factors, Malaria, Pregnant Women, Antenatal Care, WHO, Delta State, Nigeria

1. Introduction

Malaria is currently one of the world's most serious public health problems. It is a parasitic infection that is transmitted to humans through the bite of an infected female Anopheles mosquito (WHO, 2022). Malaria affects nearly half of the world's population (CDC, 2021). Globally, 241 million cases of malaria was reported in 2020, with 627,000 people dying and majority of them were children in Africa (WHO, 2022). The African Region carries a disproportionately high share of the global malaria burden (World Bank, 2022). In 2015, the Sub-

Saharan Africa accounted for 90% of malaria cases and 92% of malaria deaths (AHO, 2020). Sub-Saharan Africa is responsible for a disproportionately high percentage of the global malaria burden (CDC, 2021). Approximately 35 million pregnant women in Sub-Saharan Africa are at risk of contracting malaria each year (Gontie et al., 2020) and at least 25% of pregnant women are predicted to have the disease each year (Wagbatsoma & Omoike, 2008). In Nigeria, malaria affects 100% of the population, with at least half of the population contracting the disease once a year (CDC, 2020). Nigeria accounts for a quarter of all malaria cases in the 45 malaria-endemic countries in Africa (Chukwuocha et al., 2012). Twenty-five million pregnant women are at risk of malaria in Nigeria and according to the World Health Organization in 2015, the region experienced up to 90% malaria cases and 92% malaria deaths (UNICEF, 2017).

Globally, malaria is estimated to cause at least 10,000 maternal deaths and 200,000 newborn deaths per year (Okpere et al., 2010). In 2015, malaria was the third most common cause of death among women of reproductive age in Africa (Schantz-Dunn, & Nawal, 2009) and in 2020, children under 5 years accounted for about 80% of all the malaria deaths in the African region(WHO, 2022). Malaria contributes to an estimated 11% of maternal mortality in Nigeria (United States Embassy in Nigeria, 2011). In Nigeria, malaria affects about 70% of pregnant women (Traffina Foundation for Community Health, 2022) and it is responsible for 30% of childhood mortality, 25% of deaths in children under the age of one year, and 11% of maternal deaths (Traffina Foundation for Community Health, 2022). In 2021, Health Aid For All Initiative (HAFAI) reported the results from maternal and child health survey carried out by United Nations Children's Fund (UNICEF) in Nigeria, that Nigeria lost about 2,300 under five year-olds and 145 women of childbearing age in a day, making it the second largest contributor to the under-five and maternal mortality rates in the world (HAFAI, 2020). Also, in 2020, the four countries in Africa that accounted for over half of all malaria deaths worldwide were: Nigeria (31.9%), the Democratic Republic of the Congo (13.2%), United Republic of Tanzania (4.1%) and Mozambique (3.8%) (WHO, 2022). In terms of the actual number of maternal deaths, Nigeria was ranked second in the world behind India and in terms of the maternal mortality ratio. Nigeria is ranked eighth in Sub-Saharan Africa behind; Angola, Chad, Liberia, Niger, Rwanda, Sierra Leone and Somalia (Global one 2015, 2012).

Maternal death during pregnancy is more common in women who reside in rural regions and in impoverished populations (WHO, 2019). Rural regions in Nigeria account for roughly 51% of malaria infections and deaths, owing to a lack of effective diagnosis and treatment facilities (CDC, 2020). Malaria is responsible for approximately 60% of out-patient visits to Nigerian health facilities (Traffina Foundation for Community Health, 2022). The yearly financial loss due to malaria in Nigeria is estimated to be about 132 billion Naira (\$797 million) annually in form of treatment costs, prevention costs and loss of work-hours (CDC, 2020). During pregnancy, primigravidas, teens, and HIV-positive women have the highest risk of malaria infection and morbidity (Wagbatsoma & Omoike, 2008; CDC, 2015). Malaria in pregnancy has been estimated to cause over 40 cases of maternal anemia in Africa (United States Embassy in Nigeria, 2011). Malaria infection causes maternal anemia, spontaneous miscarriage, premature delivery, intrauterine development retardation, and the birth of low-birth-weight neonates which is a risk factor for stillbirth and neonatal death (World Bank, 2022; CDC, 2015). No wonder the Sustainable Development Goals (SDGs) and the Global Strategy for Women's, Children's, and Adolescents' Health (WHO, 2019), aims at achieving a global maternal death rate of less than 70 per 100,000 live births between 2016 and 2030.

Warri, located in the Niger-Delta region of Nigeria, characterized by frequent rains and poor environmental management, is one of the endemic zones for malaria in Nigeria (Efe & Ojoh, 2013). The communities in Warri are mostly rural and are located near rivers, together with their major occupations (farming and fishing), people of Warri are exposed to mosquito bites which could result to malaria. Furthermore, mosquito breeding places such as water bodies, farm gardens, open septic tanks, ponds, and streams are located near the residences of the people of Warri. The area's rainy season extends from January to December, with an average annual temperature of 27.4 degrees Celsius, which favors mosquito activity. Water stagnates on the ground surface due to the composition of the soil, creating a breeding place for mosquitoes (Efe & Ojoh, 2013). Although some adults in the Niger Delta region of Nigeria develop immunity to malaria as they are constantly exposed to high levels of malaria transmission, pregnant women remain vulnerable (Wagbatsoma & Omoike, 2008). Because of the area's frequent rains and poor environmental management, mosquito breeding has increased, making Warri a malaria endemic area where pregnant women and their unborn children are at risk of mosquito bites and malaria infection (Efe & Ojoh, 2013).

Pregnant women need special protective measures to ensure their survival and improved birth outcomes (Chukwuocha et al., 2012).

Though malaria is typically prevalent in poor tropical and subtropical areas of the world, where climatic variables such as temperature, humidity, and rainfall enhance the growth, survival, and multiplication of the Anopheles mosquitoes which carry the organism that causes malaria (Erhabor et al., 2007), it is preventable and curable, and its prevalence has declined in many locations (World Bank, 2022). Vector control is the primary step in preventing and reducing malaria transmission. Malaria can be prevented in pregnancy by taking the right Intermittent Preventive Treatment (sulfadoxine pyrimethamine) which is administered in the second and third trimesters of pregnancy, using insecticide-treated bed nets and keeping the surroundings clean (World Bank, 2022). Despite an increase in the number of women receiving antenatal care in health facilities and increased public awareness about malaria in pregnancy (CDC, 2015), malaria in pregnancy continues to be a major public health concern and one of the leading causes of maternal and infant morbidity and mortality in Nigeria and around the world (CDC, 2015). Therefore, the aim of this study was to determine the prevalence and risk factors associated with malaria among pregnant women receiving antenatal care in Central Hospital Warri, Delta State.

2. Materials and Methods

2.1. Study area

This study was carried out in the Obstetrics/Gynaecology section of Central Hospital Warri, located at Warri South Local Government Area (LGA) in Delta State. The LGA covers roughly 1,520 square kilometres and had a projected population of 443,175.36 people in 2021 (Warri South, 2022). Warri South's latitude and longitude are 5.1216 and 7.3733, respectively. The majority of the settlements in this LGA are rural, with many of them located near rivers (Warri South, 2022). The people's main occupations has to do with oil, agriculture (particularly fish farming), and government work (Efe & Ojoh, 2013).

Central Hospital Warri is a secondary health care institution that was established in 1906 during the colonial era. It has a capacity of 267 beds and 386 employees (Central Hospital Warri, 2017) among whom are doctors in various specialties, nurses, laboratory scientists and pharmacists. It is the largest hospital in Delta State and it oversees the activities of the other six general hospitals in the State (Central Hospital Warri, 2017). The hospital provides primary and secondary healthcare services, trainings for essential healthcare services and it is active in health researches (Warri South, 2022).

The Department of Obstetrics and Gynaecology handles cases of pregnant women at the Central Hospital Warri and it is the largest obstetric unit in Delta State. At the time of this study, the hospital had 10 Consultant Obstetricians. The hospital records an average of 25,000 antenatal cases and an average of 4000 deliveries per annum (Central Hospital Warri, 2017). The antenatal booking days are on Wednesdays, between 8am and 12pm. The hospital's antenatal clinic days are open from Monday to Thursday every week. Over 200 pregnant women attend antenatal clinic per ANC day but the Obstetricians/Gynaecologists can only see 80 pregnant women daily, on individual bases (Central Hospital Warri, 2017).

2.2. Study Design

This was a facility-based cross-sectional study carried out between May 2018 and July 2018.

2.3. Study population and sample size determination

All pregnant women presently registered and accessing antenatal care services at the Central Hospital Warri were recruited as study subjects for this study. However, only those who gave their written consents were included in this study. Pregnant women who were present, but very ill and in need of immediate medical attention were not included in this study. A sample size of 418 was calculated using Cochran's single proportion sample size formula (Cochran, 1977), with an error margin of 5%, 1.96 critical values for 95 percent confidence

level, and 57.1 percent (malaria prevalence rate for a study conducted in Andoni LGA in Rivers state, located in Nigeria's Niger Delta region, in 2010) (Chukwuocha et al., 2012).

2.4. Sampling technique

Systematic sampling technique was used to select the 418 participants. The study participants were given equal chances of being selected to participate in the study and they were selected within 8 days; Fifty-two pregnant women were selected on each ANC day and on the final day fifty-four participants were selected in order to achieve the desired sample size of 418.

From the daily attendance list, the hospital records over 200 pregnant women on each ANC day. Every 4th individual on the attendance list that gave her written consent was sampled until a total of 52 participants were achieved but on the final day, 54 individuals were selected in order to achieve the 418 expected sample size.

2.5. Data collection

Information on socio-demographic and maternal obstetric characteristics of the respondents was collected using pretested, structured, interviewer-administered questionnaires. Collecting of the blood samples, carrying out the test for the presence of malaria parasite and administering of questionnaires were done by eight research assistants who were laboratory technologists from the Central Hospital Warri Laboratory Department. They were trained for one-day on how to administer the standardized questionnaires.

2.6. Malaria testing

The malaria test was performed in the Central Hospital Warri Laboratory using the microscopy method (Giemsa thick film stain), which involves direct microscopic viewing of the malaria parasite on thick blood smears and is considered the gold standard for malaria diagnosis (Chotivanich et al., 2007).

2.7. Procedure for Microscopy

Each pregnant woman that participated in this study was given a unique identification number and the numbers were labeled on individual EDTA-containers. Venipuncture was used to obtain blood samples from the pregnant women into a labeled EDTA (Ethylenediaminetetraacetic acid)-containing tube. Blood samples were taken and analyzed on the same day they were taken. A drop of the pregnant women's blood was taken from the EDTA container using a micropipette and was spread out on a labeled, clean and grease free microscope slide to make a thick blood smear. The prepared slides were allowed to dry and then stained with Giemsa stain to give the parasite a distinguishing appearance before examining under an x100 oil immersion objective of the light microscope. The entire bulky film was scanned in its entirety. When no malaria parasite was seen on a thick film, it was considered negative. Where parasites were found, the rest of the blood film was inspected to eliminate the potential of a mixed infection being missed. All of the species and stages that were observed were identified and documented. Plasmodium falciparum was the most common Plasmodium species discovered in this investigation. They were discovered in trophozoites and ring forms.

2.8. Data analysis

The administered copies of the questionnaires were screened for completeness after collection, and the data generated from the study was collated, coded, and entered into a spread sheet before being analyzed using the Software Statistical Package for Scientific Solutions (SPSS) version 20.0 manufactured by IBM incorporated. To analyze the distribution of the variables, a preliminary univariate analysis was performed. The mean, frequencies, and proportions were used to summarize the data.

The Chi-square test and the Fisher's exact test were used to assess the association between sociodemographic/obstetric variables and malaria test results. Multiple regression analysis was used to determine the predictors of malaria infection. A p-value of <0.05 was considered statistically significant. The International Labour Organization's International Standard Classification of Occupations, volume 8 (ILO-ISCO-08) (International Labour Organization, 2012) was used to classify the participants' and spouses' employment status into skill levels;

Skill Level 1: Include jobs such as cleaning, digging, lifting and carrying materials by hand, sorting or assembling goods by hand, operating non-motorized vehicles, and picking fruits and vegetables, etc.

Skill Level 2: Examples include; butchers, bus drivers, secretaries, accounts clerks, sewing machinists, dressmakers, shop sales assistants, police officers, building electricians and motor vehicle mechanics, etc.

Skill Level 3: Includes shop managers, medical laboratory technicians, legal secretary, commercial sales representatives, diagnostic medical radiographers, computer support technicians etc.

Skill Level 4: Includes sales and marketing managers, medical practitioners, engineers, computer systems analysts etc.

2.9. Malaria prevalence

This was calculated as the proportion of the pregnant women who had a positive malaria test results.

2.10. Ethical approval

Ethical approval for this study was obtained from the Ethics and Research Committees of Central Hospital Warri (CHW/ECC VOL 1/166). Also, the management of the Central Hospital Warri gave approval before commencement of the study. A written informed consent was obtained from each participant before blood sample collection and questionnaire administration. Respondents were assured of the confidentiality of their response and that there was no risk associated with the study.

3. Results

Four hundred and eighteen pregnant women participated in this study. The mean age of the respondents was 29.9 \pm 5.7 years, with an age range of 19 and 48 years. Majority 241 (57.7%) of the respondents had at least secondary level of education while over a third 155 (37.1%) had tertiary level of education. Most of the respondents were in skill level 1 occupation, 157 (37.6%), in skill level 2. Among the respondents, 383 (91.6%) were married. The average monthly income of the respondents shows that over a third 161 (38.5%) had average monthly household income of 50,000 – 100,000 naira. (Table 1)

Variables	Frequency (n = 418)	Percent
Age (Years)		
<20	20	4.8
21 - 30	204	48.8
31 - 40	182	43.5
41 - 49	12	2.9
Marital Status		
Single	35	8.4
Married	383	91.6
Ethnicity		
Urhobo	131	31.3
Igbo	112	26.8
Isoko	40	9.6
Itsekiri	38	9.1
Ijaw	34	8.1
Yoruba	29	6.9
Benin	13	3.1

Table 1: Socio-demographic characteristics of the respondents

Hausa	11	2.6
Esan	10	2.4
Religion		
Christianity	392	93.8
Islam	21	5.0
African traditional religion	5	1.2
Residence		
Rural	77	18.4
Urban	341	81.6
Level of education		
None	4	1.0
Primary	18	4.3
Secondary	241	57.7
Tertiary	155	37.1
Occupation		
Skill Level 1	50	12.0
Skill Level 2	157	37.6
Skill Level 3	91	21.8
Skill Level 4	120	28.7
Average monthly household income (N)		
<50,000	113	27.0
50,000 - 100,000	161	38.5
101,000 - 199,000	59	14.1
200,000 - 500,000	80	19.1
>500,000	5	1.2

The highest proportion of the respondents was multigravida 185 (44.3%). Also, 209 (50%) of the respondents were in their third trimester. Thirty-six (8.6%) were first timers and over three quarter 327 (78.2%) were regular to their ANC. Three hundred and three (72.5%) of the respondents were from a family size of 1 - 6 people and 179 (42.8%) have had one or two children. (Table 2)

Table 2: Maternal obstetric characteristics of the respondents

Variables	Frequency (n = 418)	Percent			
Respondent's Parity					
Primigravidae	117	28.0			
Secundigravidae	116	27.8			
Multigravidae	185	44.3			
Number of Children					
None	128	30.6			
1 - 2	179	42.8			
>3	111	26.6			
Current Trimester					
First	85	20.3			
Second	124	29.7			
Third	209	50.0			
Regular ANC Attendant					
Yes	327	78.2			

No	55	13.2		
First timer	36	8.6		
Family size				
1 - 6.	303	72.5		
> 6	115	27.5		

Two hundred and sixty three (62.9%) of the respondents had positive malaria parasite test results. Thirty nine (78%) of the respondents who were in skill level 1 occupation tested positive to malaria parasite compared to 85 (54.1%) in skill level 2, 64 (70.3%) in skill level 3 and 75 (62.5%) in skill level 4. The association between malaria test results and respondents' occupation was statistically significant (p = 0.007). Age, residence, educational status and household income were not significantly associated with malaria test results. (Table 3)

Table 3. Association	between socio-demo	graphic charact	teristics and	malaria test	results
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Prevalence of malaria in pregnancy				
	Positive $(n = 263)$	Negative (n = 155)		
Variables	n (%)	n (%)	Test statistics	p-value
Age in years				
≤20	15 (75.0)	5 (25.0)	†=2.092	0.554
21 - 30	131 (64.2)	73 (35.8)		
31 - 40	109 (59.9)	73 (40.1)		
≥41	8 (66.7)	4 (33.3)		
Marital status				
Single	23 (65.7)	12 (34.3)	$x^2 = 0.128$	0.855
Married	240 (62.7)	143 (37.3)	df = 1	
Residence				
Rural	48 (62.3)	29 (37.7)	$x^2 = 0.014$	0.907
Urban	215 (63.0)	126 (37.0)	df = 1	
Level of education				
None	3 (75.0)	1 (25.0)	† = 1.597	0.686
Primary	9 (50.0)	9 (50.0)		
Secondary	153 (63.5)	88 (36.5)		
Tertiary	98 (63.2)	57 (36.8)		
Occupation				
Skill Level 1	39 (78.0)	11 (22.0)	$x^2 = 12.211$	0.007*
Skill Level 2	85 (54.1)	72 (45.9)	df = 3	
Skill Level 3	64 (70.3)	27 (29.7)		
Skill Level 4	75 (62.5)	45 (37.5)		
Average monthly				
household income (N)				
<50,000	70 (61.9)	43 (38.1)	† = 1.639	0.814
50,000 - 100,000	101 (62.7)	60 (37.3)		
101,000 - 199,000	37 (62.7)	22 (37.3)		
200,000 - 500,000	53 (66.2)	27 (33.8)		
>500,000	2 (40.0)	3 (60.0)		

Statistical significant, X^2 Pearson Chi-square, [†]Fisher's Exact, ATR = African Traditional religion

Two hundred and one (66.3%) of respondents who were from a family size of 1 - 6 tested positive to malaria parasite compared with 62 (53.9%) from family size of > 6 and the association was statistically significant (p = 0.023). Sixty five (76.5%) of the respondents in their first trimester of pregnancy tested positive to malaria parasite, compared with 69 (55.6%) in their second trimester and 129 (61.7%) in their third trimester tested

positive. The association between current trimester of the respondents and malaria test result was statistically significant (p = 0.008). However, respondents' parity, number of children and antenatal attendance were not significantly associated with malaria test results. (Table 4)

	Prevalence of malaria	a in pregnancy		
	Positive $(n = 263)$	Negative (n = 155)		
Variables	n (%)	n (%)	Test statistics	p-value
Respondents' Parity	7			
Primigravidae	83 (70.9)	34 (29.1)	$x^2 = 4.594$	0.103
Secundgravidae	68 (58.6)	48 (41.4)	df = 2	
Multigravidae	112 (60.5)	73 (39.5)		
Number of children				
None	89 (69.5)	39 (30.5)	$x^2 = 5.420$	0.069
1 - 2.	113 (63.1)	66 (36.9)	df = 2	
>3	61 (55.0)	50 (45.0)		
Family size				
1 - 6.	201 (66.3)	102 (33.7)	$x^2 = 5.515$	0.023*
> 6	62 (53.9)	53 (46.1)	df = 1	
Current Trimester				
First	65 (76.5)	20 (23.5)	$x^2 = 9.631$	0.008*
Second	69 (55.6)	55 (44.4)	df = 2	
Third	129 (61.7)	80 (38.3)		
Regular ANC				
Yes	204 (62.4)	123 (37.6)	$x^2 = 2.821$	0.257
No	32 (58.2)	23 (41.8)	df = 2	
First timer	27 (75.0)	9 (25.0)		

Table 4: Association between maternal obstetric characteristics and malaria test result

*Statistical significant, x²Pearson Chi-square

The secundigravida respondents were less likely by an odds ratio of 0.521 to have positive test to malaria when compared with the multigravidae. (AOR = 0.521, 95% CI: 0.28 - 0.99, p = 0.045). Also, respondents from a family size of 1-6 were more likely by an odds ratio of 2.123 to test positive to malaria parasite compared with those from family size of > 6 (AOR = 2.123, 95% CI: 1.12 - 4.04, p = 0.022). Table 5

Regression	AOR (05% CI)	n valua
coefficient(p)	AUK (9370 CI)	p-value
0.365	1.441 (0.62 - 3.37)	0.399
-0.502	0.605 (0.29 - 1.26)	0.181
0.13	1.139 (0.54 - 2.43)	0.736
	1	
-0.362	0.697 (0.33 - 1.45)	0.335
-0.651	0.521 (0.28 - 0.99)	0.045*
	1	
0.753	2.123 (1.12 - 4.04)	0.022*
	Regression coefficient(β) 0.365 -0.502 0.13 -0.362 -0.651 0.753	Regression coefficient(β)AOR (95% CI)0.3651.441 (0.62 - 3.37)-0.5020.605 (0.29 - 1.26)0.131.139 (0.54 - 2.43)11-0.3620.697 (0.33 - 1.45)-0.6510.521 (0.28 - 0.99)110.7532.123 (1.12 - 4.04)

Table 6: Logistic Regression model for determinants of malaria test results

> 6**		1	
Current Trimester			
First	0.454	1.574 (0.83 - 2.30)	0.167
Second	-0.428	0.652 (0.40 - 1.07)	0.090
Third**		1	

*Statistically significant, **Reference Category. The variance correctly classified for 65.8% of the cases.

4. Discussion

This study assessed the prevalence and risk factors associated with malaria in women receiving antenatal care in Central Hospital, Warri. The prevalence of malaria among pregnant women in this study was found to be 62.9%, as determined by malaria microscopy testing. The prevalence of malaria in pregnant women that participated in this study was higher than that obtained from a similar study carried out in Benishangul Gumuz regional State of West Ethiopia (10.2%) (Gontie et al., 2020). When compared with the prevalence of malaria in pregnancy obtained from a study that was carried out in a health institution's ANC in Bobo-Dioulass, Burkina Faso (18.1%) (Cisse et al., 2014), the malaria prevalence in this present study was relatively higher. The reason could be because the pregnant women in this present study do not practice proper malaria prevention.

The malaria prevalence of this current study relates to that of a similar study (Chukwuocha et al., 2012) carried out in parts of Niger-Delta of Nigeria which recorded a high malaria prevalence rate of 57.1% among pregnant women that participated in the study (Chukwuocha et al., 2012). The similarity (high frequency) in the malaria prevalence rate of both studies may be due to the fact that both studies were carried out in the same geographical location (Niger-Delta region of the country), which is riverine in nature and can support the breeding of mosquitoes (Erhabor et al., 2007). Poor coverage and use of LLIN and IPT, poor malaria case management, and poor environmental hygiene of study participants could also contribute to the high malaria prevalence recorded in these studies. The malaria prevalence of this present study was found to be higher than that of a similar study carried out among pregnant women in Katsina State (36.5%) (Bawa et al., 2014). The high prevalence of malaria observed in this current study could be because of the geography of Warri which is riverine. Also, this present study was conducted during the rainy season (though not at the peak of the rainy season) which is when mosquito bite is noticed to be on the increase (Wagbatsoma & Omoike, 2008).

The Prevalence of malaria in this present study is also higher than that obtained in Kebbi State where a prevalence of 41.6% was reported among pregnant women who participated in a community-based study (Fana et al., 2015) but lower than the malaria prevalence rate of 79.0% reported among pregnant women in a recent study (Damisa & Hassan, 2021) carried out in the ANC of a general hospital in Kaduna State of Nigeria. The prevalence rate of this study is also lower than the prevalence of 80.9% reported in another similar study (Ohalete, 2011) carried out among pregnant women in three major health institutions in Imo State. However, another study (Gunn et al., 2015) conducted among pregnant women in Enugu State recorded over 99.0% malaria prevalence rate. The reason this current study's malaria prevalence rate is lower than that of the above studies carried out in Kaduna State, Imo State and Enugu State could be because this present study was not carried out at the peak of rainy season which is when mosquito bite is on the increase in Warri. It could also be because this present study area is located in an oil producing state and most of the oil companies through NGOs provide treated bed nets to the residents, carry out indoor residual spraying (IRS) and free malaria testing and distribution of antimalarial medications to those who test positive to malaria. Another reason for the low prevalent rate observed in this current study could be because of the inclusion criteria of this study which exempted the very sick ones from participating in this study. Also, this study was not carried out in a community setting but among ANC attendees in a particular health institution of which some of the study participants in their second and third trimesters might have received intermittent preventive treatment (IPT) against malaria in pregnancy.

Different studies in the past have recorded different factors to be associated with malaria in pregnancy; such as young maternal age (Chukwuocha et al., 2012; Cisse et al., 2014; Bawa et al., 2014; Fana et al., 2015; Sohail et al., 2015; Agomo & Oyibo, 2013), older maternal age (Abdalla et al., 2017), rural dwellers (Sohail et al., 2015),

low level of education (Fana et al., 2015), primigravidae (Gontie et al., 2020; Cisse et al., 2014; Nega et al., 2015), non-usage of mosquito nets (Gontie et al., 2020; Chukwuocha et al., 2012; Cisse et al., 2014; Fana et al., 2015; Sohail et al., 2015), member of a family size of 1–3 (Odikamnoro et al., 2014), first trimester of pregnancy (Sohail et al., 2015), second trimester (Sohail et al., 2015; Odikamnoro et al., 2014), being HIV positive (Chaponda et al., 2015), too much farming/fishing (Chukwuocha et al., 2012), staying late-night outdoor activities (Chukwuocha et al., 2012), presence of stagnant water and overgrown bushes around the dwelling place of the pregnant woman (Chukwuocha et al., 2012), rainy season (Efe & Ojoh, 2013; Sohail et al., 2015), non-use of mosquito spray (Agomo & Oyibo, 2013), etc.

In this study, participants from family size of 1–6 had more malaria infection than those who came from a family size of > 6, this association was statistically significant. This finding could be due to people who are members of family sizes that are not too large can manage little resources, feed better and are not so much at risk of infections (Agomo & Oyibo, 2013). A similar study (Abdalla et al., 2017) conducted in Ethiopia found having a family size of \geq 3 to be less likely at risk of malaria in pregnancy. This agrees with the findings of this current study and the findings of the studies carried out in Sudan (Abdalla et al., 2017) and India (Tilla et al., 2019) which observed that the prevalence of malaria increased as the number of members of the family increased.

Also, this study found that majority of pregnant women in skill level 1 tested positive to malaria, and there was a statistically significant link between positive malaria tests result and respondents' occupation. This finding could be linked to these pregnant women's residence and their work (farming, fishing, etc) environments which predisposes them to mosquito bites, as the majority of them work in open spaces and in environments with little or no environmental care. Also, a greater number of participants in this study were in skill level 1 and earned between 50,000 and 100,000 naira per month on the average. Low monthly family income can have a negative impact on the health of both the pregnant woman and her unborn child. Low average monthly family income can result to pregnant women not being able to afford a decent meal, going to a better hospital, purchasing mosquito nets and purchasing proper malaria medications. Majority of the people who fall under the skill level 1 category most times have little or no education. Pregnant women who have little or no formal education have been linked to high chances of developing malaria in previous studies (Chukwuocha et al., 2012; Bawa et al., 2014), carried out in the past in parts of Niger Delta area of Nigeria (Chukwuocha et al., 2012), in Katsina State (Bawa et al., 2014) and in Kebbi State (Fana et al., 2015). This could be because educated people are more exposed to proper knowledge and they have better understanding of malaria, its control and prevention.

From this study also, there was a statistically significant link between the study participants' current trimester and malaria test result. The high number of positive test result in first trimester seen in this study corresponds with earlier findings from studies conducted in Katsina (Bawa et al., 2014), Parts of the Niger Delta area of Nigeria (Chukwuocha et al., 2012), Rural Surroundings of Arbaminch Town, South Ethiopia (Nega et al., 2015), Rural South-West Nigeria (Adebayo et al., 2015). But, this finding does not agree with the results of the study conducted in Ebonyi State (Odikamnoro et al., 2014) where majority of the people in their second trimester tested positive and another study carried out in Benishangul Gumuz, Ethiopia (Gontie et al., 2020) found pregnant women who were in their second trimester of pregnancy to have increased odds of developing malaria infection when compared with women in their third trimester of pregnancy. The high number of positive test result in first trimester seen in this study could be due to the fact that these pregnant women, who already reside in a malaria endemic region of the country are in their early stage of ANC attendance and have not received any IPT. Health facilities rendering ANC services usually follow the World Health Organization's recommendation that pregnant women in areas of high malaria transmission in Africa should receive Intermittent Preventative Therapy (IPT) such as Sulfadoxine-Pyrimethamine (SP) in their second and third trimesters of pregnancy as part of their ANC services, for the prevention of malaria in pregnancy. IPTp-SP is given to pregnant women at least twice after quickening regardless of the woman's malaria status. Usually, it is given at the 16th, 28th, and 34th weeks of pregnancy during routinely scheduled antenatal clinic appointments (WHO, 2018).

In this present study, when compared with multigravidae, the secondigravidae were less likely to have a positive malaria test result, and the connection was statistically significant. This agrees with the findings of a similar study carried out in Ebonyi State of Nigeria (Odikamnoro et al., 2014), where the prevalence of malaria was

higher among the multigravidae. In other similar studies conducted in Ethopia (Gontie et al., 2020) and in Lagos Nigeria (Raimi & Kanu, 2010), the results of the study showed that women who were secondigravidae had increased odds of malaria infection when compared with the multigravidae. This could be due to the fact that individuals living in areas with high malaria transmission acquire immunity to malaria over time (Fried et al., 1998). Findings from this current study shows that pregnant women living in malaria endemic regions of Nigeria are continuously at risk of malaria and the negative outcomes of malaria obth the pregnant women and their unborn children. Malaria in pregnancy is associated with maternal anemia, spontaneous miscarriage, premature delivery, intrauterine development retardation, and the birth of low-birth-weight neonates which is a risk factor for stillbirth and neonatal death (World Bank, 2022; CDC, 2015).

5. Limitations of the study

The results of this study may not be a true reflection of what is going on in the whole Warri because it is a facility-based study and not community based. Some pregnant women who may have tested positive for malaria declined to participate in the study for various reasons, and critical information may have been overlooked as a result of their refusal.

6. Conclusion and recommendation

This study has revealed a high prevalence of malaria among pregnant women attending ANC at the Central Hospital Warri. The significant predictors of malaria include; respondents' occupation (skill level 1), family size (family size of 1-6) and current trimester (first trimester).

The high prevalence of malaria in pregnancy found in this study indicates that there is need for the Delta State Government to continuous ensure the implementation of malaria control and prevention activities in the state. Also, in stable malaria transmission areas such as Warri, The State Ministry of Health should ensure that all the healthcare facilities where pregnant women receive ANC implements the WHO's three evidence-based strategies for malaria prevention and treatment in pregnancy, that is; encouraging routine use of insecticide-treated nets, scaling up of intermittent preventive treatment of malaria in pregnancy with sulfadoxine-pyrimethamine at the second and third trimesters of pregnancy, and appropriate malaria case management through prompt and effective diagnosis and treatment of malaria in pregnant women.

References

- Abdalla, E. A. M., Abdalla, L. A. M., & Eltayeb, W. A. H. (2017). Prevalence and possible risk factors of malaria among pregnant women attending to antenatal care at Umtalha Health Centre in Gezira State Sudan. Journal of Pharmacy and Biological Sciences, 12(3), 67-72. DOI:10.9790/3008-1203056772
- Adebayo, A. M., Akinyemi, O. O., & Cadimus, E. O. (2015). Knowledge of Malaria Prevention among Pregnant Women and Female Caregivers of Under-five Children in Rural South-West Nigeria. A Peer Reviewed Open Access Journal, 792(3), 1-13. https://doi.org/10.7717/peerj.792
- Africa Health Organization (AHO). (2020). *Malaria Fact Sheet*. [Accessed on 12/04/2022]. Available at: https://aho.org/fact-sheets/malaria-fact-
- sheet/#:~:text=Africa%20continues%20to%20carry%20a,cases%20and%2075%25%20deaths%20globally
- Agomo, C. O., & Oyibo, W. O. (2013). Factors associated with risk of malaria infection among pregnant women in Lagos, Nigeria. Open Access Biomedical Central Journal of Infectious Diseases, 2(19), 1-8. http://www.idpjournal.com/content/2/1/19
- Bawa, J. A., Auta, T., & Liadi, S. (2014). Prevalence of Malaria: Knowledge, Attitude and Cultural Practices of Pregnant Women in Katsina Metropolis, Nigeria. *European Scientific Journal*, 10(21), 1857-7881. DOI:10.13140/RG.2.2.31856.00003. http://eujournal.org/index.php/esj/article/viewFile/3850/3666
- Centers for Disease Control and Prevention (CDC). (2015). Intermittent Preventive Treatment of Malaria for Pregnant Women (IPTp): Why Pregnant Women Are Especially at Risk. [Accessed on 1/08/2017]. Available at: https://www.cdc.gov/malaria/malaria_worldwide/reduction/iptp.html
- Centers for Disease Control and Prevention (CDC). (2020). *Malaria. About Malaria: Where Malaria Occur*. [Accessed on 12/04/2022]. Available at: https://www.cdc.gov/malaria/about/distribution.html

- Centers for Disease Control and Prevention (CDC). (2021). *About Malaria: Malaria's Impact Worldwide*. [Accessed on 12/04/2022] Available at: https://www.cdc.gov/malaria/malaria_worldwide/impact.html
- Central Hospital Warri. (2017). [Accessed on 1/09/2017]. Available at: http://ng.wowcity.com/warri/gpid/104495738567014652336/central-hospital-warri-emergen.htm.
- Chaponda, E. B., Chandramohan, D., Michelo, C., Mharakurwa, S., Chipeta, J., & Chico, M. (2015). High burden of malaria infection in pregnant women in a rural district of Zambia: a cross-sectional study. Malaria Journal, 14(380), 1-12. DOI 10.1186/s12936-015-0866-1
- Chotivanich, K., Silamut, K., & Day, N. P. J. (2007). Laboratory diagnosis of malaria infection A short review of methods. *New Zealand Journal of Medical Laboratory Science*, 61(1): 4-7. Corpus ID: 75532110. https://www.semanticscholar.org/paper/Laboratory-diagnosis-of-malaria-infection-A-short-Chotivanich-Silamut/a2b3e000bd4a0d30702bb2eeb72e2bc92891b5a7#citing-papers
- Chukwuocha, U. M., Dozie, I. N., & Chukwuocha, A. N. (2012). Malaria and its Burden among Pregnant Women in Parts of the Niger Delta area of Nigeria. *Asian Pacific Journal of Reproduction*, 1(2), 147-151. https://doi.org/10.1016/S2305-0500(13)60066-4
- Cisse, M., Sangare, I., Lougue, G., Bamba, S., Bayane, D., & Guiguemde, R. T. (2014). Prevalence and Risk Factors for Plasmodium falciparum Malaria in Pregnant Women Attending Antenatal Clinic in Bobo-Dioulasso (Burkina Faso). Open Access Biomedical Central Journal of Infectious Diseases, 14(631), 1-7. http://www.biomedcentral.com/1471-2334/14/631
- Cochran, W. G. (1977). Sampling Techniques, 3rd Edition, John Wiley & Sons, New York.
- Damisa, S., & Hassan, S. M. (2021). Prevalence of malaria among pregnant women attending antenatal care in general hospital Saminaka, Lere Local Government Area, Kaduna State. *Bayero Journal of Medical Laboratory Sciences*, 6(2), 22 29. https://www.ajol.info/index.php/bjmls/article/view/221954
- Efe, S. I., & Ojoh., C. O. (2013). Climate Variation and Malaria Prevalence in Warri Metropolis. *Journal of Atmospheric and Climate Sciences*, 3(1), 132-140. http://dx.doi.org/10.4236/acs.2013.31015
- Erhabor, O., Ok, O., Awah, I., Uko, K. E., & Charles, A. T. (2007). The prevalence of Plasmodia parasitaemia among donors in the Niger delta of Nigeria. *Tropical Doctor*, 37(1), 32-4. PMID: 17326885. DOI: 10.1258/004947507779951998
- Fana, S. A., Bunza, M. D. A., Anka, S. A., Imam, A. U., & Nataala, S. U. (2015). Prevalence and risk factors associated with malaria infection among pregnant women in a semi-urban community of north-western Nigeria. Infectious Diseases of Poverty, 4(24), 1-5. DOI 10.1186/s40249-015-0054-0
- Fried, M., Nosten, F., Brockman, A., Brabin, B. J., & Duffy, P. E. (1998). *Maternal antibodies block malaria*. 95, 851–852. Nature
- Global one 2015. (2012). *Maternal Health in Nigeria Statistical Overview*. [Accessed on 26/09/2017]. Available at: chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/http://www.globalone2015.org/wp-content/uploads/2011/11/Maternal-Health-in-Nigeria-Statistical-Overview-.pdf
- Gontie, G. B., Wolde, H. F., & Baraki, A.G. (2020). Prevalence and associated factors of malaria among pregnant women in Sherkole district, Benishangul Gumuz regional state, West Ethiopia. *BMC Infectious Diseases*, 20, 573. https://doi.org/10.1186/s12879-020-05289-9
- Gunn, J. K., Ehiri, J. E., Jacobs, E. T., Ernst, K. C., Pettygrove, S., Kohler, L. N., Haenchen, S. D., Obiefune, M. C., Ezeanolue, C. O., Ogidi, A. G., & Ezeanolue, E. E. (2015). Population-based prevalence of malaria among pregnant women in Enugu State, Nigeria: the Healthy Beginning Initiative. *Malaria Journal*, 2015; 11 (14): 438. https://doi.org/10.1186/s12936-015-0975-x
- Health Aid for All Initiative (HAFAI). (2020). *Maternal and Child Health*. [Accessed on 12/04/2022]. Available at: http://hafai.org/works/maternal-and-child-

health/#:~:text=According%20to%20a%20UNICEF%20report,mortality%20rate%20in%20the%20world International Labour Organization. (2012). International Standard Classification of Occupations: Structure, group definitions and correspondence tables. International Labour Office, 2(8), 11-18Bottom of Form. https://ilostat.ilo.org/resources/concepts-and-definitions/classification-occupation/

- Nega, D., Dana, D., Tefera, T., & Eshetu, T. (2015). Prevalence and Predictors of Asymptomatic Malaria Parasitemia among Pregnant Women in the Rural Surroundings of Arbaminch Town, South Ethiopia. *Peer Reviewed Open Access Scientific Journal*, 10(4), 1-11. DOI:10.1371/journal.pone.012363
- Odikamnoro, O., Iganga, A., Ozowara, N. L., & Okoh, N. (2014). Prevalence of malaria among pregnant mothers and possible relationship to parity in Abakaliki, Southeast Nigeria. *European Journal of Experimental Biology*, 4(4), 15-19. https://www.semanticscholar.org/paper/Prevalence-of-malaria-among-pregnant-mothers-and-to-Odikamnoro-Iganga/533d1ba9b42e8877c48ee287e4b735182730bb42
- Ohalete, C. N., Dozie I. N. S., Nwachukwu, M. I., & Obiukwu, C. E. (2011). Epidemiology and socio-economic consequences of malaria in pregnant women in Imo State Nigeria. *African Journal of Microbiology Research*, 5(23), 3895-3900. http://www.academicjournals.org/ajmr
- Okpere, E., Enabudoso, E. J., & Osemwenkha, A. P. (2010). Malaria in pregnancy. *Nigerian Medical Journal*, 51(3), 109-113. https://www.nigeriamedj.com/article.asp?issn=0300-1652;year=2010;volume=51;issue=3;spage=109;epage=113;aulast=Okpere

- Raimi, O. G., & Kanu, CP. (2010). The prevalence of malaria infection in pregnant women living in a suburb of Lagos, Nigeria. African Journal of Biochemistry Research, 4(10), 243-245. http://www.academicjournals.org/AJBR
- Schantz-Dunn, J., & Nawal, M. N. (2009). Malaria and Pregnancy: A Global Health Perspective. Journal of Obstetrics and Gynecology, 2(3), 186–192. PMID: 19826576. PMCID: PMC2760896. https://pubmed.ncbi.nlm.nih.gov/19826576/
- Sohail, M., Shakeel, S., Kumari, S., Bharti, A., Zahid, F., Anwar, S., Singh, K. P, Islam, A. K., Lata, S., Ali, V., Adak, T., Das, P., & Raziuddin, M. (2015). Prevalence of Malaria Infection and Risk Factors associated with Anaemia among Pregnant Women in Semiurban Community of Hazaribag, Jharkhand, India. *Biomedical Research International Journal*, 2015(740512), 1-17. http://dx.doi.org/10.1155/2015/740512
- Tilla, T., Sorsa, S., & Asnake, S. (2019). Prevalence of malaria and the associated risk factors among pregnant women attending antenatal care in health institutions of Damot Woyide district, Southern Ethiopia. Journal of Parasitology and Vector Biology, 11(2), 10-18.
- Traffina Foundation for Community Health. (2022). *The burden of Malaria amongst Pregnant Mothers and Under five in Nigeria*. [Accessed on 12/04/2022]. Available at: https://traffinafoundationfch.org/the-burden-of-malaria-amongst-pregnant-mothers-under-5-in-nigeria/
- United Nations Children's Fund (UNICEF). (2017). *Child Survival: Preventing Malaria*. [Accessed on 1/08/2017]. Available at: https://www.unicefusa.org/mission/survival/malaria
- United States Embassy in Nigeria. (2011). *Nigeria Malaria Fact Sheet*. [Accessed on 2/08/2017]. Available at: http://photos.state.gov/libraries/nigeria/231771/Public/December-MalariaFactSheet2.pdf
- Wagbatsoma, V. A., & Omoike, B. I. (2008). Prevalence and Prevention of Malaria in Pregnancy in Edo State, Nigeria. African Journal of Reproductive Health, 12(3), 49-58. https://pubmed.ncbi.nlm.nih.gov/19435012/
- Warri South. (2022). [Accessed on 1/09/2017]. Available at: https://en.wikipedia.org/wiki/Warri_South
- World Bank. (2022). *Infectious Diseases and Vaccines*. [Accessed on 07/08/2022]. Available at: https://www.worldbank.org/en/topic/infectiousdiseases
- World Health Organization (WHO). (2019). *Maternal Mortality: Key facts*. [Accessed on 1/04/2022]. Available at: http://www.who.int/mediacentre/factsheets/fs348/en/
- World Health Organization (WHO). (2018). *Malaria: Intermittent preventive treatment in pregnancy IPTp.* [Accessed 21/10/2018]. Available at: http://www.who.int/malaria/areas/preventive_therapiespregnancy?en?
- World Health Organization (WHO). (2022). *Fact Sheet: Malaria*. [Accessed on 12/04/2022]. Available at: https://www.who.int/news-room/fact-sheets/detail/malaria



Indonesian Christian Community's Perspective on Pharmacists and Medicine

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Abstract

The use of drugs and the responsibility of pharmacists in the treatment process for the Indonesian Christian community face challenges in understanding biblical health doctrines especially in the use of drugs and the provision of advice by pharmacists. The view that medicine as a product of science is contrary to faith and belief in God's care for the health of the human body is a theological distortion. the pharmacist's role is equated with relying on humans and not relying on God. This study assesses perspectives on medicine and pharmacists in the Indonesian Christian community. This research is a descriptive study using questionnaire. Respondents were randomly selected from all provinces in Indonesia following the proportion of the Christian population in the province. The validity and reliability of the questionnaire were tested using the composite reliability (CR) method and Cronbach's alpha with a significance level of 5%. The questionnaire was assessed using a Likert scale and the results were classified into very good, good, and poor. This study shows the community's perspective on medicine still needs to be improved (32.6% very good, 40.6% good, and 26.8% poor). For Indonesian Christian community, the hesitation to use medicine is caused by the teaching that faith and God's promises in the Bible are sufficient to heal and maintain human health. While the perspective of pharmacists in the treatment process shows better results (45.4% very good, 36.2% good, and 18.4% poor).

Keywords: Perspective on Medicine, Perspective on Pharmacists, Christian Community

1. Introduction

1.1 Introduce the Problem

Research conducted by the Lutheran General Hospital of Park Ridge, Illinois for many years states that there is a relationship between medicine and religion. The success of treatment is strongly influenced by the faith views of the patient and his family (Marty et al., 1983). Some doctrines of faith cause patients not to believe in medicine and all things related to health sciences. The teaching of faith will cause difficulties for medical and pharmaceutical personnel in carrying out their professional practice. Indonesia is one of the countries where Christians are a minority so that their religious life becomes more serious. Therefore, this research is very important to do to get

an idea of how big the obstacles are for Christian patients and families in receiving treatment and the role of pharmacists in pharmaceutical services.

1.2 Explore Importance of the Problem

Research on the perspective of the Christian community in Indonesia on health services and health workers related to the concept of religious theology has never been done. A Christian theological review of the biblical concepts of faith and medicine has been carried out by Jacquart (2011), Keenan (1996), and Lange (2017). The research that has been done has never observed the perspective of the community directly, so the authenticity of this research is the first time that observations have been made in the community. This research is very necessary, especially for pharmacists who practice in pharmacies and are in areas where the population is mostly Christian. Pharmacists can make a strategy of approaching the community by considering their theological views and can carry out their functions as educators to support the health of the local community.

After understanding the theological objections to using drugs, pharmacists can develop educational strategies without bringing up doctrinal issues. Likewise, pharmacists who understand the community's view of their roles and functions will be able to be more active in approaching and not waiting for people to look for them at the pharmacy.

1.3 Describe Relevant Scholarship

Pharmaceutical care aims to improve the patient's quality of life or change the sick state to be healthy by means of drug treatments. As stated by WHO, health is a condition of a complete physical, mental, and social well-being, and not limited to the absence of disease (Callahan, 1973). Health is not always related to medicine and medical treatment, but also requires the role of competent people in mental and social well-being. Religion and spiritual mentoring by religious leaders are an effort to improve mental and social well-being. In comparison to other countries, developing countries have a lower standard of life, more underdeveloped industries, and a low human development index. Accessible, qualified, and responsive human resources are widely agreed upon by policymakers, researchers, and practitioners as a significant driver of population health (Huber et al., 2011).

1.4 Hypotheses and Research Design

The Indonesian government has created a public education program about drugs and the role of pharmacists in treatment called the GeMa CerMat program or the smart community movement using drugs since 2015 and has been implemented in an intensive education program for the community to the smallest regional level (Komala et al., 2019 and Mursiti et al., 2020).

Therefore, the hypothesis in this study is that the perception of the Indonesian Christian community towards drugs and the role of pharmacists is very good.

This research is a descriptive study. The measuring instrument used is a questionnaire with a Likert scale.

Variable		Indicator	Code
Perspective medicine	on	 Medicine is the result of science and is not taught in the Bible Christians should believe that only by faith (without any medicine) can they 	M1
		be healed	M2
		3. When a Christian is sick and takes the medicine, he doesn't believe that God can heal him	M3
		4. By Jesus wounds you have been healed (1 Peter 2:24b). That's why the Lord Jesus has guaranteed His children to stay healthy and don't need medicine	M4
		anymore5. The Lord Jesus during his ministry of healing the sick and even raising the dead never used medicine, therefore Christians should not use medicine either	

Table 1 : Design Research Variables and Parameters

				M5
Perspective pharmacist	on	1.	Cursed is the one who trusts in man, who draws strength from mere flesh and whose heart turns away from the Lord (Jeremiah 17:5), therefore Christians should not need a pharmacist in their treatment	Р1
		2. 3.	Pharmacists do pharmaceutical work on an intellectual basis and rely on knowledge. This is against the teachings of the Bible Pharmacists in pharmaceutical services to patients rely on drugs of good quality and clinically tested	Р2
		4.	Pharmaceutical services provided by pharmacists are intended for non-Christians	P3
		5.	I trust the advice given by the pastor at church more than the advice given by the pharmacist at the pharmacy	P4
				P5

2. Method

2.1 Subjects

The population in this study was all Indonesian Christian communities spread across 34 provinces in Indonesia. Samples were taken from each province depend on the number of Christians in the province. The more Christians there are, the more samples are taken. This research ensures that there is data from every province in Indonesia so that it can represent the population of the Indonesian Christian community.

The sample obtained at random is the result of a sampling process carried out through several stages.

2.1.1 Process at the ministry of religion in each province

Each province in Indonesia has a ministry of religion that has church data and the number of members from each church. This study obtained data on churches and the number of Christian residents in the province from the provincial ministry of religion.

2.1.2 Data collection through the church.

After getting the church data and contact persons from the church, the next step is to collect questionnaire data from the congregation who attended after the service was over.

2.1.3 Questionnaire selection process

All questionnaires collected were selected and discarded incomplete questionnaires. After that the questionnaires from each province that have been selected, are taken with the required number at random sampling.

Island	Province	Sample Data		
Sumatera	1. Nanggroe Aceh Darussalam	5		
	2. North Sumatera	96		
	3. South Sumatera	5		
	4. West Sumatera	5		
	5. Bengkulu	5		
	6. Riau	17		
	7. Riau Islands	6		
	8. Jambi	5		
	9. Lampung	5		
	 Riau Islands Jambi Lampung 	6 5 5		

Table 2 : Number of Data Each Province
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	10. Bangka Belitung	5
Kalimantan	11. West Kalimantan	17
	12. East Kalimantan	5
	13. Central Kalimantan	5
	14. South Kalimantan	5
	15. North Kalimantan	7
Java	16. Special Capital District of Jakarta	25
	17. Banten	5
	18. West Java	27
	19. Central Java	20
	20. East Java	22
	21. Yogyakarta	5
Bali and Nusa Tenggara	22. Bali	5
	23. West Nusa Tenggara	5
	24. East Nusa Tenggara	55
Sulawesi	25. Gorontalo	5
	26. West Sulawesi	6
	27. Central Sulawesi	15
	28. North Sulawesi	49
	29. Southeast Sulawesi	5
	30. South Sulawesi	5
Maluku and Papua	31. North Maluku	9
	32. Maluku	22
	33. West Papua	14
	34. Papua	64

2.2 Measurement Questionnaire

Each questionnaire statement was given a score of 1 to 5, so for each perspective variable the score range is 5 to 25. All the questionnaire statements are negative statements, therefore the highest score on the answer strongly disagrees, while the lowest score on the answer strongly agrees. The scoring classification is divided into very good, good, and poor. The score is classified to be very good if it gets score 18-25, good if gets score 11-17, and poor if gets score 5-10.

2.3 Analysis Methods

Convergent validity testing with a significance level of 0.05. The questionnaire is considered valid if the convergent validity test of the loading factor value is equal to or more than 0.7 and the AVE value is greater than 0.5. The reliability test uses two methods, Cronbach's alpha and composite reliability. Cronbach's alpha measures the lower limit of the reliability value of a construct, while composite reliability measures the actual value of the reliability of a construct. However, composite reliability is considered better in estimating the internal consistency of a construct. The rule of thumb used for Composite Reliability values is greater than 0.7 and Cronbach's alpha value is greater than 0.7.

2.4 Ethical Clearance

This study was designed and conducted following the Declaration of Helsinki. This study's aim and the measurement details were explained to the subjects orally and in writing in advance, and measurement was conducted after their written consent was obtained. The measurement was started after obtaining the consent of the member in charge of the ethical review at the Research Ethics Commission, Directorate of Research, Gadjah Mada University.

3. Results

Convergent validity relates to the principle that the manifest variables of a construct should be highly correlated.

Table 5. Validity and Renability Analysis						
Variable		Indicator	Load Factor	Composite Reliability	Cronbach's alpha	AVE
		(Statement)				
Perspective	on	M1	0.798	0.811	0.878	0.631
medicine		M2	0.935			
		M3	0.865			
		M4	0.719			
		M5	0.832			
Perspective	on	P1	0.987	0.908	0.826	0.766
pharmacist		P2	0.769			
		P3	0.841			
		P4	0.865			
		P5	0.894			

Table 3 : Validity and Reliability Analysis

Convergent validity was assessed based on the loading factor value between 0.719 until 0,987 and the AVE value 0.631 and 0.766. Meanwhile, reliability is assessed based on the Composite Reliability (CR) value 0.811 and 0.908 and Cronbach's alpha values 0.878 and 0.826. From these results it is concluded that all the questions used meet the validity and reliability requirements.

Table 4 : Normality Test				
Unstandarized residual				
Ν	531			
Kolmogorov-Smirnov Z Asymp. Sig. (2-tailed)	.939 .307			

The normality test aims to find out whether the research variables have a normal distribution or not. This study uses the Kolmogorov-Smirnov Test with = 5% if p > 0.05 then the data distribution is declared to meet the assumption of normality, otherwise if p < 0.05 it is interpreted as abnormal. The test value of p = 0.939 so that it meets the requirements in the data normality test.

The following results are a recapitulation of the entire sample who filled out the perspective questionnaire on medicine. Classification of percentages according to the method described previously.

Questionnaire Statement			Good	Poor
1.	Medicine is the result of science and is not taught in the Bible	21%	39%	40%
2.	Christians should believe that only by faith (without any medicine) can they			
	be healed	28%	45%	27%
3.	When a Christian is sick and takes the medicine, he doesn't believe that God			
	can heal him	47%	32%	21%
4.	By Jesus wounds you have been healed (1 Peter 2:24b). That's why the Lord			
	Jesus has guaranteed His children to stay healthy and don't need medicine anymore	32%	49%	19%
5.	The Lord Jesus during his ministry of healing the sick and even raising the			
	dead never used medicine, therefore Christians should not use medicine either	35%	38%	27%

Average	32.6%	40.6%	26.8%

The following results are a recapitulation of the entire sample who filled out the perspective questionnaire on pharmacist. Classification of percentages according to the method described previously.

Questionnaire Statement	Very Good	Good	Poor
1. Cursed is the one who trusts in man, who draws strength from mere flesh and whose heart turns away from the Lord (Jeremiah 17:5), therefore	43%	39%	18%
 Christians should not need a pharmacist in their treatment Pharmacists do pharmaceutical work on an intellectual basis and rely on 			
knowledge. This is against the teachings of the BiblePharmacists in pharmaceutical services to patients rely on drugs of good	52%	41%	7%
quality and clinically tested	(20/	220/	1.50/
4. Pharmaceutical services provided by pharmacists are intended for non- Christians	62%	23%	15%
5. I trust the advice given by the pastor at church more than the advice given by the pharmacist at the pharmacy	42%	46%	12%
	28%	32%	40%
Average	45.4%	36.2%	18.4%

Table 6: Perspective Result of Pharmacist

4. Discussion

The results of this study indicate that the hypothesis made previously was not fulfilled. The percentage of participants who have a very good perspective on drugs and pharmacists is not dominant. There are still many participants who have a pretty good perspective, even a few who have a less good perspective.

4.1 Community Perspective on Medicine

Questionnaire statements about the perspective on medicine consist of 5 negative statements. The results of this study indicate that most are still hesitant to determine their attitude when given the conflict between medicine as a result of science and faith and the Bible. Most of the respondents' answers are in the good group (40.6%) showing indecision in taking a stance because there are still 26.8% participants who have the wrong perspective by considering medicine as the opposite of faith and the Bible, and only 32.6% can harmonize medicine as a means of faith and cannot be contradicted by the Bible. The best response was obtained from the third statement which shows the perspective of using medicine when sick does not mean not believing in God. The worst response was obtained from the first statement which shows the view of medicine as a product of science only and is mentioned in the Bible. The teaching of practical theology in the field of health and medicine in the church is found to be lacking so that the results of this study indicate the doubts of the Indonesian Christian community regarding the position of medicine in the context of theology, especially with regard to faith and the Bible.

This misperception about medicine is the result of distortion as described by Jacquart (2011), Keenan (1996), and Lange (2017). Medicine is a product of science created by humans so that it is contrary to the faith which references that by the stripes of Jesus (1 Peter 2:24b), healing must occur. The theological view of medicine as a product of God's creation through the human mind so that it becomes a means for God to heal His people is not conveyed completely and correctly by the church, causing doubts in the Christian community in Indonesia.

4.2 Community Perspective on Pharmacist

The assessment of the perspective of the Indonesian Christian community on pharmacist shows better results than the perspective on medicine. Most of the respondents gave a very good response (45.4%) and only 18.4% gave a poor response.

The best response resulted from 3rd statement which stated the duties and responsibilities of pharmacists in providing safe and quality drugs to patients in order to meet the expected therapeutic goals. This community has understood the role of pharmacist in the health system in the field of pharmaceutical services. The worst response was obtained from 5th statement where health advice from a pharmacist was considered less than advice from a church pastor. The responsibility to provide pharmaceutical services including counseling and assistance in taking medication is the responsibility of the pharmacist and this responsibility cannot be replaced by other professions. Meanwhile, the pastor in the church is responsible for health and spiritual growth in a different field from physical health.

Pharmacist and pastor cannot be compared which one is more important and which one is less important, because both are equally important and responsible for physical and spiritual health. Pharmacy services from a pharmacist are absolutely necessary in the treatment of patients. While the pastoral care of a pastor is absolutely necessary for the growth of the church.

4.3 Limitations and Future Prospects

This study does not separate participants based on the classification of church denominations because it is still an preliminary study that takes the entire population of Christians in Indonesia. Thus further research can separate the denominations by taking the scope of the regional area so that the total population per denomination can meet good research qualifications.

The results of this study suggest that the government cooperates with local church leaders in providing education about medicine and the role of health workers in improving the health quality of Indonesian people.

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References

- Baird, A. (2021). A Faithful Man Suffering in Illness: Religious and Medical Perspectives on the Book of Job [Thesis]. https://baylor-ir.tdl.org/handle/2104/11299
- Bassett, R. L., Camplin, W., Humphrey, D., Dorr, C., Biggs, S., Distaffen, R., Doxtator, I., Flaherty, M., Jo Hunsberger, P., Poage, R., & Thompson, H. (1991). Measuring Christian Maturity: A Comparison of Several Scales. *Journal of Psychology and Theology*, 19(1), 84–93. https://doi.org/10.1177/009164719101900108
- Callahan, D. (1973). The WHO Definition of "Health." The Hastings Center Studies, 1(3), 77-87. https://doi.org/10.2307/3527467
- Conradie, E. M. (2006). Healing in Soteriological Perspective. *Religion and Theology*, 13(1), 3–22. https://doi.org/10.1163/102308012X13397496507180
- Fanning, D. (2009). Hamartiology. Bible Doctrines. https://digitalcommons.liberty.edu/cgm_bib_doc/6
- Feiler, T. (2018). Understanding responsibilization in healthcare: Differentiations in Reformation theology. *Theology*, *121*(2), 95–102. https://doi.org/10.1177/0040571X17740525
- Huber, M., Knottnerus, J. A., Green, L., Horst, H. van der, Jadad, A. R., Kromhout, D., Leonard, B., Lorig, K., Loureiro, M. I., Meer, J. W. M. van der, Schnabel, P., Smith, R., Weel, C. van, & Smid, H. (2011). How should we define health? *BMJ*, *343*, d4163. https://doi.org/10.1136/bmj.d4163
- Jacquart, D. (2011). *Medicine And Theology* (pp. 213–226). Brill. https://brill.com/view/book/edcoll/9789004192164/Bej.9789004192157.i-352_009.xml
- Kaiser, W. C. (2001). The Uses of the Old Testament in the New. Wipf and Stock Publishers.

- Keenan, J. F. (1996). Dualism in medicine, Christian theology, and the aging. *Journal of Religion and Health*, 35(1), 33–45. https://doi.org/10.1007/BF02354943
- Komala, L., Komariah, K., Novianti, E., Subekti, P., & Lukman, S. (2019). Utilizing social media for socialization of gema cermat program by public health office of west java indonesia. *Journal of Physics: Conference Series*, 1175, 012233. https://doi.org/10.1088/1742-6596/1175/1/012233
- Lange, F. de. (2017). *Public Theology and Health Care* (pp. 325–346). Brill. https://doi.org/10.1163/9789004336063_016
- Leman, A. B., Nadaweo, Y., & Montero, M. (2021). Analysis of the 100 Years of Existence of the Pentecostal Church in Indonesia (GPdI). *Jurnal Teologi & Pelayanan Kerusso*, 6(2), 1–19. https://doi.org/10.33856/kerusso.v6i2.197
- Manson, A. (2005). A Theology of Illness: Franz Kafka's "A Country Doctor." *Literature and Medicine*, 24(2), 297–314. https://doi.org/10.1353/lm.2006.0010
- Marty, M. E., Vaux, K. L., & Hiltner, S. (1983). Health/Medicine and the Faith Traditions: An Inquiry into Religion and Medicine. *Theology Today*, 40(2), 197–200. https://doi.org/10.1177/004057368304000212
- Moss, C. R. (2011). Heavenly Healing: Eschatological Cleansing and the Resurrection of the Dead in the Early Church. *Journal of the American Academy of Religion*, 79(4), 991–1017. https://doi.org/10.1093/jaarel/lfr003
- Mursiti, H., Embri, G. M., Prasanti, A., Maysha, A., Rosvita, V., Bashori, Y. M., & Farida, Y. (2020). Optimizing the Wise Use of Medicine in the Family with the Gema Cermat Program. *Pharmacon: Jurnal Farmasi Indonesia*, 0, 21–28. https://doi.org/10.23917/pharmacon.v0i0.10106
- Tambunan, D. (2021). The Church, the Poor, and Pastoral Ministry. *Character Building*. https://binus.ac.id/character-building/2021/02/gereja-orang-miskin-dan-pelayanan-pastoral/
- Thomas, J. (2005). Healing in the Atonement: A Johannine Perspective. *Journal of Pentecostal Theology*, *14*(1), 23–39. https://doi.org/10.1177/0966736905056537
- Thomas, J. C. (1998). *The Devil, Disease and Deliverance: Origins of Illness in New Testament Thought*. A&C Black.



Antibacterial Effects of Various Concentrations of Natural Ingredients Snail Mucus (*Achatina fulica*) Against Inhibition Zones of *Fusobacterium nucleatum* Causes Periodontitis In Vitro

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Abstract

Periodontitis is a microorganism that invades the subgingival area, thereby triggering an inflammatory response of the periodontal tissue. *Fusobacterium nucleatum* is one of the most abundant gram-negative bacilli colonizing the subgingival plaque and closely associated with periodontal disease. The aim of study was to calculate the inhibition of snail mucus against *Fusobacterium nucleatum* bacteria with concentrations of 12.5%, 25%, 50%, and 100%. This study used a laboratory experimental test method and was conducted at the Oral Biology Laboratory, Faculty of Dentistry, Airlangga University. The steps were performed are: plant germ stock with sterile osse on BHI broth media then incubate for 48 hours, observe the turbidity of the germs then standardize with mc farland standard 0.5, plant germs on Hilton Muller agar media with the spreading technique, treat the test sample on sterile paper disk as much as 0, 01 ml with a sterile micropipette, then paste it on the surface of the media so that it is then incubated for 48 hours. Then, observe and measure the diameter of the clear zone. The results show that the average inhibition in the control group is 25.65 mm, and the snail mucus group is 12.5%, 0 mm, 25%, 12.40 mm, 50%, 16.70 mm and 100% 19.60 mm. There is a significant difference in the diameter of the inhibitory power between the treatment groups. The conclusion of the antibacterial effect of natural ingredients of snail mucus from the 25% treatment showed strong criteria, and the greatest inhibitory power was the concentration of 100%.

Keywords: Antibacterial, Snail Slime, Fusobacterium Nucleatum

1. Introduction

Periodontitis is a disease that attacks the supporting tissues of the teeth characterized by chronic inflammation, caused by bacteria present in dental plaque. This disorder begins with the accumulation of plaque containing pathogenic bacteria and toxins. The interaction of plaque and bacteria causes ulcers in the four periodontal tissues (gingiva, periodontal membrane, cementum, and alveolar bone) (Evan Wijaksana, 2016). Figure 1 shows the condition of teeth diagnosed having periodontitis.



Figure 1: Teeth with periodontitis diagnosis

The use of natural ingredients as medicinal ingredients by the people of Indonesia has been used for tens or even hundreds of years ago, especially in rural communities. Natural ingredients are often used because people believe they can cure several diseases, such as snail slime which is used for wound healing by farmers. A research says that the use of snail mucus can reduce pain in dental caries, where the material can eliminate tooth pain (Swastini, 2011). The healing of gingivitis as well as periodontitis has been carried out, with the result that in gingivitis it can reduce the grade of gingivitis, and in healing periodontitis with parameters of levels MDA, VEGF, TGF beta and the number of fibroblasts, proved to be curative, and this is still being done in mice (Swastini, 2019). Snail mucus is known to contain several substances that are very useful for healing diseases, including the protein achasin. This protein has antibacterial activity that works by inhibiting the formation of common parts of bacterial strains such as the peptidoglycan layer and cytoplasmic membrane. The work activity of achasin against Gram-positive bacteria is by attacking the cytoplasmic membrane and causing the cell wall to peel and sink into the cytoplasm (Berniyanti & Suwarno, 2010). The absolute requirement for the growth of this bacterium is iron. It is a Gram-negative bacterium in the form of an obligatory anaerobic, non-motile, asaccharolytic stem, which forms pigmented black colonies on a blood agar plate (How et al., 2016). Figure 2 shows a step how to collect the snail slime.



Figure 2: The snail and how to collect snail slime

Fusobacterium nucleatum is an obligate, gram-negative, rod-shaped anaerobic bacterium and included in the bacterioidaceae family. *Fusobacterium nucleatum* bacteria are frequently found in dental disease, especially with periodontitis disorders, and which produce tissue irritants such as butyric acid, proteases and cytokines (Scannapieco & Dongari-Bagtzoglou, 2021). Supragingival plaque on the surface of a tooth has about 109 bacterial cells attached to it. In a healthy periodontal pocket, there are 103 bacterial cells while in a deep periodontal pocket there are about 108 bacterial cells. Biomolecular identification found about 500 types of microbes found in dental plaque (Newman et al., 2012). Dental plaque, which initiates calculus, is a complex bacterial ecosystem. Some of the bacteria that play a role in periodontal disease are gram-negative bacteria such as *Phorpyromonas gingivalis*, *Fusobacterium nucleatum, Aggregatobacter actinomycetemcomitans*, and gram-positive bacteria such as *Lactobacillus*, *Streptococcus*, *Actinomyces israelli* (Perry & Beemsterboer, 2007). *Fusobacterium* in supragingival and subgingival plaque although in small numbers. *Fusobacterium nucleatum* is one of the most abundant gram-negative bacilli colonizing the subgingival plaque and closely associated with periodontal disease. To culture these bacteria can be done on blood agar media under anaerobic conditions with colonies that are not bright, granular in shape, rhizoid edges, irregular in shape. *Fusobacterium* can remove sulfur from cysteine and methionine to produce hydrogen sulfide and methylmercaptan odors associated with halitosis (Samaranayake, 2012).

The purpose of this study was to determine the effectiveness of the antibacterial snail slime with concentrations of 12.5%, 25%, 50% and 100%, against the bacteria *Fusobacterium nucleatum*. Figure 3 shows the bacteria of *Fusobacterium nucleatum*.



Figure 3: Fusobacterium nucleatum

2. Method

This research is purely a laboratory research with a post test control group design, and was conducted in the oral biology laboratory of the Faculty of Dentistry, Airlangga University, Surabaya, in June 2021.

2.1 Snail slime collection

Snail slime was collected from a community plantation in Nyalian village, Banjarangkan, Klungkung, Bali. The weight of the snails used is in the range of 200-250 grams, then the mucus is taken using the tip of a needle to be scratched on the flesh of the snail, then the mucus is collected into a sterile bottle, followed by centrifugation for 30 minutes.

2.2 Planting the suspension of Fusobacterium nucleatum

Planting the stock of germs using sterile osse on BHI broth media then incubation for 48 hours, the turbidity of the germs was observed then standardized with the standard mc farland 0.5, then planted the germs on the Hilton agar muller media with the spreading technique, for the next treatment the test sample on paperdisk sterile 0.01 ml with a sterile micropipette, then paste it on the surface of the agar medium, then incubate for 48 hours, observe and measure the diameter of the clear zone. Bacteria stock taken from ATCC 25586 PK/5.

3. Results

In the present study, the results of the research on the inhibitory capability of mucous slime on the growth of Fusobacterium nucleatum bacteria growth can be seen in Figure 4, below:







Figure 4: Inhibitory zone of snail slime against Fusobacterium nucleatum

Subject Group	Ν	Mean ± Fusobacterium nucleatum Inhibition Zone (millimeters)	Total bacteria (CFU/m l) (No)	р
Control	4	25.65±1,04	0.5 Mc Farland	0.001*
Snail slime 12.5%	4	0 ± 0.00		
Snail slime 25%	4	12.40 ± 0.2		
Snail slime 50%	4	16.70 ± 0.73		
Snail slime 100%	4	19.60 ± 0.78		

Table 1: The width of Fusobacterium nucleatum inhibito	ry zone in the treatment group
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Post Hoc Test; Significant at p<0.05

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Table 1 illustrates that the inhibitory power of the control group is higher than the treatment group with snail slime, where in the control group the inhibitory power is 25.65±1,04 while the 100% concentration of snail slime is 19.60± 0.78. Meanwhile table 2 shows the number of difference of inhibitory zone of Fusobacterium nucleatum between the treatment groups.

Variable	Group I	Group J	Mean difference (I-J)	Р
Snail Slime	Control	12.5%	25.20	1,00
		25%	12.40	< 0.001*
		50%	9.00	<0.001*
		100%	6.00	<0.001*
	12.5%	25%	-12.40	< 0.001*
		50%	-16.20	<0.001*
		100%	-19.20	<0.001*
		control	-25.20	<0.001*
	25%	50%	-3.80	<0.001*
		100%	-6.80	<0.001*
		control	-12.80	<0.001*
		12,5%	12.40	<0.001*
	50%	100%	-3.00	<0.001*
		control	-9.00	<0.001*
		12,5%	16.20	<0.001*
		25%	3.80	<0.001*
	100%	control	6.00	<0.001*
		12,5%	19.20	<0.001*
		25%	6.80	<0.001*
		50%	3.80	<0.001*

Table 2: The difference of inhibitory zone Fusobacterium nucleatum between the treatment groups

*Analysis with Post Hoc Test; Significant at p<0.05

4. Discussion

The results shown that the research on the inhibition of snail mucus against the growth of *Fusobacterium nucleatum* bacteria can be seen in Figure 4. While the average inhibition of snail mucus against *Fusobacterium nucleatum* bacteria in this study was tested using the One Way Anova test. The results are shown in table 1. Zone diameter inhibition of snail mucus against *Fusobacterium* nucleatum bacteria has the highest concentration at 100% with an average diameter of 19.60 mm.

The results of the research that has been carried out showed that the diameter of the inhibition zone was the highest in snail slime with a concentration of 100% with an average of 19.60 mm in the strong category. According to Davis and Stout (1971) the diameter of the clear zone between 0 ± 10 mm has moderate inhibition, 11-20 mm is in the strong category, above 20 mm is very strong (Davis & Stout, 1971).

One of the soft animal species (mollusca) is snail (*Achatina fulica*). This animal was very much used by ancient farmers because the healing properties of wounds are very good. Snails produce mucus which has many health benefits. Snail mucus is produced in the body wall of the snail and is a lymph substance. Snail mucus that flows in the snail's body has the activity of eradicating bacteria and foreign objects. The components of snail mucus include analgesic, anti-septic, and antimicrobial peptides (Achasin). Achasin works by attacking or inhibiting the formation of common parts of bacterial strains such as: the peptidoglycan layer and the cytoplasmic membrane (Carranza et al., 2012).

Achasin is a protein contained in snail mucus, is a protein that has important biological functions other than those intended to prevent evaporation, assisting smooth movement is also needed to protect the body from mechanical injuries (Merglova et al., 2014). Bacteria that cause periodontitis are generally gram-negative bacterial species that colonize sub-gingival plaque, including *Porphyromynas gingivalis, Prevotella intermedia, Actinobacillus (agregibacter) actinomycetemcomitans* and *Fusobacterium nucleatum* (Merglova et al., 2014). One of the most dominant bacteria in periodontal disease is *Fusobacterium nucleatum* compared to other gram-negative bacteria. Fusobacterium nucleatum has a percentage of 55% while in Peptostreptococcus micros is 3%, Tannerella forsythia 4% and in Porphyromynas gingivalis 1% (Park et al., 2016).

Fusobacterium nucleatum is a gram-negative anaerobic bacteria that has a role in bridging the early and late colonies during plaque formation (Manson, 2013). These bacteria appear in high numbers 2 after 24 hours and can multiply for 48 hours in dental plaque. An increase in the number of *Fusobacterium nucleatum* can cause gingival inflammation, pocket deepening and periodontal tissue damage (Liu et al., 2014). *Fusobacterium nucleatum* is one of the most abundant gram-negative bacilli colonizing the subgingival plaque and closely associated with periodontal disease. This bacterium is often found in chronic gingivitis and chronic periodontitis because it plays a role in killing the normal proliferation of fibroblasts in the periodontal tissue. F. nucleatum may be an important contributor to periodontal disease either directly or by serving as a mediator of plaque colonization for other virulent anaerobes (Han et al., 2000).

The results of the data obtained were tested for normality and homogeneity as a condition for conducting the One Way ANOVA test, the Shapiro-Wilk test (p>0.05) which showed that all groups are normally distributed, and the Lavene test of all variances are homogeneous. The results of the One Way ANOVA test showed that the p-value <0.05 means that there are significant differences in the antibacterial power of various concentrations of snail mucus against *Fusobacterium nucleatum* bacteria in vitro. The difference in the results of the One Way ANOVA test was then carried out with the Post Hoc Least Significant Different (LSD) test to find out the significant differences between the treatment groups. The results showed that there were differences in the clear zone around the well which was dripped with snail mucus with various concentrations. All concentrations of snail mucus used showed significant differences in inhibition.

Antibacterial Effects of Various Concentrations of Natural Ingredients Snail Mucus (Achatina fulica) Against the Increase in the Number of Bacterial Inhibitions of Fusobacterium nucleatum that Cause Periodontitis In Vitro starting from a concentration of 25% indicating a strong diameter of inhibition zone and the highest inhibition is with a concentration of 100% snail mucus. The average inhibition in the control group was 25.65 mm, and the snail mucus group was 12.5%, 0 mm, 25%, 12.40 mm, 50%, 16.70 mm and 100% 19.60 mm. There is a significant difference in the diameter of the inhibitory power between the treatment groups. The conclusion of the antibacterial effect of natural ingredients of snail mucus from the 25% treatment showed strong criteria, and the greatest inhibitory power is the concentration of 100%.

References

- Berniyanti, T., & Suwarno. (2010). Characteristics of Local Isolated Snail Slime Protein (Achasin) as Anti-Bacterial. *Factor: Veterinary Media*, 23(3), 139–144.
- Carranza, F. A., Takei, H. H., & Newman, M. G. (2012). Clinical Periodontology (11th ed.). Saunders-Elsevier.
- Davis, W. W., & Stout, T. R. (1971). Disc plate method of microbiological antibiotic assay. II. Novel procedure offering improved accuracy. *Applied Microbiology*, 22(4), 666–670. https://doi.org/10.1128/aem.22.4.666-670.1971
- Evan Wijaksana, I. K. (2016). Infectobesity Dan Periodontitis: Hubungan Dua Arah Obesitas Dan Penyakit Periodontal [Bidirectional Relationship of Obesity and Periodontal Disease]. *ODONTO : Dental Journal*, 3(1), 67. https://doi.org/10.30659/odj.3.1.67-73
- Han, Y. W., Shi, W., Huang, G. T. J., Kinder Haake, S., Park, N. H., Kuramitsu, H., & Genco, R. J. (2000). Interactions between periodontal bacteria and human oral epithelial cells: Fusobacterium nucleatum adheres to and invades epithelial cells. *Infection and Immunity*, 68(6), 3140–3146. https://doi.org/10.1128/IAI.68.6.3140-3146.2000
- How, K. Y., Song, K. P., & Chan, K. G. (2016). Porphyromonas gingivalis: An overview of periodontopathic pathogen below the gum line. *Frontiers in Microbiology*, 7(FEB), 1–14. https://doi.org/10.3389/fmicb.2016.00053
- Liu, P., Liu, Y., Wang, J., Guo, Y., Zhang, Y., & Xiao, S. (2014). Detection of fusobacterium nucleatum and fada adhesin gene in patients with orthodontic gingivitis and non-orthodontic periodontal inflammation. *PLoS* ONE, 9(1), 3–7. https://doi.org/10.1371/journal.pone.0085280
- Manson, J. D. (2013). Buku ajar periodonti. Hipokrates.
- Merglova, V., Koberova-Ivancakova, R., Broukal, Z., & Dort, J. (2014). The presence of cariogenic and periodontal pathogens in the oral cavity of one-year-old infants delivered pre-term with very low birthweights: A case control study. *BMC Oral Health*, *14*(1), 1–8. https://doi.org/10.1186/1472-6831-14-109
- Newman, M. G., Carranza, F. A., Bulkasez, J., Quirynen, M., Teughels, W., & Haake, S. K. (2012). *Microbiology* of *Periodontal Disease in Carranza's Clinical Periodontology* (10th ed.). Saunders Elseviers.
- Park, J., Shokeen, B., Haake, S. K., & Lux, R. (2016). Characterization of Fusobacterium nucleatum ATCC 23726 adhesins involved in strain-specific attachment to Porphyromonas gingivalis. *International Journal of Oral Science*, 8(3), 138–144. https://doi.org/10.1038/ijos.2016.27
- Perry, D. A., & Beemsterboer, P. L. (2007). *Periodontology for the dental hygienist* (3rd ed.). Saunders-Elsevier. Samaranayake, L. (2012). *Essential microbiology for Dentistry* (4th ed.). Elsevier.
- Scannapieco, F. A., & Dongari-Bagtzoglou, A. (2021). Dysbiosis revisited: Understanding the role of the oral microbiome in the pathogenesis of gingivitis and periodontitis: A critical assessment. *Journal of Periodontology*, 92(8), 1071–1078. https://doi.org/10.1002/JPER.21-0120
- Swastini, I. (2011). Topical Administration of Snail Mucus Cure Gingivitis Grade 3 Faster Due to Calculus than 10% Povidone Iodine. Udayana University.
- Swastini, I. (2019). Provision of snail mucus can result in lower levels of malondialdehyde, vascular endothelial growth factor, transforming growth factor -1 and a higher number of fibroblasts in wistar rats with periodontitis. Udayana University.



Dental and Oral Care Model for Inpatients to Prevent the Occurrence of Hospital Acquired Pneumonia (HAP) in the Elderly in the Geriatric Room of Mukomuko Hospital Bengkulu Province

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Abstract

The cause of Hospital Acquired Pneumonia (HAP) is bacteria from dental plaque, colonizing with other pulmonary pathogens. This condition builds rational pathogen respiration in the oral cavity resulting in high-risk pneumonia. The risk group for HAP is over 70 years old, who have serious co-morbidities, malnutrition, decreased consciousness, and chronic obstructive pulmonary disease. Prevention of nosocomial infections requires integrated and programmed actions to reduce the transmission of organisms. This study aims to develop a model of dental and oral health care for inpatients to prevent the occurrence of HAP in the elderly. This study was conducted in the geriatric ward of Mukomuko Hospital, Bengkulu Province, Indonesia. The product test used a quasiexperimental pretest and posttest design. The sample of this study was each 16 respondents in the intervention group receiving dental and oral care and the control group receiving treatment as per the dental health national standard Indonesia Kepmenkes No.284, 2006. The results show that dental and oral care is relevant to prevent HAP with an average value of 88.4 (feasible) (p-value 0.001, and its application is effective in increasing knowledge (p=0.000), attitude (0.000), increased brushing skills (p = 0.000), gargle skills (p-value 0.000), decreased debris index score (p = 0.000), decreased CPIS respondents with (p = 0.000) and sputum culture results (p = 0.000) compared to the control group. In conclusion, dental and oral health care can increase the skills of maintaining dental oral health, reduce the debris index and CPIS (Clinical Pulmonary Infection Score) for the elderly.

Keywords: CPIS, dental and Oral Health Care, Elderly, HAP

1. Introduction

Nosocomial infections occur worldwide, both in developing and developed countries. Sources from the World Health Organization (WHO) in 2002 mentioned the results of various studies showing that nosocomial infection
is the main cause of morbidity and mortality. It can also increase the severity of illness and emotional stress that reduces the quality of life of the patients. Prevention of nosocomial infections is very important because nosocomial infections can lead to a higher incidence of morbidity and mortality in Covid-19 patients(Warganegara, 2017; Ong et al., 2021). Hospital acquired pneumonia (HAP), also known as nosocomial pneumonia, is transmitted by patients in the hospital 48 – 72 hours after being admitted. HAP extends hospital stay for 7 - 9 days and requires higher treatment costs. The risk group for HAP is over 70 years of age who have serious co-morbidities, malnutrition, decreased consciousness, and chronic obstructive pulmonary disease (Riani & Syafriani, 2019). HAP has the type of bacteria *Streptococcus pneumoniae*, *H. aemophillus influenzae* and various types of bacteria in the mouth. HAP is caused by bacteria originating from dental plaque that colonizes with other pulmonary pathogens. Bacteria in periodontal tissues such as *Porphyromonos gingivalis* bacteria, *Fusobacterium nucleatum* can be associated with respiratory pathogenic bacteria such as *Pseudomonas aeruginosa*, *Klebsiella pneumonia*. Several studies have documented that the oral cavity is a reservoir for respiration of rational pathogens that cause pneumonia in high-risk patients (Sukarman et al., 2015).

The incidence in cases of Community Acquired Pneumonia (CAP) and HAP was 60.1%, 86.7% and Aspiration Pneumonia (AP) accounted for 66.8% of the total pneumonia inpatients. This number is closely related to patients aged 65 years as many as 22 ,1% in 2018 (Suzuki et al., 2021). Nonventilator Hospital Acquired Pneumonia (NV-HAP) risk is noticed higher when the patients stay increases. Different treatments of oral care for intervention and control groups were conducted in which the intervention unit received enhanced oral care, and the control unit received usual oral care. Both of the treatments resulting in the reduction of NV-HAP incidence rate, although the enhanced oral care received better numbers than the usual oral care. In the intervention unit, the reduction of NV-HAP is 85%, and in the control unit is 56% (Giuliano et al., 2021). Meanwhile, the oral care affects 25% of HAP reduction in the patients (Talley et al., 2016). However, the exact treatment to reach the desirable change comes to obstacle. Since a proper oral disease prevention and robust design could be the way to prevent elderly contracting HAP (Kanzigg & Hunt, 2016; Mitchell et al., 2019; Pássaro et al., 2016).

This study aims to develop a model of dental and oral health care for inpatients to prevent the occurrence of HAP in the elderly. The model of dental and oral health care for inpatients to prevent the occurrence of Hospital Acquired Pneumonia (HAP) in the elderly in the geriatric ward of the hospital is carried out by adjusting the characteristics of elderly patients divided into several stages, namely Stage 1, Stage II, Stage III.

2. Method

The results of preliminary interviews data with hospitals and dental and oral health workers, especially dental and oral therapists show that so far there has been no model of dental and oral health care for inpatients to prevent HAP in the elderly in the Geriatric Ward of Mukomuko Hospital, Bengkulu Province. Therefore, the dental and oral therapists carry out more activities in outpatients, especially dental clinics. This research is expected to be able to carry out dental and oral health care activities, that with HAP prevention efforts to reduce bacteria contained in the oral cavity, plaque and periodontal tissue as the cause of HAP.

2.1 Research Design

This research design uses Research and Development (R&D). The research and development procedure include 5 (five) main steps, including: 1) Information Collection. 2) Product or model design. 3) Expert validation and revision. 4) Test product or model. 5) Data collection stage of information processed descriptively and qualitatively, in the form of a table concluded from the results of interviews for the need for dental health learning methods and media for elderly patients in the geriatric ward of the hospital. The data of the expert validation test stage and revision was carried out with the Intraclass Correlation Coefficient statistical test to find out the feasibility of the product/model. The data were tested for Shapiro-Wilk normality. The normal data was tested using a paired t-test, and for data that were not normally distributed was tested using the Wilxocon test. The comparison between the intervention group and the control group was if the data were normally distributed using the independent t-test test, while not normal using the Mann Whitney test.

The independent variable in this study is the model of dental and oral health care for elderly. Meanwhile, the dependent variables are the knowledge, attitude, skills of the elderly in performing dental and oral hygiene, the Debris Index status, and HAP occurrence shown by CPIS (Clinical Pulmonary Infection Score).

The treatment of dental and oral care comprised 3 stages. Stage I was performed by building a trust with elderly, assessment and diagnose, as well as treatment plan. Stage II was the implementation of dental and oral care in a form of 3 days of intervention. Intervention at day 1 to 3 were tooth brushing and gargling with Povidone Iodine 1%. This implementation involved internist, pulmonary specialist, nurses and dental workers. Stage III is the evaluation of the activities by filling out the questionnaire, Debris Index and CPIS checks.

2.2 Sampling Procedures and Subject Characteristics

The population in this study is all the elderly inpatients in Mukomuko Hospital, Bengkulu Province. The sample was selected using purposive sampling technique and divided into two groups, namely 16 samples for the intervention group and 16 samples for the control group. The calculation of the minimum sample adopted Lemeshow & Sastroasmoro as shown in equation (1) (Lemeshow et al., 1997).

$$n1 = n2 = \frac{2 \left(z \propto + z\beta \right)^2 s^2}{(X1 - X2)^2} \tag{1}$$

Where

N: sample in each group $z \propto$: error level type I = 5%, so $z \propto$ is 1.96 (95% power) $z\beta$: error level type II = 5%, so $z\beta$ is 1.96 (95% power) S: deviation standard x1: average value from control group effect according to previous study x2: average value from intervention group effect according to previous study

The previous study resulted in S value of 1.165, mean outcome of control group 9.07 and intervention group 10.93. Therefore, the calculation of minimum sample is as equation (2).

$$n1 = n2 = \frac{2 (1.96 + 1.96)^2 s^2}{(9.07 - 10.93)^2}$$

$$n1 = n2 = 12.05 = 13$$
(2)

So, the subject is minimum at 13. However, to anticipate drop out of 15%, the calculation was corrected to equation (3) and (4).

$$n = \frac{n}{(1-f)}$$
(3)
Where *n* is sample size, and *f* is drop out estimation.

$$n = \frac{n}{(1-0.15)} = 15.29$$
(4)

So, the sample size is 16 respondents for each group or 32 respondents for 2 groups.

The inclusion criteria of the subject are those who consented to be respondents by filling out the agreement letter. The age of respondent is 55 years old and above. Besides, the criteria are elderly who still have teeth or do not as a user of dentures, and has no defect in the body and psychology. Meanwhile the exclusion criteria are the elderly who have no inclusion criteria and a healthy elderly.

3. Results

The characteristics of the elderly are that they have varying ages. It is expected that the elderly who will carry out dental and oral health care in the inpatient geriatric ward are the elderly who are cooperative and willing to answer so that they get optimal results, the elderly are generally announced when doing most activities they need the help of other people and the elderly In general, it is difficult to change the mindset that has been ingrained for a long time so that it is difficult to provide new education. Dental and oral health problems in the elderly, especially dental caries, periodontitis, missing teeth so that the elderly mostly wear dentures. There is no policy from the

government, especially in Mukomuko Regency regarding the maintenance of dental and oral health, especially regarding dental and oral health care for the elderly in inpatient and outpatient wards. The role model method with education about dental and oral health by imitating and practicing directly can add insight and change behavior in maintaining dental and oral health. Elderly patients really need a new learning model on how to maintain oral hygiene. Such as dental and oral health care to prevent the occurrence of diseases or problems that occur in the teeth and mouth, so that it can change the mindset in dental and oral health, for example by practicing directly how to brush teeth.

3.1 Univariate Analysis

The analysis is used to analyze each variable from the results study. The results about the characteristics in this study are used to find out general description of the respondents presented in Table 1.

Charactoristics	Inter	vention	Co	ontrol	n valuo
Characteristics	п	%	п	%	
Age (mean±SD)	64.88±9.57		67.31±10.4	4	0.810*
Min-Max	55-93		55-87		
Gender					
Male	10	62.5	10	62.5	1.000**
Female	6	37.5	6	37.5	
Working status					
Working	10	62.5	9	56.3	0.719**
Not working	6	37.5	7	43.8	
Medical diagnose					
Pneumonia	5	31.3	0	0.0	
Suspect TB	4	25.	6	37.5	
Asthma bronchial	1	6.3	2	6.3	0.183**
РРОК	4	25	7	43.8	
Others	2	12.5	2	12.5	

Table 1: Characteristics of	respondents
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Table 1 shows that the age, gender and group diagnose of the respondents are homogeny, shown by the p-value of age is 0.810, gender is 1.000 and working status 0.719.

3.2 Normality Test

Table 2 shows the normality test on knowledge, attitude toward maintaining dental and oral hygiene, skills of tooth brushing and gargling, Debris Index and CPIS on intervention and control group.

Table 2: Normality	test results
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	p-value			
Variable	Intervention	Control		
	(n=16)	(n=16)		
Knowledge Pre-Test	0.061	0.332		
Knowledge Post-Test	0.065	0.115		
Attitude Pre-Test	0.026	0.001		
Attitude Post-Test	0.000	0.001		
Skill of tooth brushing Pre-Test	0.000	0.001		
Skill of tooth brushing Post-Test	0.023	0.028		
Skill of gargling Gigi lansia Pre-Test	0.240	0.073		
Skill of gargling Post-Test	0.103	0.057		
Debris Index Pre-Test	0.017	0.008		

	p-value		
Variable	Intervention (n=16)	Control (n=16)	
Debris Index Post-Test	0.001	0.000	
Clinical Pulmonary Infection Score (CPIS) Pre-Test	0.000	0.000	
Clinical Pulmonary Infection Score (CPIS) Post-Test	0.000	0.004	

The results of the normality test of the control and intervention group data for the variables of knowledge, attitude, skills of tooth brushing skill and gargling pre-test and post-test using the Shapiro-Wilk test shows different p-value comprising less and more than 0.05. The data on knowledge and skill of gargling pre-test and post-test obtain value of p > 0.05, so in conclusion, the data of knowledge and skill of gargling in pre-test and post-test are normally distributed. Hence, the parametric test was performed. While the attitude data, and skill of tooth brushing in pre-test are normally distributed. Therefore, non-parametric tests were performed.

3.3 Bivariate Analysis on The Variables

The bivariate analysis is intended to understand the difference value on the variables. As shown in table 3, the bivariate analysis was conducted based on variables of knowledge, attitude, skills of tooth brushing and gargling, Debris Index, CPIS and culture results.

 Table 3: Effectiveness test of Paired and Unpaired Data on Knowledge, Attitude, and Skills of Tooth brushing and gargling , Debris Index, and CPIS

Variable		Caracar	Mean±SD Mean±SD		Delta±SD	n nalua
		Group	Pre test	Post test	(Δ)	p-value
1.	Knowledge	Intervention	4.44 <u>+</u> 0.964	8.63 <u>+</u> 0.957	4.19 <u>+</u> 1.27	0.000*
		Control	4.56 <u>+</u> 1.209	6.75 <u>+</u> 1.183	2.19 <u>+</u> 1.42	0.000*
			p=0.749***	p=0.000***	p=0.001*****	
2.	Attitude	Intervention	24.19 <u>+</u> 0.981	30.69 <u>+</u> 1.662	6.5 <u>+</u> 1.54	0.000**
		Control	25.81 <u>+</u> 2.994	27.38 <u>+.</u> 553	1.57 <u>+</u> 1.03	0.001**
			p=0.025****	p=0.000****	p=0.000*****	
3.	Skill of tooth	Intervention	22.37 <u>+</u> 3.612	37.88 <u>+</u> 2.094	15.51 <u>+</u> 4.94	0.000**
	brushing	Control	26.81 <u>+</u> 3.885	27.38 <u>+</u> 2.630	57 <u>+</u> 1.03	0.135**
			p=0.002****	p=0.000****	p=0.000*****	
4.	Skill of	Intervention	10.19 <u>+</u> 1.328	25.19 <u>+</u> 2.167	15 <u>+</u> 2.60	0.000*
	gargling	Control	9.94 <u>+</u> 1.124	14.50 <u>+</u> 1.633	4.56 <u>+</u> 1.50	0.000*
			p=0.570***	p=0.000***	p=0.000*****	
5.	Debris Index	Intervention	2.400 <u>+</u> 0.163	0.319 <u>+</u> 0.160	2.08 <u>+</u> 6.89	0.000**
		Control	2.313 <u>+</u> 0.239	2.075 <u>+</u> 0.100	0.23 <u>+</u> 2.72	0.007**
			p=0.405****	p=0.000****	p=0.000*****	
6.	CPIS	Intervention	6.75 <u>+</u> 0.577	2.13 <u>+</u> 0.500	4.62 <u>+</u> 0.61	0.000**
		Control	6.88 <u>+</u> 0.500	3.88+0.719	3 <u>+</u> 1,13	0.000**
			p=0.325****	p=0.000****	p=0.000*****	
7.	Sputum	Intervention	2.63 <u>+</u> 1.962	1.44+0.892	1.19 <u>+</u> 2.00	0.036**
	culture results	Control	1.63 <u>+</u> 1.025	3.06+1.652	1.43 <u>+</u> 1.59	0.003**
			p=0.149****	p=0.000****	p=0.000*****	
*pai	red test		: Paired t-te	est *		
			Wilcoxon	**		
U	npaired test on pretest		: Independent t-test *			
]	Unpaired test on postte	est	: Man-Whitr	1ey *		
****Unpaired test of delta data		· Independent t-test ****				

Man-Whitney*****

Table 3 shows the different p-value. In the determination of whether the proposed model of dental and oral care for intervention group and dental and oral care based on Permenkes No.284 in 2006, p-value is considered. If the p-value is less than 0.05, then the conclusion is drawn that the model is effective, and vice versa, if the p-value is more than 0.05, the conclusion is that the model is not effective in the prevention of HAP. As shown by the p-value, the dental and oral care is effective to increase the knowledge of maintaining dental and oral health both in intervention and control groups. The same conclusion also comes to the variable of attitude, skill of gargling, Debris Index, CPIS and culture results. Meanwhile, for the skill of tooth brushing, the method is effective only in intervention group. So overall, the proposed dental and oral care show the effectivity on the increase of knowledge, attitude, skill of gargling, Debris Index, and CPIS. The sputum culture results indicate the decline of CPIS. Meanwhile the method on Permenkes No.284 2006 also show the same results, except in the skill of tooth brushing.

4. Discussion

The results of collecting information concluded that the implementation of dental and oral hygiene in the elderly in the practice of gargling and tooth brushing required special efforts through the implementation of dental and oral health care for the elderly. In its implementation, the guide from dental and oral therapists and nurses is needed. Dental and oral hygiene of the elderly need special attention because it affects general health. Good knowledge about dental and oral hygiene is one way to maintain body condition. It is necessary to assess the knowledge and attitudes of nursing staff towards oral health care. Some nursing staffs believe that the people will tell them when they need help with their daily oral hygiene. The people subsequently feel that they need interproximal help in the tooth brushing process (Croonquist et al., 2020). The role of nursing staffs is very important as nurse is the one who interact with the patient. Besides, nurse also play a role in helping patient in increasing their adaption towards the changes in their body, and motivate the patients to survive (Supriyadi et al., 2011). The increase in knowledge in the elderly is due to the fact that when the education about the maintenance of dental and oral hygiene is given, the elderly pay attention and perform the education well so that the understanding of the elderly about how to maintain oral health increases. The demonstration method is proven to be effective in increasing knowledge in providing counseling materials (Berniyanti et al., 2019; Sari & Permata Putri, 2021).

An increase in attitude occurred in the process of implementing the model of dental and oral hygiene care for the elderly who were given dental and oral health education and guidance on tooth brushing in the morning after breakfast and at night before going to bed. So, the attitude of the elderly towards dental and oral hygiene maintenance increased because of the response that had been given in line with the information. A person's dental and oral hygiene is determined by behavior because behavior is shaped by three domains, namely knowledge, attitude and action. Knowledge received by the subject through stimulation in the first place causes a closed response in the form of attitude (Hetherington, 2017).

Improved skills of tooth brushing and gargling with a model of dental and oral hygiene care of the elderly in the geriatric room of the Mukomuko Hospital, Bengkulu Province is because of the integration of education and simulation as well as demonstration of tooth brushing. It is justified by providing exercises on what the target has learned or in another word is the practice implies that something is always repeated and imprinted in mind. Nevertheless, between the first learning situation and a realistic learning situation are different, so, the upgraded ways are needed as this is intended to create a flawless skill (Deinzer et al., 2018).

The Debris Index score of elderly patients has decreased because elderly patients have been given education and practice brushing their teeth properly and correctly with the aim of removing plaque and debris so as to improve dental and oral hygiene. Dental and oral hygiene is very important to maintain. Measuring dental and oral hygiene is an attempt to determine the state of the integral part of overall body health that is not separated from general body health (Sunarjo et al., 2014). Poor oral and dental hygiene is not a very visible condition and related concerns, such as gingivitis and other dental diseases. Besides, potentially pathogenic bacteria are often present in the oral cavity and hospitalization is very risky to create conditions that favor colonization of the oropharynx (Alghamdi, 2022).

The decline in the Clinical Pulmonary Infection Score (CPIS) model of dental and oral hygiene care has successfully changed the habit of tooth brushing and gargling skills. The decline in the CPIS rate can be seen from the results of sputum culture that were examined in the Microbiology Laboratory of the Faculty of Medicine, UNAND Padang. The results of the pre-test intervention group sample examination show the growth of aerobic bacteria *Klebsiella sp., Staphylococcus aureus, Pseudomonas aeruginosa, Pseudomonas sp*, and *Candida albicans*. For the posttest, the culture examination shows no growth of *Pseudomonas aeruginosa*. It is generally a bacterium that is easily found in the environment but is an opportunistic pathogen that infects immunocompromised patients or hospitalized patients and attacks the respiratory tract. In the upper respiratory tract, especially the lungs, these bacteria are more likely to colonize the subgingival biofilm and saliva of patients with chronic periodontitis than patients without periodontitis, causing pneumonia which has the potential to cause deaths (Souto et al., 2014; Caldas et al., 2015).

The conclusion in this study relies on the model of dental and oral health care for inpatients is relevant as it is feasible and its application is effective to improve the skills of tooth brushing and gargling of elderly patients.

References

- Alghamdi, S. (2022). Isolation and identification of the oral bacteria and their characterization for bacteriocin production in the oral cavity. *Saudi Journal of Biological Sciences*, 29(1), 318–323. https://doi.org/10.1016/j.sjbs.2021.08.096
- Berniyanti, T., Kusumo, A., Bramantoro, T., Wening, G., & Palupi, R. (2019). Dental and oral health education for elderly age group: Full and partial edentulous teeth brushing method. *Journal of International Oral Health*, 11(2), 104–106. https://doi.org/10.4103/jioh.jioh_217_18
- Caldas, R. R., Le Gall, F., Revert, K., Rault, G., Virmaux, M., Gouriou, S., Héry-Arnaud, G., Barbier, G., & Boisramé, S. (2015). Pseudomonas aeruginosa and periodontal pathogens in the oral cavity and lungs of cystic fibrosis patients: A case-control study. *Journal of Clinical Microbiology*, 53(6), 1898–1907. https://doi.org/10.1128/JCM.00368-15
- Croonquist, C. G., Dalum, J., Skott, P., Sjögren, P., Wårdh, I., & Morén, E. (2020). Effects of domiciliary professional oral care for care-dependent elderly in nursing homes oral hygiene, gingival bleeding, root caries and nursing staff's oral health knowledge and attitudes. *Clinical Interventions in Aging*, *15*, 1305–1315. https://doi.org/10.2147/CIA.S236460
- Deinzer, R., Ebel, S., Blättermann, H., Weik, U., & Margraf-Stiksrud, J. (2018). Toothbrushing: To the best of one's abilities is possibly not good enough. *BMC Oral Health*, 18(1), 1–8. https://doi.org/10.1186/s12903-018-0633-0
- Giuliano, K. K., Penoyer, D., Middleton, A., & Baker, D. (2021). Oral Care as Prevention for Nonventilator Pneumonia : A Four-Unit Cluster Randomized Study. *American Journal of Nursing*, 121(6), 24–33.
- Hetherington, S. (2017). Knowledge as Potential for Action. *European Journal of Pragmatism and American Philosophy*, *IX*(2), 0–19. https://doi.org/10.4000/ejpap.1070
- Kanzigg, L. A., & Hunt, L. (2016). Oral Health and Hospital-Acquired Pneumonia in Elderly Patients: A Review of the Literature. *Journal of Dental Hygiene : JDH*, *90*, 15–21.
- Lemeshow, S., Jr, D. W. H., Klar, J., & Lwanga, S. K. (1997). *Adequacy of Sample Size in Health Studies*. World Health Organization.
- Mitchell, B. G., Russo, P. L., Cheng, A. C., Stewardson, A. J., Rosebrock, H., Curtis, S. J., Robinson, S., & Kiernan, M. (2019). Strategies to reduce non-ventilator-associated hospital-acquired pneumonia: A systematic review. *Infection, Disease and Health*, 24(4), 229–239. https://doi.org/10.1016/j.idh.2019.06.002
- Ong, C. C. H., Farhanah, S., Linn, K. Z., Tang, Y. W., Poon, C. Y., Lim, A. Y., Tan, H. R., Binte Hamed, N. H., Huan, X., Puah, S. H., Ho, B. C. H., Soon, M. M. L., Ang, B. S. P., Vasoo, S., Chan, M., Leo, Y. S., Ng, O. T., & Marimuthu, K. (2021). Nosocomial infections among COVID-19 patients: an analysis of intensive care unit surveillance data. *Antimicrobial Resistance and Infection Control*, 10(1), 1–5. https://doi.org/10.1186/s13756-021-00988-7
- Pássaro, L., Harbarth, S., & Landelle, C. (2016). Prevention of hospital-acquired pneumonia in non-ventilated adult patients: A narrative review. *Antimicrobial Resistance and Infection Control*, 5(1). https://doi.org/10.1186/s13756-016-0150-3
- Riani, & Syafriani. (2019). Hubungan Antara Motivasi dengan Kepatuhan Perawat Melaksanakan Hand Hygiene Sebagai Tindakan Pencegahan Infeksi Nosokomial di Ruang Rawat Inap Rumah Sakit AH Tahun 2019 [The Relationship Between Motivation and Compliance Nurses Implementing Hand Hygiene as a Prevention of Nosocomial Infections in the Inpatient Ward of the AH Hospital in 2019]. Jurnal Ners, 3(23), 49–59.

- Sari, M., & Permata Putri, N. I. (2021). Peningkatan Pengetahuan Kesehatan Gigi dan Mulut pada Lansia dengan Promosi Kesehatan Metode Demonstrasi [Improving Dental and Oral Health Knowledge in the Elderly with Health Promotion Demonstration Method]. *Insisiva Dental Journal: Majalah Kedokteran Gigi Insisiva*, 10(2), 26–31. https://doi.org/10.18196/di.v10i2.11311
- Souto, R., Silva-Boghossian, C. M., & Colombo, A. P. V. (2014). Prevalence of Pseudomonas aeruginosa and Acinetobacter spp. in subgingival biofilm and saliva of subjects with chronic periodontal infection. *Brazilian Journal of Microbiology*, *45*(2), 495–501. https://doi.org/10.1590/S1517-83822014000200017
- Sukarman, Adriyoso, H., & Anggi. (2015). Hubungan Pneumonia dengan Terjadinya Penyakit Periodontal pada Pasien Rawat Inap dan Rawat Jalan di RS Moehammad Hoesin dan RS AK GANI PALEMBANG [The Relationship between Pneumonia and Periodontal Disease in Inpatients and Outpatients at Moehammad Hoesin Hospital and AK GANI Hospital, PALEMBANG]. Jurnal Kesehatan, 10(1), 13–20.
- Sunarjo, L., Hendari, R., & Sulistijarso, N. (2014). Mangosteen Rind towards Mechanical Trauma Recovery in The Oral Cavity. *Jurnal Riset Kesehatan*, 3(3), 615–621.
- Supriyadi, Wagiyo, & Widowati, S. R. (2011). Tingkat Kualitas Hidup Pasien Gagal Ginjal Kronik Terapi Hemodialisis [Quality of Life of Patients with Chronic Kidney Failure Hemodialysis Therapy]. Jurnal Kesehatan Masyarakat, 6(2), 107–112.
- Suzuki, J., Ikeda, R., Kato, K., Kakuta, R., Kobayashi, Y., Ohkoshi, A., Ishii, R., Hirano-Kawamoto, A., Ohta, J., Kawata, R., Kanbayashi, T., Hatano, M., Shishido, T., Miyakura, Y., Ishigaki, K., Yamauchi, Y., Nakazumi, M., Endo, T., Tozuka, H., ... Katori, Y. (2021). Characteristics of aspiration pneumonia patients in acute care hospitals: A multicenter, retrospective survey in Northern Japan. *PLoS ONE*, 16(7 July), 1–20. https://doi.org/10.1371/journal.pone.0254261
- Talley, L., Lamb, J., Harl, J., Lorenz, H., & Green, L. (2016). HAP prevention for nonventilated adults in acute care. *Nursing Management*, 47(12), 42–48. https://doi.org/10.1097/01.numa.0000508259.34475.4c
- Warganegara, E. (2017). Pneumonia Nosokomial: Hospital-Acquired, Ventilator-Associated, dan Health Care-Associated. *Jurnal Kedokteran Unila*, *1*(3), 612–618. http://juke.kedokteran.unila.ac.id/index.php/JK/article/view/1729



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Guillain-Barré Syndrome: A Case Report of Post Covid-19 Vaccination in the Philippines

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Abstract

Guillain-Barré Syndrome (GBS) is a complex autoimmune disorder where a person's own immune system damages the nerve and is usually characterized by ascending symmetrical weakness of the upper and lower extremities (Wijdicks & Klein, 2017). It is a rare condition and the worldwide frequency of GBS is only 2 in 100,000 adult individuals (McGrogan et al., 2009). Studies have shown its association with different vaccines but in these pandemic times, there is a lack of literature on post-COVID-19 vaccination-associated GBS. We report a case of a 68-year-old male from Caloocan City with an initial complaint of febrile illness followed by distal lower extremity weakness which started seven (7) days after the patient received his first Sinovac-CoronaVac vaccine. We report a case of GBS that is related to the Sinovac-CoronaVac vaccine which adds to the body of literature that is currently available and may reflect a possible link.

Keywords: Guillain-Barre, Covid-19, Vaccination

1. Introduction

Originating in Wuhan, China last December 2019, the COVID-19 pandemic caused by the causative agent severe acute respiratory syndrome (SARS-CoV-2) dramatically affected the world population (Omer et al., 2020). To reduce the transmissibility of the disease, the role of vaccination is now taking center stage in high resource countries, especially as older and more vulnerable populations have been vaccinated. Worldwide, several patients have been reported to experience pain in the vaccination site, to a more serious anaphylactic shock (Salmon et al., 2013). Neurological complications after COVID-19 vaccinations are very rare, and only a few cases have been reported, where an article published February 2021 reported the first case of Guillain-Barré Syndrome (GBS) after the first dose of Pfizer COVID-19 vaccine in Florida (Khan et al., 2022).

GBS is a rare but serious post-infectious immune-mediated neuropathy, and it results from the auto-immune destruction of nerves in the peripheral nervous system. The disease is rare, with the incidence rate of 0.6 to 4 per 100,000 individuals (R. A. C. Hughes, 1990). Bilateral leg weakness is the most common early motor symptom, and pain is the most common non-motor symptom (Forsberg et al., 2004). Neurological adverse events following immunization may be caused by the active antigen in the vaccine or other constituents, such as adjuvants, or may merely be coincidental (Miravalle et al., 2010).

This report presents the case of a 68-year-old man from Caloocan City, with a first-ever complaint of bilateral lower extremity weakness seen last August 15, 2021, 13 days after his first dose of Sinovac-CoronaVac vaccine.

2. Case Report

We report a case of a 68-year-old man who presented at the emergency room of Jose R. Reyes Memorial Medical Center, Manila with bilateral lower extremity weakness last August 15, 2021. The patient had his first dose of Sinovac-CoronaVac vaccine on August 2, 2021. Three days after receiving the first dose, he started having undocumented febrile episodes. Four days after, the febrile episodes subsided but the patient started to have weakness of both lower extremities described as dragging of either foot upon ambulation. After two more days, there was progression of bilateral lower extremity weakness described as difficulty getting up from bed or standing up from sitting position. There was no observed weakness of the upper extremities. Few days before admission, he was unable to sit independently and was bedridden. He also had onset of cough and colds, with occasional episodes of difficulty of breathing. Consultation was sought via telemedicine to which COVID-19 reverse transcription polymerase chain reaction test was requested, and results were negative. He was then advised admission for further evaluation and management.

Upon arrival at the emergency room, the patient's blood pressure, heart rate, respiratory rate, and temperature were within normal limits. Oxygen saturation was 98-99% at room air. He was awake but follows commands inconsistently. Pertinent general physical examination included auscultatory findings of crackles on both lungs. Digital rectal examination revealed a full rectal vault and a lax sphincteric tone. Pertinent neurologic findings included a 5/5 motor strength on the upper extremities, and a 3/5 motor strength in both lower extremities using the Medical Research Council Manual Muscle Testing scale. On sensory examination, the patient perceived pain in both lower extremities however, quantification of sensory deficit by percentage cannot be fully determined as the patient was illiterate. Deep tendon reflexes for bilateral patella and Achilles were absent.

Laboratory examination yielded normal values for the complete blood count, serum sodium and potassium, and creatinine levels. An electrocardiogram revealed atrial fibrillation in controlled ventricular response. Chest x-ray was done revealing a right lower lung pneumonia with minimal pleural effusion. The patient was started on Azithromycin 500mg tablet per orem once a day and Ceftriaxone 2gm via intravenous route once a day. Lumbar puncture was done, and the Cerebrospinal Fluid (CSF) analysis revealed a colorless clear fluid, with albuminocytologic dissociation (see Table 1). A nerve conduction study was done with an impression of an acute inflammatory demyelinating (see Figures 1-4).

An impression of GBS, acute inflammatory demyelinating polyneuropathy was considered. Intravenous immunoglobulin (IVIg) infusion was requested. The patient's motor strength spontaneously improved starting three days after admission where he could eventually ambulate with assistance. He was eventually discharged on the tenth hospital stay with an Erasmus GBS Outcome Score score of 4, and was advised for follow up. Follow-up was done after three months, and the patient had no residual deficits and was independent in all activities of daily living.

3. Discussion

GBS is considered as a severe auto-immune disease that mainly affects the majority of the spinal nerve roots and peripheral nerves and may involve the cranial nerves. The initial symptoms of the disease include symmetrical weakness of the extremities, and it quickly aggravates and spreads from a distal to proximal fashion. The disease is usually preceded by infection or other immune stimulation that induces an aberrant auto-immune response, and it targets the peripheral nerves, which explains the weakness of extremities (R. A. Hughes & Cornblath, 2005). The molecular mimicry between microbial and nerve antigens is a major force that drives behind the development of the disease, the patient presents an association between GBS and his recent vaccination, as the onset of the disease started seven days after the first dose of Sinovac-CoronaVac vaccine.

The patient is a 68-year-old male with initial complaints of weakness of the distal lower extremities, and progressed up to the entire lower extremities, which had a temporal proximity from a recent vaccination with Sinovac-CoronaVac vaccine and was the likely trigger for the onset of the symptoms. In several reported cases, the presence

of diarrhea within two weeks before the disease can predict the patient's outcome for GBS (Walgaard et al., 2011). The patient however did not present with any gastro-intestinal symptoms prior to the onset of weakness. Among the triggering factors, vaccines are reported to be associated with onset of GBS (Haber et al., 2009). Vaccine-associated GBS is defined as the onset of GBS symptoms within the six-week period after receiving the vaccine (Sejvar et al., 2011). Known COVID-19 vaccines worldwide include Pfizer-BioNTech, Moderna, Oxford Astrazeneca, Johnson and Johnson, and Sinovac (Khan et al., 2022). A systematic review by Shao et al., (2021)

found only 17 publications reporting 39 cases of post-vaccination GBS. Most of the reported cases received AstraZeneca vaccine. Others included, in descending order: Pfizer, Johnson and Johnson, and Sinovac Biotech. The review also investigated the GBS rate after COVID-19 vaccination ranged from 1.8 to 53.2 cases/ 1 million doses.

In our patient, the onset of GBS started seven days after his first shot of Sinovac Vaccine, and findings also indicated a strong association between the two. The first case of GBS following COVID-19 vaccination was reported in February 2021 (Khan et al., 2022), with an elderly female from Florida, USA who presented with GBS symptoms two weeks after the first dose of Pfizer vaccine. The patient presented with fatigue and bilateral symmetric weakness of the lower limbs, and CSF analysis indicated albuminocytological dissociation. The woman was started on IVIg which contributed to the improvement of the disease and was discharged to a rehabilitation institute (Waheed et al., 2021). Considering the uncertainty of the causal relationship between vaccines and GBS and the lack of evidence that proves their relation, a temporal association is possible. However, further studies are required before establishing strong conclusions.

During the hospitalization and treatment course of the patient, the patient's motor strength spontaneously improved three days after admission owing to the self-limiting nature of GBS. IVIg infusion was deferred since the patient's condition spontaneously improved. IVIg is indicated to those who show rapid progression of weakness, respiratory insufficiency, severe autonomic or swallowing dysfunction, and unable to walk independently (Leonhard et al., 2019). According to Cochrane review of trials, within the first two weeks from the onset of weakness, GBS should be treated with IVIg or plasmapharesis if indicated. The administration of IVIg for five consecutive days was able to provide relief. The Cochrane review of trials indicated IVIg as a great replacement for plasma exchange, showing statistics on the increased use of IVIg due to its convenience and availability (R. A. Hughes et al., 2006). Although the best option for treatment depends on the patient's prognosis, eligible patients should be treated right away as lack of treatment is thought to have a prolonged immune response that causes persistent nerve damage that may require treatment for a longer period of time (Visser et al., 1998). The longer interval between the onset and treatment, as well as the longer time to nadir, can be both associated with a greater chance of relapse.

4. Conclusion

GBS is a rare, autoimmune disorder characterized by acute onset of ascending motor and/or sensory deficits and is found to be a common cause of paralysis if left untreated for a long time. GBS following COVID-19 vaccine is an adverse event of special interest due to implementation of mass vaccination in the country. Although extremely rare, the occurrence of post-COVID-19 vaccine GBS presents a potentially devastating neurologic emergency, and vigilant surveillance of this adverse event is strongly warranted as more people become vaccinated. The author hopes that this case will serve as a bridge to further research on this subject and will foster awareness on a possible onset of GBS after receiving the COVID-19 vaccine, and the emergent need for intervention should a case arise.

	1 () 5
Color	Colorless
Transparency	Clear
Volume	2 mL
RBC Count	None found
WBC Count	1 mm3
CSF Protein	245mg/dL
	(Normal: 15–50 mg/dL)

Table 1: Cerebrospinal (CSF) fluid analysis



Figure 1: The left and right median motor nerve conduction studies which showed prolonged distal latencies, normal amplitudes, and normal conduction velocities



Sensory Nerve Conduction Study

Figure 2: The left and right ulnar sensory nerve, median sensory nerve, and sural sensory nerve conduction studies showed no recordable response



Figure 3: The F-wave latencies on left and right ulnar nerves are prolonged



Figure 4: The H-reflex latencies are prolonged

References

- Forsberg, A., Press, R., Einarsson, U., de Pedro-Cuesta, J., & Widén Holmqvist, L. (2004). Impairment in Guillain–Barré syndrome during the first 2 years after onset: A prospective study. *Journal of the Neurological Sciences*, 227(1), 131–138. https://doi.org/10.1016/j.jns.2004.09.021
- Haber, P., Sejvar, J., Mikaeloff, Y., & DeStefano, F. (2009). Vaccines and Guillain-Barré Syndrome: *Drug Safety*, 32(4), 309–323. https://doi.org/10.2165/00002018-200932040-00005
- Hughes, R. A. C. (1990). Pathology of Guillain-Barré Syndrome. In M. Swash (Ed.), *Guillain-Barré Syndrome* (pp. 83–100). Springer London. https://doi.org/10.1007/978-1-4471-3175-5_4
- Hughes, R. A., & Cornblath, D. R. (2005). Guillain-Barré syndrome. *The Lancet*, 366(9497), 1653–1666. https://doi.org/10.1016/S0140-6736(05)67665-9
- Hughes, R. A., Swan, A. V., van Koningsveld, R., & van Doorn, P. A. (2006). Corticosteroids for Guillain-Barré syndrome. In The Cochrane Collaboration (Ed.), *Cochrane Database of Systematic Reviews* (p. CD001446.pub2). John Wiley & Sons, Ltd. https://doi.org/10.1002/14651858.CD001446.pub2
- Khan, Z., Ahmad, U., Ualiyeva, D., Amissah, O. B., Khan, A., Noor, Z., & Zaman, N. (2022). Guillain-Barre syndrome: An autoimmune disorder post-COVID-19 vaccination? *Clinical Immunology Communications*, 2, 1–5. https://doi.org/10.1016/j.clicom.2021.12.002
- Leonhard, S. E., Mandarakas, M. R., Gondim, F. A. A., Bateman, K., Ferreira, M. L. B., Cornblath, D. R., van Doorn, P. A., Dourado, M. E., Hughes, R. A. C., Islam, B., Kusunoki, S., Pardo, C. A., Reisin, R., Sejvar, J. J., Shahrizaila, N., Soares, C., Umapathi, T., Wang, Y., Yiu, E. M., ... Jacobs, B. C. (2019). Diagnosis and management of Guillain–Barré syndrome in ten steps. *Nature Reviews Neurology*, 15(11), 671–683. https://doi.org/10.1038/s41582-019-0250-9
- McGrogan, A., Madle, G. C., Seaman, H. E., & de Vries, C. S. (2009). The Epidemiology of Guillain-Barré Syndrome Worldwide. *Neuroepidemiology*, 32(2), 150–163. https://doi.org/10.1159/000184748
- Miravalle, A., Biller, J., Schnitzler, E., & Bonwit, A. (2010). Neurological complications following vaccinations. *Neurological Research*, 32(3), 285–292. https://doi.org/10.1179/016164110X12645013515214
- Omer, S. B., Yildirim, I., & Forman, H. P. (2020). Herd Immunity and Implications for SARS-CoV-2 Control. JAMA, 324(20), 2095. https://doi.org/10.1001/jama.2020.20892
- Salmon, D. A., Proschan, M., Forshee, R., Gargiullo, P., Bleser, W., Burwen, D. R., Cunningham, F., Garman, P., Greene, S. K., Lee, G. M., Vellozzi, C., Yih, W. K., Gellin, B., & Lurie, N. (2013). Association between Guillain-Barré syndrome and influenza A (H1N1) 2009 monovalent inactivated vaccines in the USA: A meta-analysis. *The Lancet*, 381(9876), 1461–1468. https://doi.org/10.1016/S0140-6736(12)62189-8
- Sejvar, J. J., Kohl, K. S., Gidudu, J., Amato, A., Bakshi, N., Baxter, R., Burwen, D. R., Cornblath, D. R., Cleerbout, J., Edwards, K. M., Heininger, U., Hughes, R., Khuri-Bulos, N., Korinthenberg, R., Law, B. J., Munro, U., Maltezou, H. C., Nell, P., Oleske, J., ... Wiznitzer, M. (2011). Guillain–Barré syndrome and Fisher syndrome: Case definitions and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine*, 29(3), 599–612. https://doi.org/10.1016/j.vaccine.2010.06.003
- Shao, S.-C., Wang, C.-H., Chang, K.-C., Hung, M.-J., Chen, H.-Y., & Liao, S.-C. (2021). Guillain-Barré Syndrome Associated with COVID-19 Vaccination. *Emerging Infectious Diseases*, 27(12), 3175–3178. https://doi.org/10.3201/eid2712.211634
- Visser, L., van der Meche, F., Meulstee, J., van Doorn, P., & the Dutch Guillain-Barre study group. (1998). Risk factors for treatment related clinical fluctuations in Guillain-Barre syndrome. *Journal of Neurology*, *Neurosurgery & Psychiatry*, 64(2), 242–244. https://doi.org/10.1136/jnnp.64.2.242
- Waheed, S., Bayas, A., Hindi, F., Rizvi, Z., & Espinosa, P. S. (2021). Neurological Complications of COVID-19: Guillain-Barre Syndrome Following Pfizer COVID-19 Vaccine. Cureus. https://doi.org/10.7759/cureus.13426

- Walgaard, C., Lingsma, H. F., Ruts, L., van Doorn, P. A., Steyerberg, E. W., & Jacobs, B. C. (2011). Early recognition of poor prognosis in Guillain-Barre syndrome. *Neurology*, 76(11), 968–975. https://doi.org/10.1212/WNL.0b013e3182104407
- Wijdicks, E. F. M., & Klein, C. J. (2017). Guillain-Barré Syndrome. *Mayo Clinic Proceedings*, 92(3), 467–479. https://doi.org/10.1016/j.mayocp.2016.12.002



A Study on Distribution and Association of CD320, a Receptor for Active Vitamin B12, in Pregnant Mothers and their Newborns Cord Blood

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Abstract

Objective: Our objective was to study the levels and relation between CD320 receptors in anaemic mothers in their last trimester of pregnancy and their newborns after birth. Methods: Association between CD320, VitB12, active B12 levels in mothers with anaemia were analysed before they underwent labor and in blood collected from their newborns after labor. Regression analysis was performed in order to assess the relation between the mothers and their neonatal vitamin CD320 levels in relation to their Vit B12 status. Results: Among the 200 pregnant mothers recruited, 59% were anaemic. CD320 levels were significantly higher in anaemic mothers (414.09 \pm 10.75 pg/ml) when compared with cord blood (372.25 \pm 7.81 pg/ml). CD320 levels were higher in both mother and cord blood with Vit B12 deficiency, however, were lower in mothers with active B12 deficiency. CD320 levels in cord blood were positively associated with haemoglobin (r value 0.8, *p* value 0.42) of mothers and negatively with serum folate (r value -0.15; *p* value 0.23), Vit B12(r value -0.21; *p* value0.28) and active Vit B12 levels (r value -0.25; *p* value0.03) in mothers. Conclusion: CD320 levels in cord blood positively correlated with haemoglobin of mothers and negatively with folic acid and B12 status. A negative trend (*p*=0.0024) was observed between maternal and neonatal CD320 levels.

Keywords: CD320, Anaemia, Pregnant Mothers, Cord Blood, Vitamin B12, Active B12

1. Introduction

Deficiency of Vitamin B12 (Vit B12) is common in India and is found to be more widespread than it is assumed, especially among pregnant women (Pathak et al. 2007; Laxmaiah 2015). Apart from beneficial effects of Vit B12 in mothers, it also helps in the development of the foetus (Pepper 2011 & Greibe 2011). Newborns with Vit

B12 deficiency are known to develop neural tube defects and intrauterine growth retardation (Molloy et al 2009).Vit B12 after absorption in the intestinal cells of the mother (Nielsen et al 2012), gets partially bound to the protein transcobalamin (TC), is called holo- transcobolamin or holo-TC and the remaining with apoTC (Nielsen et al 2012). HoloTC is also called active B12 and is the form through which cellular uptake of B12 takes place with the help of a receptor (Nielsen et al 2012) called CD320, which was recently purified from human placenta. This holoTC receptor mediates holoTC uptake in most cells in the body (Quadros et al 2009). The CD320 receptor was found to bind mostly to holoTC, only to a lesser degree to apoTC and is a low-density lipoprotein receptor. Abuyaman et al 2013, had reported for the first time that sCD320 was found in both serum and urine, its urinary concentration exceeded those of the serum and its levels increased with progression of pregnancy.

Apart from the discovery of the new Vit B12 receptor, there are no further studies on it and whose novelty lies in the fact that it is expressed both in serum as well as urine. Hence, there is an unmet need to explore the diagnostic potential of this new receptor. We thus hypothesized that Vit B12 deficiency in the mothers leads to Vit B12 deficiency in the cord blood and thus leading to increased expression of the CD320 receptors in the placenta to compensate for the deficiency in the cord blood and replenish Vit B12 levels in the foetus. As total Vit B12 levels are not reliable indicator of B12 status and also Vit B12 deficiency is very common in our country, especially in pregnant women we intended to study this newly discovered receptor of active Vit B12 both in the mothers and newborns blood, its expression in the placentas and their association with total Vit B12 levels.

2. Methods

Ours was a cross-sectional study that was carried out in accordance with Ethical committee clearances from both our Institute and the collaborating Hospital, which is a tertiary level hospital where patients from both lower as well middle strata of the society come for their treatment. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Institutional Ethical committee. A written informed consent was obtained from all subjects. We recruited 200 mothers who were either in labor or admitted for delivery and who agreed to participate in the study. Upon admission and after undergoing routine complete blood picture (CBP) estimation, the mothers were requested to participate in the study. They were made to understand the details of the study including its benefit for the society and science, before asking for their consent. The study was initiated after we gave information about the study to the subjects. Mothers in the reproductive age group of 18–45 years, primiparous, were recruited, while those suffering from high blood pressure or high blood sugar and positive for hepatitis or AIDS were excluded from the study.

Information on the mother's sociodemographic profile, clinical and obstetric history, dietary intake was obtained through a questionnaire, by trained staff recruited for the study. Maternal anthropometry was also performed in which, the height, weight and BMI of mothers were noted. The body mass index (BMI) of the mothers was calculated in kg/m².

2.1. Study groups

There were two study groups based on mother's hemoglobin (Hb) levels. All the mothers whose Hb values were less than 11g/dl were assigned to anaemic group and those whose Hb levels were $\geq 11g/dl$ were assigned to normal group (WHO).

2.2. Sample collection and processing

2.6.1. Blood samples

After enrollment into the study, about 5ml of non-fasting sample of blood was drawn from the ante-cubital vein of mothers in labor or who was about to deliver, while 5ml of blood from the umbilical cord of the newborns was collected after delivery, in lavender cap ethylene diaminetetraacetate (EDTA) and red cap vacutainer tubes, which were transported in ice. In the lab, blood in EDTA tubes, was processed within 6 hours of collection, in automated hematology analyzer (ADVIA 120, Seimens) and analyzed for Hb and red cell indices. The serum after separation from blood, was stored in a -80° C refrigerator till it was analyzed for ferritin, vit B12, holo-transcobolamin (holo-TC) or active B12 and soluble CD320 receptors.

2.6.1.1 Serum ferritin was estimated by using SA ELISA kit from Calbiotech, Inc. based on the streptavidinbiotin principle.

2.6.1.2 Serum Vit B12 and folic acid analysis was performed by using the RIA kit from MP Biomedicals (USA).

2.6.1.3 Serum holo-TC (Active B12) was estimated by using ELISA method (Axis-Shield Diagnostics, Dundee, Scotland, UK).

2.6.1.4 CD320/8D6A ELISA Kit was used to determine serum CD320 receptor levels by sandwich ELISA method.

2.6.2 Placentas

After delivery, placentas were collected in 10% buffered formalin solution. In the lab, they were weighed after removing the umbilical cord (UC) and the amniotic and chorionic membranes and their morphology along with weight and size were noted. After overnight fixation in the buffered formalin they were grossed and 4 bits were taken from the four quadrants, about 2-5cms from the center, away from the margins and including both maternal and fetal surfaces. Two sections from the UC and one from the membranes were also taken. The tissues were next processed overnight in an automatic tissue processor, their paraffin blocks prepared, sectioned to 5 microns and stained with hematoxylin and eosin stain (H&E). The stained placental tissue sections were viewed under a brightfield microscope and the histopathological findings were noted.

2.6.2.1 Immunohistochemical expression of the placental tissues for CD320 protein expression was studied on the paraffin embedded tissues by using the CD320 primary antibody from CUSABIO technology Ltd (Product code: CSB-PA865096DSR1HU). The secondary antibodies used were 2-step plus Poly-HRP Anti-Mouse/Rabbit IgG Detection system with DAB solution by Elabscience (Catalog No. E-IR-R217). The placental tissue sections after dewaxing and hydration, incubated with E-IR-R217 C (3% H₂O₂) to eliminate endogenous peroxidase activity and washed in phosphate buffered saline (PBS) followed by incubation in E-IR-R217 A normal goat serum. Next the primary CD320 antibody at 1:900 dilution, raised in rabbit was added to the slides and incubated at 4° C overnight. This was followed by incubation at room temperature in E-IR-R217 B (Polyperoxidase antirabbit IgG), followed by DAB solution.

2.6.2.2 Immunohistochemical stained sections analysis

The immunohistochemical analysis was performed by counting the percent (P) of trophoblastic cells of placenta, exhibiting positive stain for the marker, which was graded from not-detected with grade 0%, to total or homogenous staining graded as 100%. The intensity of immunohistochemical staining (I) was also graded as follows: grade 1 for weak intensity of stain, 2 was given for moderate intensity and 3- for strong intensity of staining. The final results were then obtained by multiplying the percentage of stained cells (P) with staining intensity (I), which was called quick score (Q) where Q was = $P \times I$ with as maximum score of 300) (Abuyaman et al 2013).

2.7 Cut-off values for defining deficiency of parameters

Serum ferritin deficiency was stated as <15ng/ml (WHO), Vit B12 deficiency was stated, after using the standards of Centre for Disease Control and Prevention (CDC) definition, as <150 pmol/L or 203 pg/ml (Yetley 2011). Folate deficiency as <6.8 nmol/L or 4.4ng/ml (10), holo-TC deficiency as <35 pmol/l (Lindgren 1999). CD320 being a relatively newly discovered receptor, we could not come across any cut off values for defining deficiency state both for the mothers and cord blood. Maternal BMI was ranged from 18.5 to 30.0 kg/m², based on classification by World Health Organization (WHO) and CDC(WHO 1995).

2.8 Statistical Analyses of data

Processing of the data and its analyses was carried out with SPSS software (19.0 version). Constant or continuous data were expressed in means \pm standard deviation or SD and absolute or definite or categorical data as numbers (%). We examined the relation between mothers and newborn cord blood Vit B12 levels including CD320 levels. All the mean values of constant or continuous data variables were compared in the mother's blood and newborn cord blood by using Student's *t* test. Non-parametric *t*-test was used to compare the data between serum ferritin, VitB12, active B12, CD320 levels and CD320 expression level score by immunohistochemistry in placentas. Multiple comparisons within the groups were carried out with the help of ANOVA. Correlations between various data variables in mother's blood and newborn cord blood were examined with Spearman's test. Multivariable backward regression analyses was performed to study the different variables that could predict change in the levels of CD320 in maternal and newborn cord blood. All the variables were compared with low CD320 levels. The characteristics found significant in the univariate analysis with p < 0.2 were analysed for multivariate analysis. Variables which had p<0.05 were analysed further by final adjusted regression model if they had a p value <0.05. Statistical significance was given to a *p* value of less than 0.05.

3. Results

Table 1 shows the general features of the pregnant mothers included in our study. The average age of the subjects was 23 years while BMI was 24.53 ± 2.39 . While 45.5% of them were illiterate, majority were non-working mothers (90%).61.5% of the mothers were gravida >1 with 32.6% being multiparous. The mean placental weight was 416.35 ± 89.45 grams and mean newborn birth weight was 2.82 ± 0.43 kgs.

Variables	Values	
Maternal		
Age (years) Mean \pm SD (Range)	23.84 ± 3.10 (18-35)	
Height, (feet) Mean \pm SD (Range)	5.31±0.20 (4.11-5.80)	
Weight (kg) Mean \pm SD (Range)	63.23 ±7.47 (38-86)	
BMI (kg/m ²) Mean \pm SD (Range)	24.53±2.39(17.2-30.6)	
Education % (n/total)		
Illiterate	45.5 (91/200)	
Schooling	45 (90/200)	
College	9.5 (19/200)	
Occupation % (n/total)		
Working	10 (20/200)	
Non-working	90 (180/200)	
Monthly income % (n/total)		
<rs 5000<="" td=""><td>6.5 (13/200)</td></rs>	6.5 (13/200)	
Rs 5000-10,000	70 (140/200)	
Rs10,000-50,000	23.5 (47/200)	

Table 1: Characteristic features of pregnant mothers and their newborns

Gravida >1 % (n/total)	61.5 (123/200)
Parity >1 % (n/total)	32.6 (45/138)*
Previous place of delivery % (n/total)	
Home	7.2 (15/158)*
Hospital	68.4 (143/158)*
Number of antenatal visits $\geq 5\%$ (n/total)	73.5(147/200)
Placenta Weight(gm) Mean \pm SD (Range)	$416.35 \pm 89.45(223-703)$
Newborn	
Birth Weight(kg) Mean \pm SD (Range)	2.82 ±0.43(1.8-5.1)
Birth C-R length(cms)Mean \pm SD (Range)	30.62 ±1.71(27-38)
Skinfold thickness(cms)Mean ± SD (Range)	1.24 ±0.24 (0.8-2.5)
Head circumference(cms)Mean ± SD (Range)	30.8 ±1.64 (26-36)
Mid circumference(cms)Mean ± SD (Range)	12.75 ±1.32 (8-17)

*History not available for the remaining cases

Kg-Kilograms, BMI-Body mass index, kg/m² -Kilograms per meter square, SD- Standard deviation, Rs- Rupees, gm- grams, C-R- Crown-rump , cms-centimeters

Figure 2 shows distribution of the study subjects according to Hb levels. While 41% were non-anaemic, 59% were anaemic, among whom 16% had mild, 35% moderate and 8% severe anaemia.



Figure 2: Pie chart showing distribution of subjects with different grades of anaemia.

Table 2 shows the different hematological and biochemical parameters in maternal and cord blood. Hb showed significant (p<0.001) and higher levels in the cord blood than mother's blood. Similarly, micronutrients like folate, total Vit B12, Active B12 except CD320 receptor levels (levels significantly lower in cord blood), were found to be higher (p<0.001) in cord blood when compared to maternal blood.

Table 2: Comparison of Hb, Vit B12, its active form and its receptors and folate levels in mother's and cord blood

Parameter	Mothers blood Mean ± SE (Range)	Cord blood Mean ± SE (Range)	<i>p</i> -value
Hb (g/dl)	$\begin{array}{c} 10.30 \pm 0.15 \\ (3.7\text{-}15.4) \end{array}$	$ \begin{array}{r} 15.75 \pm 0.15 \\ (7.40-23.30) \end{array} $	<0.001
CD320 receptors (pg/ml)	414.09 ±10.75 (155.7-736.2)	372.25 ± 7.81 (231.80-554.70)	0.0024 (<0.01)
Folate (ng/ml)	5.66 ± 0.97 (0.55-74.3)	$10.84 \pm 0.53 \\ (1.33-23.93)$	<0.001
Vitamin B12 (pg/ml)	147.23 ± 26.54 (28.6-1472.2)	$286.06 \pm 30.18 \\ (21.9-1684.9)$	<0.001
Active B12 (Holo-TC)	48.48 ± 3.35	106.67 ± 6.44	< 0.001

(pmol/l)	(11.1-228.30)	(19.10-315.10)	
Hb- Hemoglobin, SE- Standard Erro	or, Holo-TC- Holo-transcobolamin,	fl- fentolitre, ng/ml-nanogram/millilite	r, pg- picogram, pmol-
picomoles.			

Figure 3 shows the number of mothers and newborns cord blood with different micronutrient levels. While 59.3% mothers were anaemic with Hb levels<11g/dl, only 7.8% of newborns cord blood was deficient in Hb. 54% mothers showed low ferritin levels while only 1.6% of newborns were ferritin deficient. In case of folate, 66% mothers were folate deficient while 7.2% of newborns cord blood was folate deficient.89% of mothers showed Vit B12 deficiency while 53% of newborns cord blood was Vit B12 deficient. With Holo-TC samples, 36.7% mothers showed deficient levels while cord blood samples showed only 9.3% deficiency.



Figure 3: Bar diagram shows the number, in percentage, of mothers and newborns with different micronutrient levels.

Table 3 shows that CD320 levels were higher, albit non-significantly (p=0.19), in mothers who had anaemia when compared those who were normal, but in cord blood the CD320 levels were observed to be low in newborns of mothers suffering from anaemia (p=0.18). Similarly CD320 levels were higher in both mothers and cord blood with Vit B12 deficiency in comparison to those with normal B12 levels. However, CD320 levels were lower in mothers with Active B12 deficiency while in cord blood the levels were higher.

Parameter	Status	CD320 in Mothers (pg/ml)			CD32	0 in Cord bl (pg/ml)	lood
		Mean± SE	<i>p</i> - value	Median	Mean± SE	<i>p</i> - value	Median
Hb Mothers	Normal	391.09±17.68	0.199	357.70	389.25 ±15.14	0 177	394.70
(g/dl)	Anaemic	422.84± 13.14	0.188	412.50	365.67± 9.05	0.1//	365.10

Table 3: CD320 levels in mothers and cord blood in relation to other micronutrient status in mothers blood

Ferritin Mothers	Normal	398.97 ±15.19	0.204	381.15	371.83± 12.10	0.009	372.30
(ng/ml)	Deficient	421.21±14.63	0.294	405.75	373.68± 10.38	0.908	366.65
Folate Mothers	Normal	404.84±20.58	0.(2	369.95	365.13 ±15.68	0.561	364.95
(ng/ml)	Deficient	416.45 ±13.64	0.63	412.50	376.19± 11.12	0.561	373.65
Vit B12 Mothers	Normal	397.94 ±46.64	0.625	332	336.29 ±30.44	0.115	328.30
(pg/ml)	Deficient	415.31 ±11.62	0.635	407.50	379.42 ±9.19	0.115	374.40
Active B12/Holo-	Normal	422.1 ±14.69	0.001	412.05	359.30± 12.62	0.170	363.10
TC Mothers (pmol/l)	Deficient	399.98 ±15.43	0.321	393.50	385.16± 12.17	0.163	387.10

Hb- Hemoglobin, RBC- Red blood cells, PCV- Packed cell volume /hematocrit, MCV – Mean corpuscular volume, RDW- Red cell distribution width, SE- Standard Error of mean, Holo-TC- Holo-transcobolamin, fl- fentolitre, ng/ml- nanogram/milliliter, pg- picogram, pmol- picomoles

Table 4 shows spearman correlation between different blood parameters in mothers and cord blood. CD320 levels in the mothers correlated positively with age, height and weight of mothers but negatively with BMI.CD320 levels of cord blood showed positive correlation with age and height of mothers but negative correlation with weight and BMI of mothers.CD320 levels of mothers showed a negative correlation with Hb, B12, active B12 and folate of the mothers.CD320 levels of the cord blood although showed a positive correlation with Hb of mothers, it showed a significant negative correlation with folate and Vit B12 and a significant negative correlation with active B12 of mothers.Similarly,CD320 levels of cord blood positively correlated with Hb and negatively with folate, Vit B12 and active Vit B12 in cord blood.

		Hb (M)	Folate (M)	Vit B12 (M)	Active B12 (M)	CD32 0 (M)	Hb (B)	Folate (B)	VitB12 (B)	Active B12 (B)	CD320 (B)
Age (M)	Correlation	.032	.120	071	002	.009	050	049	121	.092	.048
(111)	Significance	.644	.280	.529	.985	.930	.492	.661	.277	.369	.662
	Ν	209	83	82	98	98	192	83	83	97	86
Ht (M)	Correlation	.051	078	153	129	.071	006	051	307**	122	.026
()	Significance	.467	.484	.169	.206	.486	.937	.649	.005	.234	.815
	Ν	209	83	82	98	98	192	83	83	97	86
Wt	Correlation	.067	.061	.029	.045	.000	055	050	029	.052	138
(M)	Significance	.337	.582	.797	.663	.997	.448	.652	.797	.612	.204
	Ν	207	83	82	98	98	191	83	83	97	86
BMI	Correlation	.044	.110	.126	.109	075	114	033	.126	.077	195
(M)	Significance	.523	.322	.258	.286	.463	.116	.768	.257	.452	.072
	Ν	209	83	82	98	98	192	83	83	97	86
Hb	Correlation	1.00	.536**	.286**	.346**	060	.058	.511**	.250*	.174	.088
(M)	Significance		.000	.009	.000	.559	.427	.000	.023	.089	.423
	N	209	83	82	98	98	192	83	83	97	86
Folate (M)	Correlation		1.000	.271*	.418**	060	.074	.667**	.214	.322**	150
()	Significance			.014	.000	.592	.514	.000	.062	.005	.231

Table 4: Spearman correlation between maternal and cord blood parameters

	Ν	83	82	82	81	81	77	77	76	66
B12	Correlation		1.000	.686**	036	.075	.101	.516**	.496**	141
(M)	Significance			.000	.754	.508	.384	.000	.000	.262
	Ν		82	81	80	80	76	76	75	65
Activ e B12	Correlation			1.000	016	.093	.229*	.593**	.463**	256*
(M)	Significance				.877	.377	.045	.000	.000	.036
	N			98	95	93	77	77	78	67
CD320 (M)	Correlation				1.000	.064	.126	051	050	.189
(111)	Significance					.545	.278	.661	.665	.125
	N				98	93	76	76	78	67
Hb(B)	Correlation					1.000	065	.219*	.137	.130
	Significance						.564	.050	.191	.246
	Ν					192	81	81	93	82
Folate (B)	Correlation						1.000	.180	.172	033
(D)	Significance							.104	.121	.787
	Ν						83	83	82	71
Vit B12	Correlation							1.000	.508**	078
(B)	Significance								.000	.517
	Ν							83	82	71
Activ	Correlation								1.000	027
(B)	Significance							.000		.806
	Ν							82	97	83
CD320 (B)	Correlation									1.000
	Significance									
	Ν									86

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Hb- Hemoglobin, M- Mother, B- baby, Wt- weight, Ht- height, BMI- Body mass index.

Linear regression (Table 5) analysis was performed to study the predictors of CD320 levels in mothers and cord blood. Maternal CD320 levels were found to be associated with several maternal and neonatal features like education status, occupation and Active B12 status of mothers and MCV, MCH, MCHC, HDW and folate of cord blood. In the multivariate analyses, after adjusting for confounding factors like age, height, weight, BMI, education, occupation, income etc, it was found that poor education, better occupation and higher active B12 values were significantly associated with higher CD320 values in mothers and lower HDW values in cord blood were significantly associated with higher CD320 levels in mothers.

	CD320 of mothers							
Parameters	Univariate (<i>p</i> <0.2)		Multivariat	e (<i>p</i> <0.05)				
i ai ainetei ș	β (95% CI)	<i>p</i> -value	β (95% CI)	<i>p</i> -value				
Age of mothers	-2.93 (-9.49-3.63)	0.378	-	-				
Height of mothers	26.8 (-71.94-125.54)	0.591	-	-				
Weight of mothers	-0.302 (-2.87-2.27)	0.816	-	-				

Table 5: Multivariate regression analysis

BMI mothers	-4.04 (-12.31-4.24)	0.335	-	-
Education status	-33.17		-46.52	
mothers	(-65 111 22)	0.042	(-80 2-12 82)	0.008
Occupation mothers	(-0 <i>J</i> .111.22) 88 53		(-00.2-12.02)	
Occupation motifers	(25, 24, 151, 83)	0.007	(23 17 143 0)	0.007
Monthly family	1 70		(23.17-143.0)	
incomo	(10, 65, 37, 25)	0.931	-	-
Donity	12.52			
ranty	(20.96.55.90)	0.526	-	-
The of model one	(-28.80-33.89)			
rib of mothers	(207,0.21)	0.929	-	-
DDC	(-0.97-9.01)			
RBC mothers	-2.21	0.863	-	-
MCV oth ora	(-27.32-23.11)			
MC v motners	0.91	0.417	-	-
MCH	(-1.30-3.11)			
MCH mothers	0.195	0.933	-	-
MORE	(-4.41-4.80)			
MCHC mothers	-1.50	0.516	-	-
	(-6.0/-3.0/)			
HDW mothers	7.83	0.341	-	-
	(-8.46-24.13)			
Ferritin mothers	-0.18	0.654	-	-
	(-0.96-0.60)			
Folate mothers	-1.32	0.306	-	-
Dia u	(-3.88-1.23)			
B12 mothers	-0.03	0.461	-	-
	(-0.13-0.06)		0.74	
Active B12 mothers	0.442	0.177	0.76	0.033
	(-0.20-1.09)		(0.06-1.45)	
Cord blood Hb	2.76	0.601	-	-
	(-7.66-13.17)			
Cord blood RBC	-5.81	0.784	-	-
	(-47.91-36.29)			
MCV cord blood	2.55	0.026	_	-
	(0.31-4.78)			
MCH cord blood	5.84	0.176	_	-
	(-2.67-14.35)			
MCHC cord blood	-6.91	0.069	-	-
	(-14.36-0.54)			
HDW cord blood	-7.29		-8.13	0.040
	(-16.55 -1.97)	0.121	(-16.23—0.031	0.049
	(10000-1000))	
Ferritin cord blood	-0.14	0.302	_	-
	(-0.41-0.13)			
Folate cord blood	3.86	0.112	_	-
	(-0.92-8.65)			
B12 cord blood	-0.002	0.971	_	-
	(-0.09-0.08)			
Active B12 cord	-0.07	0.717	-	_
blood	(-0.45-0.31)			
CD320 cord blood	0.19	0.300	-	_
	(-0.17-0.56)	0.500		

Values in bold are statistically significant

BMI- body mass index, Hb- Hemoglobin, RBC- Red blood cells, PCV- Packed cell volume /hematocrit, MCV – Mean corpuscular volume, MCH- mean corpuscular hemoglobin, MCHC- mean corpuscular hemoglobin concentration, HDW- hemoglobin distribution width.

Table 6 and Figures 4 & 5 shows the results of CD320 immunohistochemical expression scores in placentas in the background of different micronutrients levels in mothers. Placental CD320 expression was found to be

higher in mothers (Fig 4) with deficient Hb, folate, Vitamin B12 and active B12 levels when compared to those with normal levels(Fig 3), albeit non-significantly.

Table 6: CD320 Immunohistochemistry score i	in relation to different micronutrients statu
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Parameter	Status	Minimum	Maximum	Mean(SD)	95% CI	<i>p</i> -value
		score	score			
Hb status	Normal	2	12	6.42 (3.8)	-0.48 to 4.33	0.45
	Anaemia	4	12	8.34 (3.17)]	
Folate	Normal	3	12	6.75 (2.60)	-0.17 to 4.44	0.06
	Low folate	3	12	8.89 (3.41)]	
Vit B12	Normal	4	12	7.75 (3.45)	-2.40 to 3.08	0.8
	Low Vit	3	12	8.09 (3.32)]	
	B12					
Active B12	Normal	3	12	7.59 (3.15)	-1.14 to 3.63	0.6
	Low	4	12	8.83 (3.56)		
	Active			. ,		
	B12					

Hb- haemoglobin, SD- standard deviation, CI- confidence interval



Figure 4: Shows the placental chorionic villi lined by the trophoblasts and which show mild intensity of the immunohistochemical staining for CD320. The bold black arrow points to the syncytiotrophoblasts which are stained mildly, while the bold orange arrows point to the cytotrophoblasts showing similar mild staining. The thin black arrows show mild staining of the fetal capillaries for CD320 immunostain. Original magnification X20.



Figure 5: Shows the placental chorionic villi lined by the trophoblasts showing intense immunohistochemical staining for CD320. The bold black arrow points to the syncytiotrophoblasts which are stained intensely, while the bold yellow arrow points to the cytotrophobblasts showing similar strong staining. The bold red arrow shows mild staining of the fetal capillaries for CD320 immunostain. Original magnification X20

4. Discussion

In the present study, pregnant mothers in last trimester of pregnancy were recruited along with their corresponding newborns. They were evaluated for important micronutrients like Vit B12, folate and ferritin with specific reference to the newly discovered Vit B12 receptor CD320.

Anaemia among pregnant women is highly prevalent in our country with the latest National Family Health Survey (NFHS-5) data showing 53.2% pregnant mothers being anaemic (Hb<11g/dl) (NFHS-5 2019-21). Our study too showed an almost similar prevalence of 59% of anaemia in the study participants with 35% of them having moderate anaemia, thus indicating that anaemia was highly prevalent in this vulnerable population despite the distribution of free iron and folic acid tablets by the Indian Government. Figure2 shows the distribution of various micronutrient deficiencies. Although the role played by iron deficiency is considered to be most important in development of nutritional anemia and is also the commonest micronutrient deficiency in developing countries (Krafft 2003), in our study, ferritin deficient mothers were lesser (54%) when compared to folate deficient (66.4%) and total VitB12 deficient (89%). Thus, a high prevalence of Vit B12 deficiency was a notable finding along with folate deficiency in our study. Literature search of different studies from the world and also from India showed that Vit B12 deficiency was highly prevalent in the range of 40-70% [Pathak 2007, Van den Broek 2000, Milman 2006, Katre 2010, Veena 2010). Dr Yajnik et al, 2008 have also found folic acid deficiency to be highly prevalent in their study conducted in pregnant women (Yajnik et al, 2008). This could be due to low Vit B12 intake in our country due to vegetarian diet and also that pregnant women are administered only iron and folic acid tablets in pregnancy. But in our study, compared to total VitB12 deficiency, lesser number of women (36.7%) were deficient in active B12. However, the figure shows that in contrast to mother's micronutrient status, except for total VitB12, cord blood showed sufficient levels of Hb, ferritin, folate and active B12 in majority of subjects which is in corroboration with other studies [Rima Obeid 2006, Rima Obeid 2005, Muthayya 2006, Adaikalakoteswari 2015, Murphy 2007, Koc A 2006) and thus indicating that like iron, Vit B12 and folate are transferred across placenta against a concentration gradient to maintain sufficient levels in the fetus.

CD320 has been recently discovered as the receptor for active B12. As VitB12 deficiency is very common in our country, and despite extensive literature search we couldn't come across any studies till date on the CD320 receptors in the world, we intended to study these receptors in both mothers and cord blood. Total Vit B12 is not considered to be a suitable predictor of Vit B12 levels in pregnant mothers as it gradually declines during pregnancy (Milman 2006, Koebnick 2002). CD320 circulates in the blood mostly bound to active B12 or holo-TC and which is available for tissue uptake and is also suggested to be more sensitive to early changes in the levels of total Vit B12 (Nexo 2002). Our study found higher CD320 levels in mothers with anaemia in comparison to normal mothers but was reverse in cord blood where the CD320 levels were lower in newborns of anaemic mothers. This increased levels of CD320 could be to meet increased requirement due to anaemia in mothers while cord blood had sufficient Hb levels. In case of total Vit B12, folate and ferritin, in both mothers and cord blood, CD320 levels were higher in deficiency state, which could be the compensatory mechanism to meet the increased requirement. However, in contrast, CD320 levels were lower in mothers with active B12 deficiency while in cord blood, the levels were higher from deficient mothers which could be due to the fact that active B12 deficiency was observed to be more in mothers in comparison to cord blood which showed normal active B12 levels in majority. However, there were no other studies elsewhere to compare our study results.

Spearman correlations were performed to study the relation between CD320 levels and various maternal and cord blood parameters. CD320 levels in mothers and cord blood correlated positively with age, height and weight, but negatively with BMI of mothers.CD320 levels of the cord blood were negatively correlated with weight of mothers thus indicating the role of maternal anthropometrical parameters on cord blood CD320 levels although not significantly. With respect to blood parameters, CD320 levels of mothers negatively correlated with Hb , total B12, active B12 and folate of the mothers while those of cord blood positively correlated with Hb of mothers. However, one study on non-pregnant women showed that the sCD320 levels were positively related to both total B12 and active B12 (Arendt 2012 & Arendt 2012). This is in contrast to our study which was conducted in pregnant women. Similarly, CD320 levels of cord blood positively correlated with Hb and negatively with folate, Vit B12 and active B12 of cord blood. These findings indicate that CD320 levels are mostly negatively related with micronutrient levels, which could be the response to deficiency state probably by increasing the expression of genes encoding CD320 receptors in order to meet the deficient levels. Studies on the genes coding CD320 receptors could be planned to throw light on this matter.

Better occupation of the mothers and higher active B12 values were significantly associated with increased CD320 values in mothers and lower HDW values in cord blood were significantly associated with higher CD320 levels in mothers in multivariate regression analyses while adjusting for maternal age, BMI, gravida, parity, BMI, occupation, family income, Vit B12 and folate levels. Our study being the only study on CD320 in both pregnant women and cord blood, there were no other studies to compare and analyze these findings. However, our study definitely shows the effect of both maternal and cord blood parameters on CD320 levels of both mothers and their newborns.

Expression of CD320 in placentas by immunohistochemistry shows increased expression of CD320, albeit nonsignificantly, in mothers with deficiency of iron, Vit B12, folate and active B12, thus pointing to definite role played by CD320 in their uptake and transfer from mothers to the developing foetus. CD320 receptors have been stated to be associated with cellular active B12 uptake and their association in our study with all the other micronutrients however point to their non-specific role in relation to active B12 and in turn point to the role of another receptor. Even Arendt et al could not find any association of CD320 levels with clinical findings and they also they did not find any evidence with regard to sCD320 acting as a novel biomarker for VitB12 deficiency [29]. Instead a study by Moestrup et al shows megalin to be the receptor having a high-affinity for the TC-B12 complex. They found that cells take up the Vit B12 after the TCB12 complex binds to megalin, which is followed by uptake of TC by the cells and degradation of TC. Thus, this finding proves the novel role of megalin receptor in Vit B12 homeostasis (Moestrup 1996). Thus, it needs to be followed up by further studies. There are however a few limitations in our study. Unfortunately, we could not measure urine CD320 levels and also the metabolite of Vit B12, methylmalonic acid (MMA). As CD320 levels are supposed to be more in urine in comparison to serum, its study in urine and also in relation to MMA would have added strength to our study.

To conclude, we found that Vit B12 deficiency was highly prevalent in the pregnant women in our study. CD320 receptors levels were for first time studied in the cord blood and although not significant, a definite correlation was observed between CD320 levels in the mothers and newborn cord blood which needs further evaluation.

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References

- Abuyaman, O., Andreasen, B.H., Kronborg, C., Vittinghus, E., Nexo, E. (2013). The soluble receptor for vitamin B12 uptake (sCD320) increases during pregnancy and occurs in higher concentration in urine than in serum. *PLoS One*,8(8):e73110. doi:10.1371/journal.pone.0073110
- Adaikalakoteswari, A., Vatish M., Lawson A., Wood C., Sivakumar K., McTernan P.G., Webster C., Anderson N., Yajnik C.S., Tripathi G., et al. (2015). Low maternal vitamin B12 status is associated with lower cord blood HDL cholesterol in white Caucasians living in the UK. *Nutrients*,7:2401–2414. doi: 10.3390/nu7042401
- Arendt, J.F., Nexo, E. (2012).Cobalamin related parameters and disease patterns in patients with increased serum cobalamin levels. *PLOS ONE*, 7: e45979 PubMed: 23029349
- Arendt, J.F., Quadros, E.V., Nexo, E. (2012). Soluble transcobalamin receptor, sCD320, is present in human serum and relates to serum cobalamin establishment and validation of an ELISA. *Clin Chem Lab Med*, 50: 515–519.
- Greibe, E., Andreasen, B.H., Lildballe, D.L., Morkbak, A.L., Hvas, A.M. et al.(2011). Uptake of cobalamin and markers of cobalamin status: a longitudinal study of healthy pregnant women. *Clin Chem Lab Med*, 49: 1877–1882.
- http://www.rchiips.org/nfhs/index.shtml
- Katre. P., Bhat, D., Lubree, H., Otiv, S., Joshi, S., Joglekar, C., Rush, E., Yajnik, C. (2010). Vitamin B12 and folic acid supplementation and plasma total homocysteine concentrations in pregnant Indian women with low B12 and high folate status. *Asia Pac J Clin Nutr*, 19(3):335–43.
- Koc, A., Kocyigit, A., Soran, M., Demir, N., Sevinc, E., Erel, O., Mil, Z. (2006). High frequency of maternal vitamin B12 deficiency as an important cause of infantile vitamin B12 deficiency in Sanliurfa province of Turkey. *Eur. J. Nutr*,45:291–297. doi: 10.1007/s00394-006-0598-7
- Koebnick, C., Heins, U.A., Dagnelie, P.C., Wickramasinghe, S.N., Ratnayaka, I.D., Hothorn, T., Pfahlberg, A.B., Hoffmann, I., Lindemans, J., Leitzmann, C. (2002). Longitudinal concentrations of vitamin B(12) and vitamin B(12)-binding proteins during uncomplicated pregnancy. *Clin Chem*, 48(6 Pt 1):928-33.
- Krafft, A., Huch, R., Breymann, C. (2003). Impact of parturition on iron status in non-anemic iron deficiency. *Eur J Clin Invest*,33:919–923.
- Laxmaiah, A. (2015). Vitamin B12 and folic acid: significance in human health. Indian Pediatr, 52(5):380-381.
- Lindgren, A., Kilander, A., Bagge, E., Nexo, E. (1999). Holotranscobalamin—a sensitive marker of cobalamin malabsorption. *Eur J Clin Invest*,29:321–9.
- Milman, N., Byg, K.E., Bergholt, T., Eriksen, L., Hvas, A.M. (2006). Cobalamin status during normal pregnancy and postpartum: a longitudinal study comprising 406 Danish women. *Eur J Haematol*, 76(6):521-5.
- Moestrup, S.K., Birn, H., Fischer, P.B. et al. (1996). Megalin-mediated endocytosis of transcobalamin-vitamin-B12 complexes suggests a role of the receptor in vitamin-B12 homeostasis. *Proc Natl Acad Sci U S A*, 93(16):8612-8617. doi:10.1073/pnas.93.16.8612
- Molloy, A.M., Kirke, P.N., Troendle, J.F., Burke, H., Sutton, M., Brody, L.C., et al. (2009). Maternal vitamin B12 status and risk of neural tube defects in a population with high neural tube defect prevalence and no folic acid fortification. *Pediatrics*,123:917–23. http://dx.doi.org/ 10.1542/peds.2008-1173.
- Murphy, M.M., Molloy, A.M., Ueland, P.M., Fernandez-Ballart, J.D., Schneede, J., Arija, V., Scott, J.M. (2007). Longitudinal study of the effect of pregnancy on maternal and fetal cobalamin status in healthy women and their offspring. *J. Nut*, 7;137:1863–1867. doi: 10.1093/jn/137.8.1863

- Muthayya, S., Dwarkanath, P., Mhaskar, M., Mhaskar, R., Thomas, A., Duggan, C., Fawzi, W.W., Bhat, S., Vaz, M., Kurpad, A. (2006). The relationship of neonatal serum vitamin B12 status with birth weight. *Asia Pac. J. Clin. Nutr*, 15:538–543.
- Nexo, E., Hvas, A.M., Bleie, O., Refsum, H., Fedosov, S.N., Vollset, S.E., Schneede, J., Nordrehaug, J.E., Ueland, P.M., et al. (2002). Holo-transcobalamin is an early marker of changes in cobalamin homeostasis. A randomized placebo-controlled study. *Clin Chem*, 48:1768–71.
- Nielsen, M.J., Rasmussen, M.R., Andersen, C.B., Nexo, E., Moestrup, S. K. (2012). Vitamin B12 transport from food to the body's cells—a sophisticated, multistep pathway. *Nat Rev Gastroenterol Hepatol*,9: 345–354. doi:10.1038/nrgastro.2012.76
- Pathak, P., Kapil, U., Yajnik, C.S., Kapoor, S.K., Dwivedi, S.N., Singh, R. (2007). Iron, folate, and vitamin B12 stores among pregnant women in a rural area of Haryana state, India. *Food Nutr Bull*, 28(4):435-438.
- Pepper, M.R., Black, M.M. (2011). B12 in fetal development. Semin Cell Dev Biol 2011; 22: 619–623. doi:10.1016/j.semcdb.2011.05.005.
- Quadros, E.V., Nakayama, Y., Sequeira, J. M. (2009). The protein and the gene encoding the receptor for the cellular uptake of transcobalamin-bound Cobalamin. *Blood* ,113: 186–192. doi:10.1182/blood-2008-05-158949.
- Rima, Obeid., Anne, L. Morkbak., Winfried, Munz., Ebba, Nexo., Wolfgang, Herrmann. (2006). The Cobalamin-Binding Proteins Transcobalamin and Haptocorrin in Maternal and Cord Blood Sera at Birth. *Clinical Chemistry*,52(2):263–269. https://doi.org/10.1373/clinchem.2005.057810
- Rima, Obeid., Winfried, Munz., Monika, Jäger., Werner, Schmidt., Wolfgang, Herrmann. (2005). Biochemical indexes of the B vitamins in cord serum are predicted by maternal B vitamin status. AJCN ,82(1):133–139. https://doi.org/10.1093/ajcn/82.1.133
- Van, den, Broek N.R., Letsky, E.A. (2000). Etiology of anemia in pregnancy in south Malawi. *Am J Clin Nutr*, 72(1):247S-256S.
- Veena, S.R., Krishnaveni, G.V., Srinivasan, K., Wills, A.K., Muthayya, S., Kurpad, A.V., Yajnik, C.S., Fall, C.H. (2010). Higher maternal plasma folate but not vitamin B12 concentrations during pregnancy are associated with better cognitive function scores in 9- to 10- year-old children in South India. J Nutr, 140(5):1014–22.
- WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity VMNIS | Vitamin and Mineral Nutrition Information System WHO/NMH/NHD/MNM/11.1 Microdeficiencies: Iron deficiency anaemia, http://www.who.int/ nutrition/topics/ida/en/
- WHO. (1995). Physical Status: The Use and Interpretation of Anthropometry. World Health Organization; Geneva, Switzerland: p. 329. Report of a WHO Expert Committee.
- Yajnik, C.S., Deshpande, S.S., Jackson, A.A., Refsum, H., Rao, S., Fisher, D.J., Bhat, D.S., Naik, S.S., Coyaji, K.J., Joglekar, C.V., Joshi, N., Lubree, H.G., Deshpande, V.U., Rege, S.S., Fall, C.H. (2008). Vitamin B12 and folate concentrations during pregnancy and insulin resistance in the offspring: the Pune Maternal Nutrition Study. *Diabetologia*,51(1):29–38.
- Yetley, E.A., Pfeiffer, C.M., Phinney, K.W., Bailey, R.L., Blackmore, S., Bock, J.L., Brody, L.C., Carmel, R., Curtin, L.R., Durazo-Arvizu, R.A., et al. (2011). Biomarkers of vitamin B-12 status in NHANES: A roundtable summary. *Am. J. Clin. Nutr*, 94:3138–3218. doi: 10.3945/ajcn.111.013243



Epidemiological Distribution of Risk Factors Contributing to Symptomatic Helicobacter Pylori Infection in Diyala Province, Iraq

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Abstract

Helicobacter pylori is a worldwide spread bacterium, that invades the submucosal membrane of the stomach via feco-oral or feco-fecal transmission, causing several gastric and duodenal diseases. The study aims to assess risk factors for the symptomatic *Helicobacter pylori* infection and their epidemiological distribution. A cross-sectional study was conducted from October 2020 to May 2021 in Baquba, Iraq. It was conducted on a sample of outpatient patients who complain of various symptoms of gastrointestinal disorders, where the sample size was 194 patients, their ages ranged from 15 to 70 years, the main characteristics taken up in the study included age, sex, and place of residence, smoking status. H. pylori infection was recognized by identifying anti-H. Pylori (IgG) in patient serum using the ELISA technique. Chi-squared and Binomial tests were adopted to assess the difference between study variables. The study shows that the highest rate of infection was among patients aged 15-30 years (45.7%), most patients were females (66.3%), and the majority of them (68.5%) were rural dwellers. In conclusion, younger ages, female gender, rural dwellers, non-smokers, and those who complained of stomach pain were significantly associated with the helicobacter pylori infection.

Keywords: Helicobacter Pylori, Epidemiological Distribution, Risk Factors, Symptomatic Infection

1. Introduction

Helicobacter pylori, is a worldwide spread a pathogenic bacterium present in the gastric mucosa of infected humans, with a spiral shape, was first isolated by Warren and Marshall in 1982 and soon after with chronic gastritis, the global prevalence of *H. pylori* is over 50%, mostly in developing countries (Shurooq et al., 2014; Aqeel et al., 2020; Frenck et al., 2003; Peleteiro et al., 2014). As reported by primary care physicians, 78% of physicians believe that contaminated water and foods are the commonest sources of the spread of infection causing chronic gastritis that may turn into gastric carcinogenesis later (Ahmed et al., 2009; Cohen et al., 2021). This bacterium invades the submucosal membrane of the stomach as well as secretes the enzyme urease, which converts the acidic medium of the stomach to alkaline and thus is safe from stomach acids (Shiota et al., 2013).

50% of general population carries these bacteria without symptoms, which makes detection of infection late and then it either transmitted through oral-oral or fecal-oral route, it has been isolated from dental plaque, saliva and feces (Plummer et al., 2007). The clinical outcome of *H. pylori* infection may depend on several factors, such as age, environmental factors, immune response, and genetic factors (Plummer et al., 2007). And it varies in different societies and geographical locations, as social and demographic characteristics, economic status, health practices and lifestyles help spread these bacteria (Kamholz., 2006; Olokoba et al., 2013; World gastroenterology organization global guideline., 2011). The incidence of infection among the elderly who do not show symptoms of the disease is about 40-60%, Helicobacter pylori have been identified as a Class A carcinogenesis of the gastric mucosa (Nicoline et al., 2013; Schistosomes., 1994). It could be because of that mismanagement of H. pylori infection thus contributes to tumors in a different oorgansof the gastrointestinal tract, most commonly stomach, duodenal, pancreatic and liver cancers, especially in cases with a past history of hepatitis virus infection, which increases the development of the disease (Rabelo-Gonçalves et al., 2015).

2. Methods

A cross-sectional study conducted from October 2020 to May 2021 in Baquba, Iraq. This city is the center of Diyala province, whose population is made up of heterogeneous groups of various spectra and sects.

2.1 Sample collection

This study was conducted on a sample of outpatient patients/ Doctors Street in Baquba city who complained of various symptoms of gastrointestinal disorders, where the sample size was 184 patients. The sample size was based on total patients behaving the sample. Where, the level of confidence was 95% and margin of error was 5%, using the following formula:

Unlimited population:
$$n = \frac{z^2 \times \hat{p}(1-\hat{p})}{\varepsilon^2}$$

Finite population: $n' = \frac{n}{1 + \frac{z^2 \times \hat{p}(1-\hat{p})}{\varepsilon^2 N}}$
where
 z is the z score
 ε is the margin of error
 N is the population size
 \hat{p} is the population proportion

2.2 Eligibility and laboratory assessment

The study included persons suspected of contracting helicobacter pylori infection in the 15-70 age groups. Individuals who used antimicrobials 2 weeks before the date of detection were excluded from the study. Pregnant and nursing mothers were also excluded. The investigators invited patients to participate voluntarily in the study with an explanation and clarification of the study protocol. The characteristics taken up in the study included age, sex and place of residence, smoking status, the main complaint that prompted the patient to request medical assistance, the duration of an early or late diagnosis of the infection and the statement of the previous history of gastrointestinal symptoms through the use of a simple questionnaire constructed.

The intravenous blood sample was collected from each participating patient by 6-8 ml. Each blood sample was immediately divided into two containers, one containing anticoagulants, EDTA and another without anticoagulants. Normal container samples are allowed to clot at room temperature, then undergo centrifuge at 3,000 rpm for 5 minutes to obtain the serum, which has been transferred to a sterile Eppendorf tube and stored at -20°C until further analysis. H. pylori infection was recognized by identifying anti-H. pylori (IgG) in patient serum using the-ELISA Indirect Enzyme-Linked Immune Sorbent Assay corresponding to the company's protocol (Abcam, UK). ELISA Kit has 94.4% sensitivity and 92% specificity. All ELISA conditions were

achieved in duplicate and each sample was recorded with a mean IgG titter of over 20 U/ml as positive (reactive) for infection.

2.3 Statistical analysis

To analyze the data statistically, the information for each questionnaire was entered into Microsoft excel. Statistical analysis of the results of the study was carried out using the Statistical Package of Social Sciences (SPSS) program, Version 24. Search results were expressed as numbers and percentages. Chi-squared and Binomial tests were adopted to assess the difference between study variables. A P-value of less than 0.05 was considered significant.

3. Results

Table1 Expresses the rate of helicobacter pylori infection of patients based on their demographic characteristics, showing the highest rate (45.7%) significantly among patients aged 15-30 years and the lowest rate (20.7%) among those over 55 years of age, most patients are significantly females (66.3%), and a majority of whom are significantly rural dwellers (68.5%).

Demographic characteristics	Variables	Ν	º⁄0	p-value
Age/years	15-35 years	84	45.7	0.000
	35-55 years	62	33.7	
	55+	38	20.7	
	Total	184	100.0	
Gender	Males	62	33.7	0.000
	Females	122	66.3	
	Total	184	100.0	
Place	Urban	58	31.5	0.000
	Rural	126	68.5	
	Total	184	100.0	

Table 1: Distribution of study subjects based on their demographic characteristics

Table 2 shows that vast majority patients with helicobacter pylori infection are significantly non-smokers and account for 87% of the sample (p=0.000).

Table 2. Distribut	ion of study	subjects based (Shi then shioking status	
Table 2. Distribut	ion of study	subjects based	on their smaking status	

Smoking status	No.	%	p-value
Smoker	24	13.0	0.000
Non-smoker	160	87.0	
Total	184	100.0	

Most patients with helicobacter pylori infection at the beginning of symptoms mainly complained of epigastric pain with nausea and vomiting at a rate of 76.1%, as for the duration of complaints of these symptoms until going to the physician, the highest rate of them 43.5% significantly complained of symptoms for less than one month, while the lowest 16.3% complained for more than a year, as shown in the table 3.

Table 3: Distribution of study	v subjects based	on chief complaint
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Patient's complaint	Variables	Ν	%	p-value
Chief complaint	Only epigastric pain	44	23.9	0.000
	Epigastric pain associated with nausea and vomiting	140	76.1	
	Total	184	100.0	
Early detection of HP	Yes	80	43.5	0.090
infection (< 1 months	No	104	56.5	
from	Total	184	100.0	

the begging of symptoms)

Table 4 Study demonstrates that most patients with helicobacter pylori infection (78.3%) reported a previous history of gastrointestinal disturbances.

Table 4. Distribution of study subjects based on previous instory for of symptoms				
History of previous gastrointestinal symptoms	Ν	%	p-value	
Yes	144	78.3	0.000	
No	40	21.7		
Total	184	100.0		

Table 4: Distribution of study subjects based on previous history for GI symptoms

4. Discussion

H. pylori infection is one of the most prevalent chronic and common bacteriological diseases lead to many upper stomach and duodenal diseases (Seid & Demsiss., 2018). Although many researches (15-19) have reported that infection rates with helicobacter pylori increase with age (Pilotto & Franceschi., 2014; Neri et al., 1996; Salles., 2007; Hou et al., 2019; Huang et al., 2021). And yet our study has proven otherwise, as the rate of H. pylori infection increases significantly at younger ages, especially at 15-30 years, and decreases as age increases. This is consistent with other Iraqi and Arabic studies, because the highest rate of infection was among patients aged 20-30 years in Sulaimani, Iraq (Al-Windi., 2013), 16-18 years in Duhok, Iraq (Al-Brefkani et al., 2021), and age group of 30 years old in Emirates Arabia (Khoder et al., 2019). Other non-Arab studies have also found that rates of H. Pylori infections are rising in younger age groups (Syam et al., 2015; Namyalo et al., 2021). In this study, infections rise significantly among younger ages, and this is consistent with other investigations (Khoder et al., 2019; Namyalo et al., 2021). This may be because H. pylori bacterium is often acquired in childhood, especially when one of family members becomes infected with this bacterium then transmits it to others at an early age as a result of sharing food tools, and it can last for life [Namyalo et al., 2021; Weyermann et al., 2009; O'Ryan et al., 2015). This study showed that rates of H pylori infections are significantly higher in females and this is in line with other studies [(Al-Brefkani et al., 2021; Khoder et al., 2019, 28]. It may be because females are more interested in preparing food and spend more time in the kitchen than males (Majeed et al., 2020). In addition to that, rates of gender-related H. pylori infections are often vary based on the country's development index and geographical area location (Al-Brefkani et al., 2021). For the place of residence, most patients infected with H pylori were significantly from rural areas. This finding is similar to two other Iraqi studies (Al-Brefkani et al., 2021; Majeed et al., 2020). The cause may be due to several factors, the most important of which are poor living conditions, lack of personal hygiene, poor environmental conditions, big families, and lack of education in rural areas compared to urban (Al-Brefkani et al., 2021). In this study, smoking has not played a significant role in high rates of H pylori infection among patients with symptoms, but the contrary has been shown because most patients were significantly non-smokers. These findings are close to other studies (Al-Brefkani et al., 2021; (Khalifa et al., 2014; Módena et al., 2007), as cigar smoking is not associated with H pylori infection. The study showed that helicobacter pylori infection was significantly higher among non-smokers. The reason for this is that most patients in the study sample are females, and smoking among the Iraqi women's community is known to be limited. Three quarters of patients with helicobacter pylori infection at the beginning of symptoms mainly complained of epigastric pain associated with nausea and vomiting. These results are comparable to an Egyptian study (Gomaa et al., 2019), and another Brazilian study (Faintuch et al., 2014). The latter revealed that most patients with H pylori (68%) reported complaints of epigastric pain. This explains that individuals with these symptoms due to indigestion are twice more likely to develop H pylori infection than those who have not shown symptoms (Aminde et al., 2019). Early detection of H pylori infection can prevent the development of the disease and its transformation into chronic inflammation and also reduce the atrophy of the mucosa of the stomach and thus prevent stomach cancer (Yang & Hu., 2021). However, in less than half of patients, the study detected H pylori infection with no significant difference (p=0.090). This may be due to the effect that some patients practice self-medication or alternative medicine to treat symptoms of the disease without regard to the type and causes of the disease. The results of this study showed that patients with a history of gastrointestinal symptoms were significantly more likely to develop Helicobacter pylori infection. That's consistent with other studies (Melese et al., 2019; Hojati et al., 2021). This could be due to bacteria adapting to changes in the stomach in terms of pH, gastric lining wall and stomach ulceration in patients with a previous history of gastrointestinal symptoms.

Conclusion

In conclusion: younger ages, female gender, rural dwellers, non-smokers, who complain of stomach pain associated with vomiting and nausea, those with a previous history of gastrointestinal symptoms were significantly associated with the helicobacter pylori infection.

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References

- Ahmed, S., Salih, M., Jafri, W., Ali Shah, H. & Hamid, S. (2009). Helicobacter pylori infection: approach of primary care physicians in a developing country. *BMC Gastroenterology*, 9(1), 23. https://doi.org/10.1186/1471-230X-9-23
- Al-Brefkani, A. M. T., Naqid, I. A., Yahya, N. B., & Hussein, N. (2021). Seroprevalence and risk factors associated with Helicobacter pylori infection among children aged less than 18 years old in Duhok Province, Iraq: Prevalence and risk factors for H. pylori infection. *Journal of Contemporary Medical Sciences*, 7(3), 152–157.
- Al-Windi, A., Centre for Family and Community Medicine, Stockholm, Karolinska Institute, Huddinge, Sweden, Hussain, A. H., Salih, N., Sulaimani Technical College & Kurdistan institution for strategic studies and scientific research. (2013). Seroprevalence of anti-Helicobacter pylori antibodies in population of Sulaimani governorate/Kurdistan Region/Iraq. *Journal of Zankoy Sulaimani - Part A*, 15(3), 175–185. https://doi.org/10.17656/jzs.10267
- Aminde, J. A., Dedino, G. A., Ngwasiri, C. A., Ombaku, K. S., Mahop Makon, C. A. & Aminde, L. N. (2019). Helicobacter pylori infection among patients presenting with dyspepsia at a primary care setting in Cameroon: seroprevalence, five-year trend and predictors. *BMC Infectious Diseases*, 19(1), 30. https://doi.org/10.1186/s12879-019-3677-0
- Aqeel, A., & Luma, A. (2020) Seropositivity in Helicobacter Pylori Asymptomatic Infection and Its Correlation with Intensity And Duration of Cigarette Smoking. *Biochemical and Cellular Archives*, 20 (1), 0000-000
- Cohen, D. & Muhsen, K. (2012). Association between Helicobacter pylori colonization and glycated hemoglobin levels: is this another reason to eradicate H. pylori in adulthood? *The Journal of Infectious Diseases*, 205(8), 1183–1185. https://doi.org/10.1093/infdis/jis110
- D Majeed, P., Department of Nursing-Erbil Medical Technical Institute- Erbil Polytechnic University-Erbil -Iraq & Jwan Saleh Khoshnaw, K. (2020). Seroprevalence of Helicobacter pylori infection among patients with gastroduodenal disorders in Erbil city. *Diyala Journal of Medicine*, 18(2), 91–101. https://doi.org/10.26505/djm.18014880818
- Faintuch, J. J., Silva, F. M., Navarro-Rodriguez, T., Barbuti, R. C., Hashimoto, C. L., Rossini, A. R. A. L., Diniz, M. A. & Eisig, J. N. (2014). Endoscopic findings in uninvestigated dyspepsia. *BMC Gastroenterology*, 14(1), 19. https://doi.org/10.1186/1471-230X-14-19
- Frenck, R. W., Jr & Clemens, J. (2003). Helicobacter in the developing world. *Microbes and Infection*, 5(8), 705–713. https://doi.org/10.1016/s1286-4579(03)00112-6
- Gomaa, A., Hassan, E. & Youssef, H. (2019). Prevalence of Helicobacter pylori in dyspeptic patients and outcome of load regimen on its eradication. Fayoum *University Medical Journal*, 4(1), 11–20. https://doi.org/10.21608/fumj.2019.65930
- Hojati, S. A., Kokabpeyk, S., Yaghoubi, S., Joukar, F., Asgharnezhad, M. & Mansour-Ghanaei, F. (2021). Helicobacter pylori infection in Iran: demographic, endoscopic and pathological factors. *BMC Gastroenterology*, 21(1), 355. https://doi.org/10.1186/s12876-021-01931-1
- Hou, B., Zhang, M., Liu, M., Dai, W., Lin, Y., Li, Y., Gong, M. & Wang, G. (2019). Association of active Helicobacter pylori infection and anemia in elderly males. *BMC Infectious Diseases*, 19(1). https://doi.org/10.1186/s12879-019-3849-y
- Huang, Q., Jia, X., Chu, Y., Zhang, X. & Ye, H. (2021). Helicobacter pylori infection in geriatric patients: Current situation and treatment regimens. *Frontiers in Medicine*, 8, 713908. https://doi.org/10.3389/fmed.2021.713908

- Kamholz, S. L. (2006). Pulmonary and cardiovascular consequences of smoking. *Clinics in Occupational and Environmental Medicine*, 5(1), 157–171, x. https://doi.org/10.1016/j.coem.2005.10.001
- Khalifa, M. A. A., Khodiar, S. E.-F. & Almaksoud, A. A. (2014). Cigarette smoking status and Helicobacter pylori infection in non-ulcer dyspepsia patients. *The Egyptian Journal of Chest Diseases and Tuberculosis*, 63(3), 695–699. https://doi.org/10.1016/j.ejcdt.2014.03.007
- Khoder, G., Muhammad, J. S., Mahmoud, I., Soliman, S. S. M. & Burucoa, C. (2019). Prevalence of Helicobacter pylori and its associated factors among healthy asymptomatic residents in the United Arab Emirates. *Pathogens*, 8(2), 44. https://doi.org/10.3390/pathogens8020044
- Melese, A., Genet, C., Zeleke, B. & Andualem, T. (2019). Helicobacter pylori infections in Ethiopia; prevalence and associated factors: a systematic review and meta-analysis. *BMC Gastroenterology*, 19(1), 8. https://doi.org/10.1186/s12876-018-0927-3
- Módena, J. L. P., Acrani, G. O., Micas, A. F. D., Castro, M. de, Silveira, W. D. da, Módena, J. L. P., Oliveira, R. B. de & Brocchi, M. (2007). Correlation between Helicobacter pylori infection, gastric diseases and life habits among patients treated at a university hospital in Southeast Brazil. *The Brazilian Journal of Infectious Diseases: An Official Publication of the Brazilian Society of Infectious Diseases*, 11(1), 89–95. https://doi.org/10.1590/s1413-86702007000100020
- Namyalo, E., Nyakarahuka, L., Afayoa, M., Baziira, J., Tamale, A., Atuhaire, G. C. & Kungu, J. M. (2021). Prevalence of Helicobacter pylori among patients with gastrointestinal tract (GIT) symptoms: A retrospective study at selected Africa air rescue (AAR) clinics in Kampala, Uganda, from 2015 to 2019. *Journal of Tropical Medicine*, 2021, 9935142. https://doi.org/10.1155/2021/9935142
- Neri, M. C., Lai, L., Bonetti, P., Baldassarri, A. R., Monti, M., De Luca, P., Cunietti, E. & Quatrini, M. (1996). Prevalence of Helicobacter pylori infection in elderly inpatients and in institutionalized old people: correlation with nutritional status. *Age and Ageing*, 25(1), 17–21. https://doi.org/10.1093/ageing/25.1.17
- Nicoline, F. T., Rol & N, N. (2013). A South African perspective on Helicobacter pylori: Prevalence, epidemiology and antimicrobial chemotherapy. *African Journal of Microbiology Research*, 7(21), 2430–2437. https://doi.org/10.5897/ajmr2013.5594
- Olokoba, A. B., Gashau, W., Bwala, S., Adamu, A. & Salawu, F. K. (2013). Helicobacter pylori infection in Nigerians with dyspepsia. *Ghana Medical Journal*, 47(2), 79–81.
- O'Ryan, M. L., Lucero, Y., Rabello, M., Mamani, N., Salinas, A. M., Peña, A., Torres-Torreti, J. P., Mejías, A., Ramilo, O., Suarez, N., Reynolds, H. E., Orellana, A. & Lagomarcino, A. J. (2015). Persistent and transient Helicobacter pylori infections in early childhood. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 61(2), 211–218. https://doi.org/10.1093/cid/civ256
- Peleteiro, B., Bastos, A., Ferro, A. & Lunet, N. (2014). Prevalence of Helicobacter pylori infection worldwide: a systematic review of studies with national coverage. *Digestive Diseases and Sciences*, 59(8), 1698–1709. https://doi.org/10.1007/s10620-014-3063-0
- Pilotto, A. & Franceschi, M. (2014). Helicobacter pylori infection in older people. *World Journal of Gastroenterology*: WJG, 20(21), 6364–6373. https://doi.org/10.3748/wjg.v20.i21.6364
- Plummer, M., van Doorn, L.-J., Franceschi, S., Kleter, B., Canzian, F., Vivas, J., Lopez, G., Colin, D., Muñoz, N. & Kato, I. (2007). Helicobacter pylori cytotoxin-associated genotype and gastric precancerous lesions. Journal of the National Cancer Institute, 99(17), 1328–1334. https://doi.org/10.1093/jnci/djm120
- Rabelo-Gonçalves, E. M., Roesler, B. M. & Zeitune, J. M. (2015). Extragastric manifestations of Helicobacter pylori infection: Possible role of bacterium in liver and pancreas diseases. *World Journal of Hepatology*, 7(30), 2968–2979. https://doi.org/10.4254/wjh.v7.i30.2968
- Salles, N. (2007). Infection à Helicobacter pylori chez la personne âgée. *La Revue de medecine interne*, 28(6), 400–411. https://doi.org/10.1016/j.revmed.2007.01.017
- Schistosomes, liver flukes and Helicobacter pylori. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. (1994). *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, 61, 1–241.
- Seid, A. & Demsiss, W. (2018). Feco-prevalence and risk factors of Helicobacter pylori infection among symptomatic patients at Dessie Referral Hospital, Ethiopia. BMC Infectious Diseases, 18(1). https://doi.org/10.1186/s12879-018-3179-5
- Shiota, S., Murakawi, K., Suzuki, R., Fujioka, T. & Yamaoka, Y. (2013). Helicobacter pylori infection in Japan. *Expert Review of Gastroenterology & Hepatology*, 7(1), 35–40. https://doi.org/10.1586/egh.12.67
- Rasha, R. (2014). Detection of Helicobacter Pylori IgG and IgA, Serum Biomarkers CA19-9 and CEA in Patients with Gastrointestinal Diseases. *Iraqi Journal of Pharmaceutical Sciences*, 23(1), 68-72
- Syam, A. F., Miftahussurur, M., Makmun, D., Nusi, I. A., Zain, L. H., Zulkhairi, Akil, F., Uswan, W. B., Simanjuntak, D., Uchida, T., Adi, P., Utari, A. P., Rezkitha, Y. A. A., Subsomwong, P., Nasronudin, Suzuki, R. & Yamaoka, Y. (2015). Risk factors and prevalence of Helicobacter pylori in five largest islands of Indonesia: A preliminary study. PloS One, 10(11), e0140186. https://doi.org/10.1371/journal.pone.0140186

- Weyermann, M., Rothenbacher, D. & Brenner, H. (2009). Acquisition of Helicobacter pylori infection in early childhood: independent contributions of infected mothers, fathers, and siblings. *The American Journal of Gastroenterology*, 104(1), 182–189. https://doi.org/10.1038/ajg.2008.61
- World gastroenterology organisation global guideline: Helicobacter pylori in developing countries. (2011). *Journal of Clinical Gastroenterology*, 45(5), 383–388. https://doi.org/10.1097/mcg.0b013e31820fb8f6
- Yang, H. & Hu, B. (2021). Diagnosis of Helicobacter pylori Infection and Recent Advances. Diagnostics (Basel, Switzerland), 11(8), 1305. https://doi.org/10.3390/diagnostics11081305