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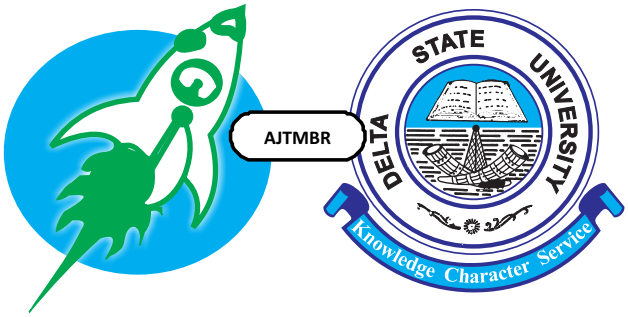

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Focus and Scope

The African Journal of Tropical Medicine and Biomedical Research is a multidisciplinary and international journal published by the College of Health Sciences, Delta State University of Abraka, Nigeria. It provides a forum for Authors working in Africa to share their research findings on all aspects of Tropical Medicine and Biomedical Sciences and to disseminate innovative, relevant and useful information on tropical medicine and biomedical sciences throughout the continent. The journal will publish original research articles, reviews, editorials, commentaries, short reports, case reports and letters to the editor. Articles are welcome in all branches of medicine and dentistry including basic sciences (Anatomy, Biochemistry, Physiology, Pharmacology, Psychology, Nursing etc) and clinical sciences (Internal Medicine, Surgery, Obstetrics and Gynaecology, Dental surgery, Child Health, Laboratory Sciences, Radiology, Community Medicine, etc). Articles are also welcome from social science researchers that document the intermediating and background social factors influencing health in countries of Africa. Priority will be given to publication of articles that describe the application of the principles of primary health care in the prevention and treatment of diseases.

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Original Articles

Original Articles

Blood pressure and associated risk factors of hypertension in patients attending a Family Medicine Clinic in Delta State, Nigeria.

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Abstract

Introduction: Hypertension is a significant public health issue globally. In recent times, its prevalence in Nigeria is experiencing an upsurge due to the increase in the risk factors associated with hypertension and many persons are unaware of their status. The study assessed the blood pressure pattern and associated risk factors of hypertension in patients attending a Family Medicine Clinic in Delta State, Nigeria.

Materials and methods: It was a cross-sectional study conducted in the Family Medicine Clinic, Delta University Teaching Hospital, Oghara, Nigeria. Participants were recruited using systematic random sampling method. Interviewer administered questionnaire was used to collect data and data was analyzed using Statistical Product and Service Solution version 23. The level of significance of analysis was set at $p < 0.05$.

Results: The study had 235 adults with a mean age of 45.1 ± 13.7years, with more females (63.0%) than males (37.0%). The prevalence of hypertension was 29% and 30.2% had prehypertension. Increasing age was significantly associated with hypertension, as hypertension occurred more in the elderly (p= <0.001). Also, hypertension occurred more in participants who were separated (71.4%) and widowed (62.5%) than those who were married. This was statistically significant. Obesity(p=0.002) and a positive family history (p=0.001) were significantly associated with increased risk of hypertension.

Conclusion: Increasing age, obesity and a positive family history were associated with increased risk of hypertension. There is need to educate the public on hypertension risk factors and to adopt healthy lifestyle practices to promote prevention and control of hypertension.

Keywords: Blood pressure**,** Hypertension, Patients, Risk factors**.**

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INTRODUCTION

Commonly referred to as a "silent killer", hypertension is often asymptomatic and many hypertensive patients remain undiagnosed and untreated and only present following a complication. [1] It has been identified as a significant modifiable risk factor for cardiovascular diseases.[2] Globally, about 1.4billion people have hypertension and is responsible for 10 million premature deaths annually.[3] A World Health Organization survey reported that about 44% of men and 25% of women had never checked their blood pressure, thus the prevalence of hypertension may be higher than what is reported.[3] The prevalence of hypertension is rising globally especially in the low- and middle-income countries at a rate of 8% per decade.[3] This is due to the upswing of the risk factors of hypertension and many persons are unaware of their status.[4] Unfortunately, the upsurge of hypertension in Sub-Saharan Africa has made it a significant public health problem.[5]

In Nigeria, the estimated age-adjusted prevalence of hypertension between 1995 and 2020 rose from 8.5% to 32.5%.[6]

The development of hypertension results from the interplay of complex and inter-related factors known as risk factors.[7] A risk factor can be described as an attribute, characteristic or exposure of a person that is significantly associated with the development of a disease.[8] The risk factors associated with hypertension can be classified into modifiable and non-modifiable risk factors.[7] The modifiable risk factors of hypertension are attributes, characteristics, exposure or lifestyle patterns that can be changed or altered to prevent the development of the disease.[9] They include tobacco use, high salt intake, consumption of saturated fat, alcohol consumption, physical inactivity, obesity and environmental stress.[8, 9] While the non-modifiable risk factors are traits or characteristics in a person that cannot be changed or adjusted and so almost nothing can be done to control these factors.[9] These factors include age, gender, family history, genetic composition and race. [9, 10] A population based cross-sectional study in Dubai revealed that obese persons were five times more likely to have hypertension while those that were physically active were less likely to develop hypertension. [10] Also, low physical activity and alcohol consumption were associated with hypertension in a national survey on hypertension conducted in Nigeria.[6]

Moreover, the burden of hypertension is likely to be influenced by the progressive increase in the ageing population, obesity, sedentary lifestyle and high sodium consumption, hence attention need to be focused on these risk factors, so as to curb their effect on hypertension.[11] In recent times, the low- and middle- income countries, Nigeria inclusive have experienced rapid westernization of their lifestyle leading to adoption of unhealthy dietary and lifestyle habits.[1,12] Also, associated with this is the urbanization process that has led to changes in the type of jobs and means of transportation utilized by people resulting in sedentary living. [1] All these can lead to increased vulnerability to the modifiable risk factors of hypertension and hence a rise in the prevalence of hypertension. [12]

The emerging epidemic of hypertension and the ensuing cardiovascular diseases can therefore be controlled by identifying risk factors, promoting routine blood pressure screening and adoption of healthy lifestyle measures to curtail the risk factors of hypertension and thus control blood pressure. [1,3] This in the long term is more cost-effective, considering the high clinical and economic cost attributed to hypertension.[3] Thus, the study seeks to assess the blood pressure and associated risk factors of hypertension in patients seen in a Family Medicine clinic in Delta state, Nigeria.

MATERIALS AND METHODS

A hospital based cross sectional study carried out from November, 2023 to January, 2024.

It was conducted at the Family Medicine Clinic in Delta State University Teaching Hospital, Oghara (DELSUTH). The hospital is a 180-bed ultra-modern facility with many clinical specialties and provides primary, secondary and tertiary healthcare services to Delta indigenes and its adjoining states. It is located in Oghara, Ethiope West Local Government Area of Delta State, South-South Nigeria.

Adults (age 18 years and above) attending the Family Medicine Clinic in DELSUTH. Clinical records of the clinic reported that a total of 4,997 adult patients attended the clinic in the year 2022.

Adults aged 18 years and above willing to participate in the study with the ability to give informed consent. Critically ill patients.

The sample size was determined using the formula, $n = z^2 pq/d^2$, [13] using z as 95% of confidence level, d as 5% margin of error and p the estimated proportion with the attribute of interest (21.0%) from a previous study,[9] and n the minimum sample size was 255. The number of patients seen in the clinic in the previous year was < 10,000, the sample size was adjusted using; $nf = n/(1+ n/N)$,[13] where, n was the desired sample size when the population is >10,000, and N was population of patients. Thus, nf, the desired sample size when the population is <10,000, was 243. So, the minimum sample size was 243.

Participants were recruited using systematic random sampling method. Using $K= N/n$,[13] where K was the sampling interval, N, the sampling frame (4997 patients aged 18 years and above were seen in 12 months, so 1666 patients were seen in 3 months) and n was 243. A sampling interval of 6 was obtained. The first subject was selected by simple random and every 6th participants was selected by systematic random sampling until the required sample size was met.

An interviewer administered questionnaire was used to collect data. The questionnaire was adopted from previous studies.[14,15] The questionnaire consists of socio-demographic characteristics, history of alcohol consumption, cigarette smoke, physical activity and family history of hypertension, anthropometric and blood pressure measurements. Participants who smoke at least one stick of cigarette per day in the last previous year before the study were grouped as smokers while those who had quit cigarette smoke for at least one year or had never smoked were termed non-smokers.[15] Alcohol use was assessed with the question; have you consumed alcohol in the past 12 months?[16] Physical activity was assessed based on work, leisure and sports. Participants with sedentary or light daily physical activities were classified as physically inactive while those whose physical activities can be described as moderate and vigorous were categorized as physically active.[15]

Hypertension was defined as systolic and/ or diastolic blood pressure (≥140/90mmHg) during the study on two occasions using a standardized mercury sphygmomanometer or a patient on treatment for hypertension.[15] The blood pressure was classified based on The Seventh Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC7).[17]

Body Weight, Height and Body Mass Index: The combined Weight Scale and Stadiometer was standardised and tested before daily measurements. Participants were measured standing upright with face forward in normal clothing and without footwear, head-tie, or accessories such as a purse, cell phone, keys etc. Weight was recorded in Kilogrammes (Kg) and height was in Metres (m). These were measured to the nearest 0.1kg and 0.1cm respectively.

Body mass index (BMI) was calculated using the formula; BMI = weight/ $(height)^2$ (kg/m^2). Body mass index was categorised as underweight (< 18.5kg/m^2), normal weight (18.5-24.9kg/m^2), overweight (25.0-29.9kg/m^2), obese (≥30.0kg/m^2).[5]

Data was entered into excel spreadsheet and coded into the Statistical Product and Service Solution (SPSS) version 23 (IBM, Chicago) for analysis. Demographic variables and categorical variables were presented using frequency tables as

appropriate. Statistical association was analyzed using the Chi square test and Fisher's test as applicable in contingency tables. Continuous variables such as age were presented using means and standard deviation. Statistical significance was evaluated at $p<0.05$ at the 95% confidence interval.

Ethical consideration: Approval was obtained from the Research and Ethics Committee of Delta State University Teaching Hospital, Oghara. Informed consent was obtained from all the participants before data collection. All information was treated confidentially and participants could withdraw at any point without prejudice to their future care.

RESULTS

Two hundred and forty-three participants were recruited in the study of which eight were excluded for incomplete data, leaving a total of 235 participants, giving a response rate of 96.7%.

The sociodemographic characteristics of the participants as shown in Table 1. The mean age of participants was 45.1 ± 13.7, with many (41.3%) of the participants within the middle age group of 45 to 59 years old. There were more females (63.0%) than males (37.0%), majority of the participants were married (68.1%) and over half (56.6%) had tertiary level of education.

Table 1: Socio-demographic characteristics of respondents.

Variable	Frequency	Percentage
Gender		
Male	87	37.0
Female	148	63.0
Age (in years)		
Young adult (18 – 26)	25	10.6
Adult (26 – 44 years)	73	31.1
Middle age (45 – 59)	97	41.3
Old age (≥ 60)	40	17.0
Mean age	45.1 ± 13.7	
Religion		
Christianity	228	97.0
Muslim	4	1.7
Traditionalist	1	0.4
Others	2	0.9
Education		
None	5	2.1
Primary	30	12.8
Secondary	67	28.5
Tertiary	133	56.6
Marital status		
Single	57	24.3
Married	160	68.1
Widowed	9	3.8
Separated	7	3.0
Divorced	2	0.9
Residence		
Urban	141	60.0
Semi-urban	71	30.2
Rural	23	9.8
Family history of hypertension		
Yes	100	42.6
No	135	57.4

Less than half 91(41%) had normal blood pressure and about a third 67(30.2%) had pre-hypertensive blood pressure. While those who had hypertension were; stage 1 hypertension 36 (16.2%) and stage 2 hypertension 28(12.6%). The overall prevalence of hypertension in the study population was 64(29.0%). (Table 2)

Table 2: Blood pressure pattern of participants.

Variable	Frequency	Percent
Blood pressure grade		
Normal	91	41.0
Pre-hypertension	67	30.2
Stage 1 hypertension	36	16.2
Stage 2 hypertension	28	12.6
Overall prevalence of hypertension		
Hypertensive	64	29.0
Non-hypertensive	158	71.0

The association between sociodemographic characteristics and the prevalence of hypertension showed that hypertension occurred more in males 30(36.1%) than females 34(24.5%), which was not statistically significant. Likewise, the prevalence of hypertension decreased with the possession of a higher educational level even though it was not statistically significant. However, the prevalence of hypertension increased with increasing age, as it was more prevalent in the elderly 23(59.0%) and was statistically significant. Also, hypertension occurred more in participants who were separated 5(71.4%) and widowed 5(62.5%) than those who were married and this was statistically significant. (Table 3)

Table 3: Socio-demographic factors associated with prevalence of hypertension

Variable	**Hypertension status**		**Test statistics**
	Hypertensive	**Non-hypertensive**	
Gender			
Male	30 (36.1)	53 (63.9)	$X^2 = 3.458$
Female	34 (24.5)	105 (75.5)	p = 0.063
Age (in years)			
Young adult (18 – 26)	0 (0.0)	25 (100.0)	$X^2 = 31.372$
Adult (26 – 44 years)	12 (18.2)	54 (81.8)	p = **<0.001**
Middle age (45 – 69)	29 (31.5)	63 (68.5)	
Old age (≥ 60)	23 (59.0)	16 (41.0)	
Education			
None	3 (60.0)	2 (40.0)	$X^2 = 6.466$
Primary	10 (33.3)	20 (66.7)	p = 0.092
Secondary	23 (35.9)	41 (64.1)	
Tertiary	28 (22.8)	95 (77.2)	
Marital status			
Single	6 (10.9)	49 (89.1)	Fisher's exact test
Married	47 (31.3)	103 (68.7)	p = **<0.001**
Widowed	5 (62.5)	3 (37.5)	
Separated	5 (71.4)	2 (28.6)	
Divorced	1 (50.0)	1 (50.0)	
Residence			
Urban	37 (27.4)	98 (72.6)	$X^2 = 0.421$
Semi-urban	21 (31.8)	45 (68.2)	p = 0.812
Rural	6 (28.6)	15 (71.4)	

Also, the association between risk factors of hypertension and the prevalence of hypertension revealed that the prevalence of hypertension increased with increase in the body mass index of participants. More participants who were obese 25(46.3%) had hypertension compared with those who had normal body mass index and this was statistically significant. Similarly, the presence of a family history of hypertension was significantly associated with increase in the prevalence of hypertension. However, those who had moderate and high physical inactivity levels had a higher prevalence of hypertension compared with those with low level of physical inactivity, although this was not statistically significant. Also, hypertension occurred more in those who did not consume alcohol 34(30.6%) than those who did 30(27.0%), but was not statistically significant. (Table 4)

Table 4: Risk factors association with prevalence of hypertension

Variable	**Hypertension status**		**Test statistics**
	Hypertensive	**Non-hypertensive**	
Smoking status			
Yes	0 (0.0)	8 (100.0)	$X^2 = 3.362$
No	64 (29.9)	150 (70.1)	p = 0.067
Alcohol intake			
Yes	30 (27.0)	81 (73.0)	$X^2 = 0.351$
No	34 (30.6)	77 (69.4)	p = 0.553
BMI			
Underweight	0 (0.0)	8 (100.0)	$X^2 = 15.008$
Normal	15 (19.0)	64 (81.0)	p = **0.002**
Overweight	23 (30.7)	52 (69.3)	
Obese	25 (46.3)	29 (53.7)	
Level of Physical activity			
Physically inactive	14 (23.0)	47 (77.0)	$X^2 = 1.640$
Physically active	48 (31.8)	103 (68.2)	p = 0.440
Family history of hypertension			
Yes	38(40.4)	56(59.6)	$X^2 = 10.686$
No	26(20.3)	102(79.7)	P= **0.001**

DISCUSSION

The overall prevalence of hypertension in this study was 29%. This was similar to findings reported in a previous study in Akwa Ibom state, South-South, Nigeria and Ethiopia.[18,19] However, it was lower than 22% reported in a population based study in Oyo state, South-West, Nigeria and 27.4% in Kenya. [12,20] While a higher prevalence was reported in Enugu state, South-East, Nigeria (37.2%) and India (32.9%). [4,21] In recent years, the prevalence of hypertension in Nigeria have been on the rise.[6] Report from a systematic review conducted in Nigeria showed an increase from 8.2% in 1990 to 32.5% in 2020.[22] The probable factors responsible for this rise include adoption of unhealthy lifestyle habits, increase in the ageing population, urbanization leading to change in the kind of jobs people engage in and the type of transport system utilized resulting in sedentary living.[1,5,22] Also, the lack of effective preventive strategies targeted at hypertension can be a contributory factor.[5]

It is worthy to note that about a third of the participants had prehypertension blood pressure. This finding falls within the range reported by a systematic review on the prevalence of prehypertension in Africa of between 32.9% to 56.6%.[23] Also, Raimi and Odusan in Ogun state, Nigeria reported similar finding.[24] A lower prevalence was reported by Sharma et al in South Africa while a higher prevalence was found by Olack et al in Kenya. [5,25] The high rate of prehypertension in this study is worrisome. Although, prehypertension does not necessarily progress to hypertension.[26] Previous studies have reported that its presence can increase the risk of developing hypertension, cardiovascular complications and damage to target organs by 30% in the absence of modification of lifestyle habits.[26] Hence the need to enlighten the public on the need to adopt healthy lifestyle behaviours to reduce the risk of hypertension.

Furthermore, the prevalence of hypertension increased with increasing age, as it was more prevalent in the elderly and was statistically significant. This was in congruent with previous studies in Nigeria and globally. [4,5,6,19] Age have been identified as one of the non-modifiable risk factor that have a positive association with hypertension.[27] This occur due to increase in systolic blood pressure following increase in age, resulting from decrease in the elasticity of arteries and arterioles.[4,27] The current trend experienced globally in population structure which has led to a growing increase in the ageing population, with Nigeria not left out, as the population of the elderly is expected to rise from 9 million in 2016 to 26 million by 2050.[16,28] The implication of this is an increase in the occurrence of chronic diseases including hypertension associated with ageing, thus there is need to develop health policies and strategies that will promote healthy ageing.[16] In addition, hypertension occurred more in participants who were separated, divorced and widowed than those who were married and single and this was statistically significant. This finding was consistent with a study conducted in Saudi Arabia.[20] Also, a population based study conducted in Kenya noted that participants who were widowed had a 20% greater risk of developing hypertension compared to those who were married.[25] In contrast, a national survey carried out in Nigeria (REMAH study) found out that those who were married and widowed had an increased risk of having hypertension.[6] It has been discovered that marriage promotes the uptake of healthy lifestyle practices and better mood which confer various positive health benefits on an individual.[29,30] Whereas, divorce which is regarded as the most stressful life experience is associated with increased risk of early mortality.[29] This could be due to the loss of

this protective benefits of marriage together with the stress of separation which can result in aberration from healthy lifestyle behaviours that may predispose to long-term consequences for disease processes.[29] Also, those who are widowed, separated or divorced have to take up more responsibility to carter for the needs of their children which may give rise to stress that may increase the risk of hypertension.

A significant and well documented non-modifiable risk factor of hypertension is the presence of a family history of hypertension.[1,31] The presence of a family history of hypertension was significantly associated with increase in the prevalence of hypertension in this study. Similar finding was reported by previous studies.[1,12] In contrast, population based studies conducted in Rivers state, Nigeria and in Ethiopia reported no association between family history of hypertension and the prevalence of hypertension.[19,31] A higher risk of hypertension have been described in offspring of first- and second-degree parents with hypertension compared to the general population.[1] Also, the development of hypertension before the age of 55years (early onset) in parents and grandparents is a greater predictor of the risk of hypertension in their offspring.[1] In defiance of the established role played by genetic predisposition in the development of hypertension, it has been documented that the uptake of healthy lifestyle practice can reduce this risk.[1]

Also, increase in the body mass index of participants was associated with increase in the prevalence of hypertension, as more of those who were obese had hypertension compared to those who had normal body mass index. A cohort study carried out in Thailand revealed that an increasingly higher body mass index was positively associated with an increased incidence of hypertension over a 4-year period.[26] Several previous studies in Nigeria and globally reported that overweight and obesity were associated with increased risk of hypertension.[1,4,5,11,12,19,26] The World Health Association (WHO) reports that the prevalence of obesity globally is on the increase.[32] It describes the situation as alarming as diseases associated with obesity are soaring especially cardiovascular diseases which is currently the leading cause of mortality globally.[32] Dietary habits and pattern of physical activity play an important role in obesity. Despite the well-established relationship between obesity and hypertension, the mechanism by which obesity cause hypertension is complex as it involves interactions between renal, metabolic and neuroendocrine pathways.[33] The probable pathogenesis of obesity-related hypertension include over activation of the sympathetic nervous system, alteration of adipose-derived cytokines, insulin resistance, structural and functional renal changes and the stimulation of renin-angiotensin-aldosterone system.[32,33] Weight reduction have been identified as the primary measure to address obesity-related hypertension, as it reverses the pathophysiological mechanism that lead to hypertension.[33] This can be achieved through lifestyle changes such as healthy dietary habit and increased physical activity.[33]

CONCLUSION

In the study, about a third of the study participants had prehypertension and hypertension respectively. Increasing age, being separated, obesity and a positive family history were associated with increased risk of hypertension. There is need to provide health education and health promotion activities that address awareness of the risk factors of hypertension and to encourage the adoption of healthy lifestyle habits targeted at controlling the risk factors of hypertension and hence hypertension. This is necessary because adoption

of healthy lifestyle practices has been found to contribute to the reduction of blood pressure. Also, controlling these risk factors can counterpoise to some extent the genetic predisposition for hypertension and the development of its ensuing sequelae.

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Phytochemical, Acute Toxicity, and Anti-plasmodial Potential of Concomitant Extracts of *Azadirachta indica* and *Mangifera indica* on Liver Function and Microscopic Anatomy in Swiss Mice

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Abstract

Introduction: In sub-Saharan Africa, malaria is the predominant contributor to mortality and there is significant dependence on phytotherapy for its treatment. This study investigated comparative phytoconstituents, acute toxicity, anti-plasmodial activities on liver function and microanatomical perturbations following the concomitant administration of ethanol extracts of *Azadirachta indica* leaves and *Mangifera indica* bark in *Plasmodium berghei*-infected Swiss mice.

Materials and Methods: Sixty experimental mice were allotted into 12 groups (n = 5) and inoculated with 1 x 10^6 *P. berghei* two weeks post- acclimatization. Group one served as the normal control; group two [parasitized-non-treated] and groups 3 to 11 were low, medium, and high doses of the extracts singly and concomitantly, while group 12 received artemether-lumefantrine. All administrations were via oral route for three days, respectively. Phytochemical screening, parasite density, serum liver enzymes and microanatomical alterations were analyzed.

Results: Phytochemistry showed that *A. indica* possessed abundant alkaloids that were absent in *M. indica*. The median lethal dose (LD50) of *A. indica* leaf and *M. indica* bark extract was 3240.37 and 2738.61 mg/kg, respectively. The single administration of *A. indica* outperformed *M. indica* via mitigated parasite progression, reduced *P. berghei*-induced hepatotoxicity and elevated liver enzymes.

Conclusion: *Azadirachta indica* surpasses *Mangifera indica* in alleviating hyperparasitemia, parasite-associated hepatotoxicity, and hepatic microanatomical changes in *in vivo* rodentia malaria model. *A. indica* also mild to moderately improved hepatic collagen and glycogen storage than *M. indica*. It possessed a better synergistic effect than *M. indica* alone.

Keywords: *Azadirachta indica*, *Mangifera indica, Plasmodium berghei*, anti-plasmodial, liver function

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INTRODUCTION

Malaria is an infectious disease that can be life-threatening and is spread by mosquitoes to humans and other animals. It is caused by the parasite protozoans that belong to the genus Plasmodium[1]. The disease is spread to people through the bites of infected female *Anopheles* mosquitoes. There are about four species of malaria parasites that can infect rodents namely, *Plasmodia berghei, vinckei, yoelli,* and *chabaudi,* respectively. Amongst these parasites, *Plasmodium berghei* is preferably used as model for experimental studies. It preferentially infects normocytes and reticulocytes and usually produces infections in mice that induce severe pathology[2].

Artemisua anua, is a Chinese plant whose major component forms the current gold-standard treatment for malaria is the artemisinin-based

combination therapy (ACT). It is worth noting that combination therapies, in which two or more antimalarial drugs with different mechanism of action are given together in fixed dose are quite effective for improved efficacy and reduced drug resistance by the parasites[3].

Several pharmacological studies have been reported in a recent review literature to demonstrate antimalarial plants either as crude extracts, or in purified metabolites for malaria treatment. Interestingly most of these plants show no evidence of parasite resistance because of their synergistic action of phytochemical components representing the great potential of the plant extract against malaria resistance[4]. Amongst several antimalarial plants used in folklore are; neem plant (*Azadirachta indica*) popularly called dongoyaro, and mango plant (*Mangifera indica*) found in many rural residences and now popular in tea mixture sold at malls and supermarkets based on preference.

Azadirachta indica is a tree in the Mahogany family Meliciceae[5,6]. It is native to tropical and semi-tropical climates and thrives in arid and savanna parts of Nigeria. Neem and its derivatives have been employed in ethnomedicine as antimalarials. The antimalarial effect of neem has been documented and is thought to act by redox perturbation in the form of imposition of substantial oxidant stress during malaria infection[7,8].

Mangifera indica is a tree from the *Anacardieceae* family that is widely found in tropical countries. This tree produces very sweet fruits. All the parts of a mango are very useful and thus a pharmacologically, ethnomedical, and phytochemically diverse plant[9]. The bark of a mango tree contains an active substance called mangiferin. It is known for its use in natural medicine, not only as a health enhancing panacea or adjunct therapeutic, but also for brain function improvement[10].

The liver is a key organ in the pathogenesis of malaria infection. It serves an important part of the life cycle of the parasite, being the organ where asexual reproduction (schizogony) of the mosquito infected sporozoites takes place[11,12]. Furthermore, certain clinical characteristics associated with severe malaria, including hypoglycemia, hyperparasitemia, jaundice, and bruising, are entirely or partially explained by liver damage caused by parasitemia or the immunological response to the parasite. This study investigated the phytochemical constituents, acute toxicity, and anti-plasmodial activity of the concomitant administration of *Azadirachta indica* and *Mangifera indica* extracts on the hepatic function and microanatomical alterations in Swiss Mice.

MATERIALS AND METHODS

The medicinal plant farm of the Department of Pharmacology and Natural Medicine, Faculty of Pharmacy, University of Uyo, Akwa Ibom State, Nigeria, provided fresh mature *Azadirachta indica* leaves and *Mangifera indica* bark. Both plants were washed to remove dirt and sun-dried for one week before being recognized and verified by taxonomists and placed in the Herbarium Unit of the Department of Botany, Faculty of Science, University of Uyo, Nigeria, with specimen voucher number (U.U.H. 63/19).

The dried plant materials were thereafter separately blended into a powdery form, and preserved separately in containers with appropriate covering, at room temperature. About 2.5 liters of absolute ethanol was used to mix 700 g of each powdered plant material and the mixture was kept for 72 hours at room temperature. They were filtered using linen and then filter paper. After 30 minutes of post-

filtration, the filtrates were evaporated to dryness using a water bath at 40 °C, and the extracts were stored in a sealed container in the freezer (2 – 8 °C) until it was used.

Extracts from both the *Azadirachta indica* leaf and bark of *Mangifera indica* were used for the phytochemical tests as described by Harborne[13], Sofowora[14] and Trease and Evans[15]. This procedure was done in the Pharmacology Department, Faculty of Pharmacy, University of Uyo, Nigeria.

This experiment involved sixty Swiss mice weighing between 20 and 30 g. The mice were sourced from the Animal House of the Faculty of Pharmacy at the University of Uyo in Akwa Ibom, Nigeria. The protocols for this study were approved by the Department of Human Anatomy, University of Uyo, Nigeria, which aligns with the globally accepted guideline for the use and handling of laboratory animals[15]. Additionally, an ethical approval was granted by the Health Research Ethics Committee of the Akwa Ibom State Ministry of Health with Ref: MH/PRS/99/Vol.IV/715. The study was conducted in accordance with the Basic and Clinical Pharmacology and Toxicology policy for experimental studies[16]. All animals were weighed, identified, and placed in a standard plastic cage for one week of acclimatization in an optimum pathogen-free environment, with a 12hour light/dark cycle of 25 – 27 °C and relative humidity of 40 - 60% measured using a CEM hydrometer (DT 615, Shenzhen China). The animal cages were maintained adequately by changing sawdust, leftover feed and drinking water, daily. All animals were fed with pelletized grower mash (Grand Cereal Vital® Feed Ltd. Jos) and provided drinking water *ad libitum*. The ARRIVE guidelines 2.0 as checklist of relevant information for animal research documentation of *in vivo* experiments was consulted.

Blood stage *Plasmodium berghei* samples were collected from a laboratory stock via serial blood passage from one mouse to another. This was sourced from the Faculty of Pharmacy at the University of Uyo.

Leaf extract of *Azadirachta indica* and bark extract of *Mangifera indica* were investigated to determine their medium lethal doses (LD_{50}) in the Department of Pharmacology, Faculty of Pharmacy, University of Uyo. This was done to establish the different amounts of the extracts to be administered to the mice using the method of Lorke[17].

Fifteen (15) animals were separated into five (5) groups of three (3) each. Each group received increasing doses of leaf/bark extracts (1000, 1500, 2000, 2500, and 3000 mg/kg). The animals were carefully monitored for 24 hours to assess their behavioural activities as well as the possibility of fatality. Where there was no mortality, another group of three (3) animals was given greater doses and monitored for 24 hours.

The inoculum was prepared as described by Edagha et al[18]. The blood donor with high parasitemia was acquired by anaesthetizing the mouse with ketamine hydrochloride and collecting blood via heart puncture with a sterile syringe into a sterile heparinized tube. The percentage parasitemia was calculated by dividing the number of parasitized red blood cells by the total number of red cells, and a desired volume of blood was obtained from the donor mouse and diluted with sterile normal saline so that the final inoculum (0.2 mL) for each mouse contained the required number of parasitized red blood cells (1.0×10^6 parasitized red blood cells), which is the standard inoculum for infection of a single mouse.

Infection of Swiss Mice with *P. berghei* and

administration of the Extracts of *Azadirachta indica* **and** *Magnifera indica*

The sixty Swiss mice were allotted into twelve (12) groups (n = 5) mice each. All the mice were infected two weeks after acclimatization with 1 x 10^6 *P. berghei* parasitized erythrocytes intraperitoneally, except the healthy control group that is, the normal control (NC). The infection of the recipient mice was initiated by needle passage of the inoculum parasite preparation from the donor to healthy animals via an intraperitoneal route as described by Edagha *et al*., (2014). The presence of parasites was confirmed by a daily parasitemia determination using direct enumeration via a Giemsa-stained thin blood smear from the mice's lateral caudal vein, which demonstrated the number of PRBCs per 100 RBCs of parasitemia as outlined by WHO in malaria microscopy in WHO[19]. The experimental groups commenced treatment when parasitemia reached 5% of the initial inoculum. The parasite density was calculated using the formula below:

$$\% \text{ parasitemia} = \frac{Number\ of\ parasitized\ RBC}{Total\ number\ of\ RBC} \text{ X } 100$$

Also the percentage inhibition of the parasite for each day was calculated by the formula:

$$\frac{Mean\ \%\ parasitemia\ of\ untreated\ group - Mean\ \%\ parasitemia\ of\ treated\ group}{Mean\ \%\ parasitemia\ of\ untreated\ group} \text{ X}100$$

Experimental Design

S/N	Group	Treatment	Duration (days)
1.	NC	DW 5 mL/kg bwt	3
2.	PNT	DW 5 mL/kg bwt	3
3.	PAI_{LD}	324 mg AI/kg bwt	3
4.	PAI_{MD}	648 mg AI/kg bwt	3
5.	PAI_{HD}	972 mg AI/kg bwt	3
6.	PMI_{LD}	274 mg MI/kg bwt	3
7.	PMI_{MD}	548 mg MI/kg bwt	3
8.	PMI_{HD}	822 mg MI/kg bwt	3
9.	$PAIMI_{LD}$	324 mg AI + 274 mg MI /kg bwt	3
10.	$PAIMI_{MD}$	648 mg AI + 548 mg MI /kg bwt	3
11.	$PAIMI_{HD}$	972 mg AI + 822 mg MI /kg bwt	3
12.	PAL	8 mg/kg bwt	3

DW – Distilled water, NC – Normal control, PNT – Parasitized non-treated, PAI_{LD} – Parasitized and treated with *Azadirachta indica* extract – low dose, PAI_{MD} – Parasitized and treated with *Azadirachta indica* extract – middle dose, PAI_{HD} – Parasitized and treated with *Azadirachta indica* extract – high dose, PMI_{LD} – Parasitized and treated with *Mangifera indica* – low dose, PMI_{MD} – Parasitized and treated with *Mangifera indica* – middle dose, PMI_{HD} – Parasitized and treated with *Mangifera indica* – high dose, $PAIMI_{LD}$ –

Parasitized and treated with *Azadirachta indica* extract and *Magnifera indica* extract – low dose, $PAIMI_{MD}$ – Parasitized and treated with *Azadirachta indica* extract and *Magnifera indica* extract– middle dose, $PAIMI_{HD}$ – Parasitized and treated *Azadirachta indica* extract and *Magnifera indica* extract– high dose, PAL – Parasitized and treated with Arthemeter Lumefantrine (ACT). All extracts treatment was administered orally using oro-gavage tube every day for 3 days.

Termination of the Experiment

Twenty-four hours following the last dose, the animals were anesthetized intraperitoneally with 50 mg per kg body weight of ketamine hydrochloride (Rotex Medica, Germany). The thoracoabdominal wall was dissected to examine the heart, and blood was taken from the left ventricle. The heparinized blood was collected immediately and centrifuged at 3000 g for 15 minutes. The plasma was refrigerated and processed as a single batch for determination of aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) levels within 12 hours[20]. A cannula was utilized to administer a guided intracardiac perfusion of phosphate-buffered saline (PBS, 2M. PH 7.0) to the animals, which were then perfused-fixed with 10% buffered formalin. The livers of all animals were extracted and fixed in 10% buffered formalin for 48 hours.

Histopathological Assessment

The fixed tissue was dehydrated using graded alcohol to eliminate excess water found in tissues, as follows: two changes of 70% and 90% alcohol for two hours each, and two changes of 100% alcohol for two hours each. After dehydration, tissue was cleared with two changes of xylene (5 minutes each). This was followed by impregnation with two changes of paraffin wax in the oven at 60°C for one hour and thirty minutes each to allow for infiltration. Tissue was put into molds filled with molten paraffin wax. Once the paraffin wax had cooled and set, the mold was removed, revealing a small paraffin block containing the tissue sample. After freezing the tissues on ice, the paraffin blocks were sectioned with a microtome at a thickness of 5 μm.

Tissue sections were taken to water by deparaffinizing in two changes of xylene for 2 minutes each, followed by rehydration in 100% alcohol, 95% alcohol, 70% alcohol twice for 5 minutes each, and finally rinsed in tap water. Sections were stained with Hematoxylin for 10 minutes, washed briefly in tap water, differentiated in acid alcohol, washed well in running tap water till it turned blue. The blued sections were counterstained in Eosin solution for 1 minute[21]. The tissues were air dried and mounted with dipolycysteine xylene (DPX) before being cover-slipped for viewing under a light microscope. Three independent histopathologists unrelated to the study evaluated the tissues to mitigate study bias. The processed slides were examined under a light microscope (Olympus, USA), and photomicrographs were taken.

Statistical Analysis

The data obtained from this study was analyzed using the IBM Statistical Package for Social Science (SPSS) version 25 software, with the "One-Way" analysis of variance (ANOVA) and the Tukey post-hoc test to establish significance. Data was presented as mean ± Standard Error of Mean. Values were statistically significant ($p < 0.05$).

RESULTS

Phytochemical Analysis of Extracts of *Azadirachta indica* Leaf and *Mangifera indica* Bark

The different phytochemical constituents present in *Azadirachta indica* leaf extract and *Mangifera indica* bark extract are shown in Table 1.

The qualitative phytochemical analysis of the

extract of *Azadirachta indica* revealed the presence of alkaloids, tannins, flavonoids, polyphenols and terpenoids in high concentration, saponins, carbohydrate, glycoside, and cardiac glycoside in moderate concentrations, while steroid was present in trace concentration. On the other hand, the phytochemical constituents of extract of *Mangifera indica* were tannins, flavonoids, polyphenols, and glycosides, all present in high concentrations; saponins and carbohydrate were present in moderate concentrations; cardiac glycosides, steroids and terpenoids were also in trace concentration while alkaloid was absent.

Median Lethal Dose (LD_{50}) of Extracts of *A. indica* **Leaf and** *M. indica* **Bark**

There was no toxicity or mortality seen as the dose of *Azadirachta indica* leaf extracts increased to 3000 mg/kg body weight. The median fatal dose of *Azadirachta indica* extract was found to be more than 3000 mg/kg, (Table 2a).

The ethanolic extract of *Mangifera indica* bark revealed no toxicity or death at doses as high as 2500 mg/kg body weight. The median fatal dose was predicted to be more than 2500 mg/kg body weight, (Table 2b).

Effect of Extracts of *A. indica* **Leaf and** *M. indica* **Bark on Body and Liver Weights of** *P. berghei*-**infected Swiss Mice**

The post passage weight showed a significant ($p < 0.05$) reduction of the weights in groups 4, 5, 7, and 10 respectively, when compared to the NC. Although, group 9 was significantly ($p < 0.05$) increased in weight when compared to the groups 4 and 7, group 8 also was significantly ($p < 0.05$) increased when compared to the groups 4, 5, and 7.

Final body weight demonstrated a significant ($p < 0.05$) reduction of weight in groups 2, 3, 4, 5, 7, 9, and 10 respectively when compared to the control group. Although, observations showed a significant ($p < 0.05$) decrease in the groups 7 and 9, there is a significant ($p < 0.05$) increase in the groups 6, 8, 11 and 12 when compared to the diseased group (group 2).

Analysis also observed that there were negative changes in weight in all the treatment groups except in group 12, which showed a positive change in weight when compared to the control and other treatment groups, as shown in Table 3.

Liver weight analysis demonstrated no significant ($p > 0.05$) differences across treatment groups when compared to the control group, as shown in Table 3.

Effect of Extracts of *A. indica* **Leaf and** *M. indica* **Bark on Serum Liver Enzymes of** *P. berghei*-**infected Swiss Mice**

The serum AST concentration showed a significant ($p < 0.05$) increase in groups 2 and 6 when compared to the control group. There was a significant ($p < 0.05$) decrease in AST concentration of group 12 when compared to the parasitized non-treated (PNT) group.

Assessment of the ALT presented a significant ($p < 0.05$) increase in ALT concentration across all treatment groups except for groups 4, 5, and 12 when compared to the control group. Whereas, groups 3, 4, 5, 7, 8, 10, 11 and 12 showed a significant ($p < 0.05$) decrease in ALT concentration when compared to the PNT group, group 5 and 12 showed a significant ($p < 0.05$) reduction when compared to group 9 and a significant ($p < 0.05$) reduction in groups 5 and 12 when compared to the group 7.

Result presentation showed a significant ($p < 0.05$) elevated ALP concentration in the groups 2 and 6 when compared to the control group. However, all other treatment groups showed a significant ($p < 0.05$) reduction in ALP concentration when compared to the PNT group (group 2), as shown in Table 4.

Effect of Extracts of *A. indica* **Leaf and** *M.*

***indica* Bark on Parasitemia of *P. berghei*-infected Swiss Mice**

The assessment of initial parasitemia level across all treatment groups showed no significant ($p > 0.05$) difference when compared to the PNT group (group 2), as presented in Table 5. Final parasitemia showed a significant ($p < 0.05$) decrease in all the treatment groups when compared to the PNT group (group 2). However, a more significant ($p < 0.05$) decrease is observed in the groups 11 and 12 when compared to other treatment groups as well as the PNT group (group 2).

Effect of Extracts of *A. indica* Leaf and *M. indica* Bark on the Histology of the Liver of *P. berghei*-infected Swiss Mice

The section of the liver of group 1 (NC) showed a regular histoarchitecture with normal array of hepatocytes (HP) and portal triad as shown in Figure 1. The liver section of group 2 animals (PNT) revealed severely distorted histoarchitecture demonstrating the presence of degenerating hepatocytes (D), numerous hepatic vacuolations (V), widespread microvascular steatosis (St) and widely populated parasites (p). Groups 3 to 11 demonstrated mild to moderate reversal of liver distortions when compared to NC, and group 12 treated with standard anti-malarial drug (PAL).

Collagen expression was demonstrated in Figure 2, showing intense Masson's trichrome uptake in the *Mangifera indica* bark extract treatment groups particularly medium dose (group 7), the concomitant extract groups exhibited moderate expressions (groups 9 to 11) as well as groups 1, 3, 4, and 5, the low expression groups were PNT and particularly the PAL group.

Glucose deposition demonstrated by periodic acid Schiff was most expressed in groups NC and PAL. Other test groups expressed low to slightly moderate glucose storage, as presented in Figure 3.

Table 1: Phytochemical Screening for the Ethanolic Leaf Extract of *Azadirachta indica* and Bark Extract of *Mangifera indica*

S/N	Phytochemical Constituents	*Azadirachta indica*	*Mangifera indica*
1.	Alkaloids: (a). Dragendoff Reagent	+++	-
	(b). Mayer's Reagent	+++	-
2.	Saponin	++	++
3.	Tannin	+++	+++
4.	Carbohydrate	++	++
5.	Flavanoid	+++	+++
6.	Polyphenol	+++	+++
7.	Cardiac Glycoside	++	+
8.	Steroids	+	+
9.	Terpenoids	+++	+
10.	Glycoside	++	+++

+ = present (+ = trace; ++ = moderate concentration; +++ = high concentration); - = absent

Table 2a: Median Lethal Dose for the Ethanolic Leaf Extract of *Azadiractha indica*

Group (n = 3)	Dose of *Azadirachta indica* extract (mg/kg)	Mice Mortality
1.	1000	None
2.	2000	None
3.	3000	None
4.	3500	1

$LD_{50} = \sqrt{AB}$; Where A = The maximum dosage that produce 0 % mortality and
B = The minimum dosage that produces 100 % mortality.

A. *indica* = $\sqrt{3000X3500}$ = $\sqrt{10,500,000}$ = 3240.37 mg/kg

10 %, 20 % and 30 % of the value was used for the main experiment

Table 2b: Median Lethal Dose for the Ethanolic Extract of *Mangifera indica* Bark

Group (n = 3)	Dose of *Mangifera indica* extract (mg/kg)	Mice Mortality
1.	1000	None
2.	2000	None
3.	2500	None
4.	3000	1

LD_{50} is over 2500 mg/kg body weight of *Mangifera indica.*

Likewise using the above formula *M. indica* = $\sqrt{2500X3000}$ = $\sqrt{7,500.000}$ = 2738.61 mg/kg.

10 %, 20 % and 30 % of the value was used for the main experiment

Table 3: Effect of Extracts of *A. indica* Leaf and *M. indica* Bark on Body Weight of *P. berghei*-infected Swiss Mice

S/N	Group	Initial BW (g)	Post Passage W. (g)	Final BW (g)	Change in W (g)	% Change in Weight	Liver Weight (g)
1.	NC	25.74 ± 0.75	26.56 ± 0.56[a]	25.65 ± 0.53	0.09	0.35	1.41± 0.07
2.	PNT	23.53 ± 1.33	23.85 ± 1.44	17.65 ± 3.97	- 5.88	33.31	1.66± 0.13
3.	PAI_{LD}	24.16 ± 0.99	26.40 ± 0.86[a]	20.83 ± 3.06	- 3.33	15.98	1.64± 0.12
4.	PAI_{MD}	21.60 ± 0.68	20.43 ± 2.98	18.50 ± 2.70	- 3.10	16.70	1.47± 0.14
5.	PAI_{HD}	22.56 ± 0.64	21.48 ± 3.15	16.88 ± 3.71	- 5.68	33.61	1.75± 0.33
6.	PMI_{LD}	25.70 ± 0.89	26.63 ± 1.19[a]	25.85 ± 1.00	- 0.19	0.58	1.61± 0.12
7.	PMI_{MD}	21.33 ± 0.66	19.80 ± 2.99	14.85 ± 3.30	- 6.48	43.63	1.36± 0.14
8.	PMI_{HD}	30.45 ± 1.31	30.13 ± 1.07[a]	24.43 ± 3.55	- 6.02	24.59	1.89± 0.03
9.	$PAIMI_{LD}$	27.09 ± 1.22	29.51 ± 1.64[a]	23.09 ± 3.19	- 3.52	16.82	1.78± 0.00
10.	$PAIMI_{MD}$	22.33 ± 1.14	22.19 ± 1.01	17.99 ± 2.68	- 4.34	24.12	1.58± 0.20
11.	$PAIMI_{HD}$	23.69 ± 0.73	25.03 ± 0.99	23.16 ± 0.77	- 0.53	2.28	1.61± 0.08
12.	PAL	23.35 ± 0.97	25.13 ± 0.52	24.33 ± 0.68	0.98	4.07	1.39± 0.24
	P value	-	0.0005	-	-	-	0.471

Values are expressed as Mean ± SEM; n = 5; a = $p < 0.05$ relative to PMI_{HD}.

Table 4: Effect of Extracts of *A. indica* Leaf and *M. indica* Bark on Serum Liver Enzymes of *P. berghei*-infected Swiss Mice

S/N	Group	AST (U/L)	ALT (U/L)	ALP (U/L)
1.	NC	146.7 ± 2.59	57.76 ± 2.59	52.86 ± 2.70
2.	PNT	169.1 ± 2.32[a]	84.75 ± 2.68[a]	75.6 ± 2.38[a]
3.	PAI_{LD}	157.8 ± 6.76	72.71 ± 3.08[a,b]	61.87 ± 5.10
4.	PAI_{MD}	154.1 ± 2.47	66.41 ± 2.57[b]	60.11 ± 1.34
5.	PAI_{HD}	153.1 ± 1.31	64.09 ± 2.27	59.09 ± 0.74
6.	PMI_{LD}	167.1 ± 2.20[a]	78.20 ± 1.38[a,b,c]	65.11 ± 1.46[a]
7.	PMI_{MD}	163.9 ± 3.33	70.13 ± 0.85[a,b]	58.64 ± 0.77
8.	PMI_{HD}	163.8 ± 2.50	68.44 ± 1.28[a,b]	57.16 ± 1.88
9.	$PAIMI_{LD}$	155.2 ± 2.11	75.08 ± 2.58[b]	60.56 ± 2.00
10.	$PAIMI_{MD}$	155.8 ± 8.04	73.54 ± 0.80[a,b]	59.04 ± 2.40
11.	$PAIMI_{HD}$	151.9 ± 4.31	70.61 ± 1.69[a,b]	56.47 ± 1.59
12.	PAL	148.1 ± 2.75	64.91 ± 1.07	56.20 ± 2.08
	P value	0.0027	0.0001	0.0001

Values are expressed as Mean ± SEM; n = 5; a = $p < 0.05$ relative to NC; b = $p < 0.05$ relative to PNT; c = $p < 0.05$ relative to PAI_{HD} and PAL.

Table 5: Effect of *A. indica* Leaf Extract and *M. indica* Bark Extract on Parasitemia of *P. berghei*-infected Swiss Mice

S/N	Group	% Initial Parasitemia	% Final Parasitemia
1	NC	0.00 ± 0.00	0.00 ± 0.00
2	PNT	48.00 ± 2.08	53.00 ± 1.95[a,b]
3	PAI_{LD}	47.75 ± 1.54	36.00 ± 0.91[a,b]
4	PAI_{MD}	48.00 ± 1.95	31.50 ± 1.55[a,b]
5	PAI_{HD}	47.75 ± 1.37	27.50 ± 1.25[a,b]
6	PMI_{LD}	47.75 ± 2.59	36.75 ± 1.75[a,b]
7	PMI_{MD}	48.50 ± 1.84	33.50 ± 1.70[a,b]
8	PMI_{HD}	48.75 ± 3.63	29.50 ± 2.10[a,b]
9	$PAIMI_{LD}$	48.50 ± 2.32	32.25 ± 3.35[a,b]
10	$PAIMI_{MD}$	48.75 ± 3.17	29.75 ± 2.86[a,b]
11	$PAIMI_{HD}$	48.75 ± 2.17	15.75 ± 2.62[a]
12	PAL	50.00 ± 1.08	8.50 ± 0.64[a]
	P value	-	0.0001

Values are expressed as Mean ± SEM; n = 5; a = $p < 0.05$ relative to NC; b = $p < 0.05$ relative to $PAIMI_{HD}$ and PAL.

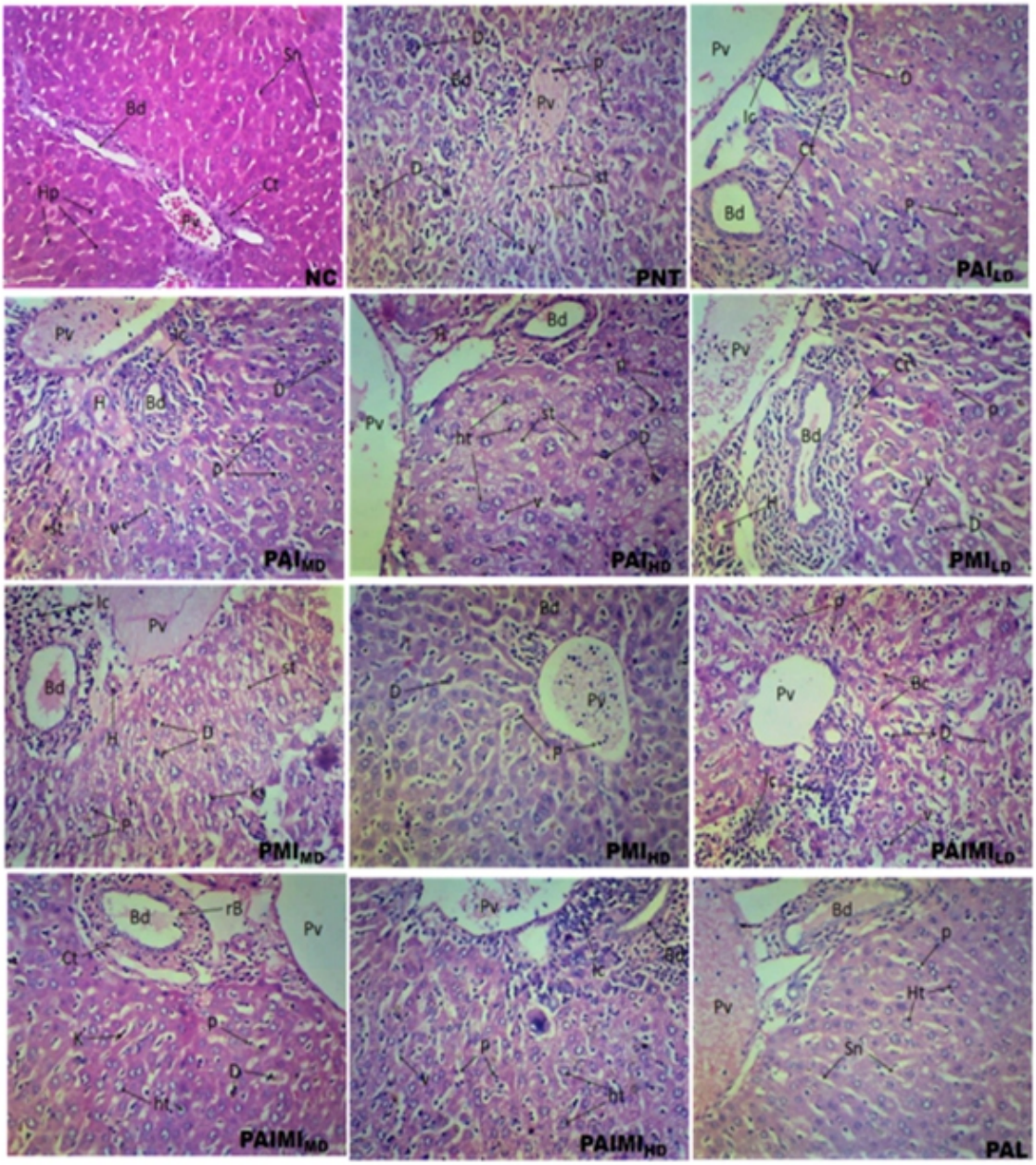

Figure 1: Representative photomicrographs of the cross section of the liver showing normal control (NC), that is group 1 and test groups 2 to 12 respectively. H&E x400. Legend: **S**inusoidal (Sn) arrays within the hepatic lobules, populated hepatocytes (Hp), Portal vein (Pv), Bile duct (Bd), and connective tissue cells (Ct) within the portal area.

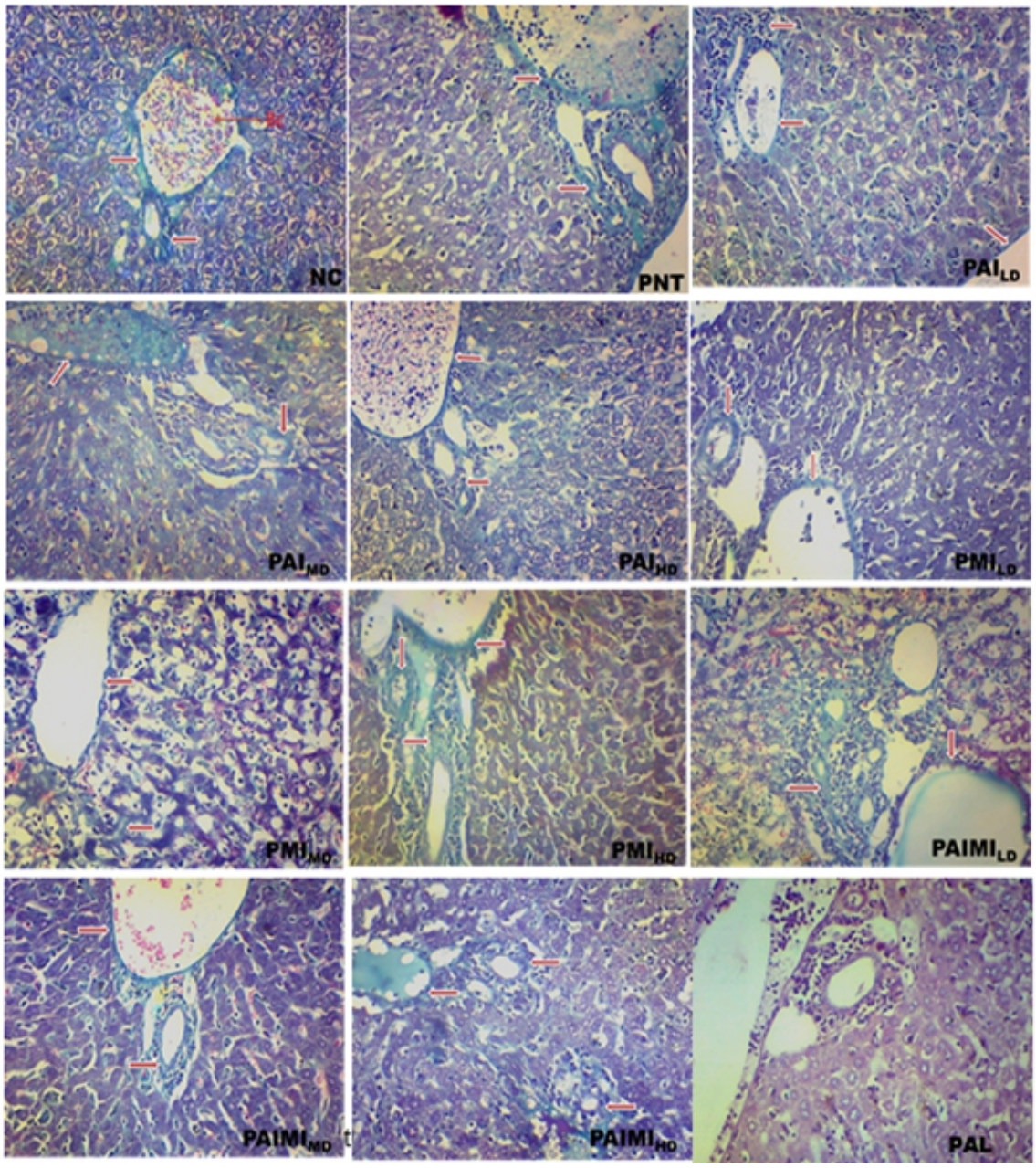

Figure 2: Representative photomicrographs of the cross section of the liver showing normal control (NC), that is group 1 and test groups 2 to 12 respectively. Masson's trichrome x400. Legend: Red arrow = collagen expression.

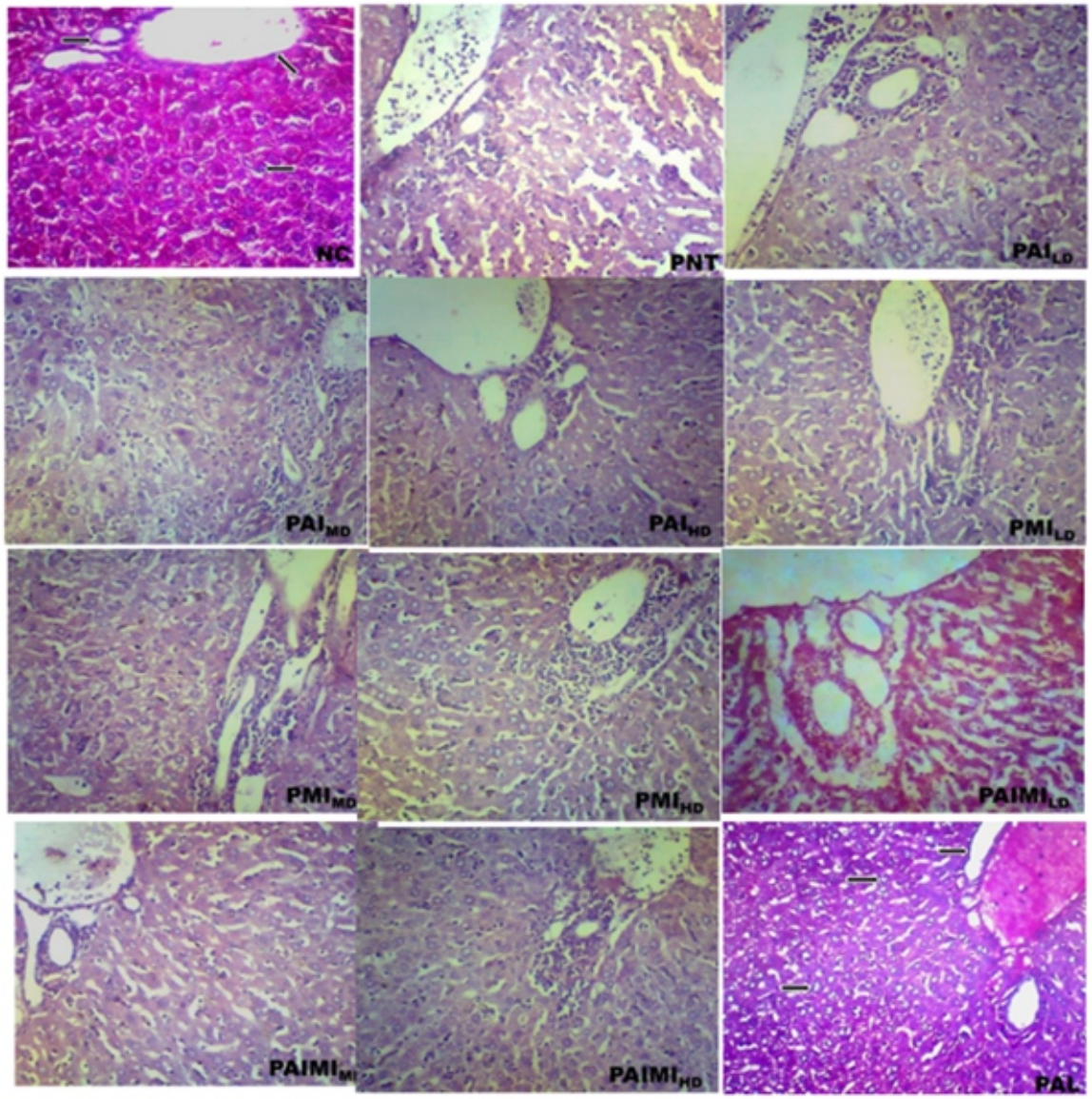

Figure 3: Representative photomicrographs of the cross section of the liver showing normal control (NC), and the test groups, respectively. Periodic acid Schiff, x400. Legends: NC: showing normal glycogen expression. Parasitized groups demonstrated depleted glycogen expression except in PAIMI$_{LD}$ and PAL which had up-regulated glycogen contents.

DISCUSSION

Herbal tea mixtures are now a popular trend, and many contain two or more blends of plants, mostly consumed for their health benefits. Here we investigated the concurrent administration of two popular anti-malarial plants; ethanol leaf extract of *Azadirachta indica* and *Mangifera indica* bark extract.

The phytochemical screening of the extracts

revealed that both extracts were rich in saponin, tannin, carbohydrates, flavonoid, polyphenol and glycoside and there was moderate concentration of steroids. *A. indica* was also rich in alkaloids but no trace of alkaloids was found in *M. indica,* also, *A. indica* contains more cardiac glycosides, and terpenoids than *M. indica.* The phytochemicals present in these extracts may support their medicinal efficacy and established pathways of action against many diseases. Several of these phytochemicals have been shown to have anti-plasmodial effects. Alkaloids are reported to block parasitic protein synthesis[22,23]. In this study, parasitized mice treated with *A. indica* showed impeded parasite growth compared with PNT group. Flavonoids are one of the most important phytochemicals detected in both extracts. Flavonoids are capable of impairing *Plasmodium* nucleic acid base pairing[23]. Saponins have antimalarial, cytotoxic, and anti-tumor activities[23,25] and plants containing saponins have been used for medicinal purposes for decades[26]. Saponins, tannins and phenols also pose some anti-plasmodial activity. *A. indica* possesses a higher concentration of terpenoids and terpenoid possesses effects detrimental to infective protozoans, including *Plasmodium*[27,28]. The phytochemicals screening also showed that the extracts of *A. indica* had alkaloids which were absent in *M. indica,* and more terpenoids. This could indicate that the *A. indica* extract has greater biological activity.

Acute toxicity study on the ethanolic leaf extracts of *Azadirachta indica* showed that the median lethal dose of the extract is over 3000 mg/kg body weight, this value is like those obtained by Achi et al[28]. While the median lethal dose of the ethanolic extract of *Mangifera indica* bark was calculated to be over 2500 mg/kg body weight, this value is like those obtained by Reddeman et al[29]. These values demonstrate that *A. indica* has lower acute toxicity than *M. indica.* According to Tabuti et al[30] both plants are potent antimalaria plants and are slightly toxic, although safe for human consumption.

Conventionally, body weight gains often result from physiological variation such as food intake, and metabolism[31]. Therefore, changes in body weight can thus be used to examine individual response to pharmacological effects (both herbal and conventional). Also, it may indicate the side effects of drug[32]. As earlier reported by Farah et al[33] significant changes in body and internal organ weights are considered sensitive indices of toxicity after exposure to toxic substances. A gradual loss of appetite and weight are usually seen in most if not all clinical manifestations of established malaria infection. Thus, the change in body weights of the experimental individuals indicated that malaria parasitemia was associated with considerable weight loss. The observed weight loss in the PNT is most likely owing to the negative consequences of malaria parasitemia. Treatment with Artemether/Lumefantrine - Coartem® (group 12) was associated with a significant weight gain. This agrees with the findings of Uraku[34] and Ozoko et al[35]. The rebound rise in body weight after treatment with Coartem® (ACT) is most likely attributable to a reversal of the negative effects of malaria parasitemia in mice. This recovery is interesting especially when compared to the findings by Samuel et al[36] who reported that administration of ACT or artesunate in unparasitized rats resulted in significant weight loss. It looks odd that untreated malaria parasitemia would be associated with weight loss, whereas parasitized treated with ACT was still associated with weight loss. However, the persistent weight loss observed in the treatment groups that received ethanol extracts of *M. indica* and *A. indica,* may be because of the presence of saponins and tannins (due to bitterness and astringent properties of

these respective phytochemicals), as previously reported by Yemitan and Adeyemi[37].

This study indicates that *A. indica* had better anti-plasmodial effect compared to *M. indica* when administered separately. However, there is a remarkable anti-plasmodial effect following the combination of both *A. indica* and *M. indica* on the parasite. This may be because of the chemical interaction between the phytochemical constituents of both plants' extracts, as well as their synergistic anti-plasmodial effect. This corroborates the conclusion drawn by Ofori-Attah et al[38] that reported the use of these concoctions in folkloric medical settings in the treatment of malaria. A combination of both single plant extract increased the concentration of polyphenols, thus improving their anti-plasmodic activities. Polyphenols elevate red blood cell oxidation and inhibit the parasites protein synthesis[39]. This activity nullifies the oxidative damage induced by the malaria parasite[40]. Thus, the strong antimalarial activity observed may be due to the presence of numerous polyphenols.

The commonest enzymes regarded as indicators of liver damage are aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT). The damage to hepatocellular cells results in the increase in these enzymes[41]. This research showed that there is a significant ($p < 0.05$) increase in AST level especially in the group with PNT animals as compared to the normal control (NC). However, extracts caused significant reduction in AST, ALT, and ALP activities in treated groups.

This research work has demonstrated the effect of aqueous extract of *A. indica* and *M. indica* on the liver. This claim has been supported by Prasad et al[42] who reported on the hepatoprotective potentials of these herbal compounds. Histopathological assessment using H&E technique was employed to study the hepatic tissue alterations induced by material parasites. The PNT group showed varying levels of hepatotoxic injuries and degenerations of hepatocytes, cellular vacuolations, and widespread micro-vesicular steatosis and evidence parasites present in the blood vessels and within sinusoids of the hepatic lobules. Result also showed varying levels of this inflammatory and degenerative condition in the extract administered. Liver involvement in severe malarial infection is commonly a significant cause of mortality among humans[43]. It was also documented in the study that hepatopathological changes of severe malarial parasite cases were associated with total bilirubin levels, apoptosis of Kupffer cells and portal tracts lymphocyte activation[43]. It is therefore an established fact based on findings that *Plasmodium* strains are localized in the liver of the first phase of the infection causing varying levels of hepatotoxic effects[44], as shown in this study.

A. indica and *M. indica* exhibit hepatoprotective activities[45,46]. Although the hepatic pathological features observed in the PNT group were also obvious in some of the extract treated groups, both in the single and in combination groups, the intensity of these features were mild, and reversed in the standard drugs treatment group when compared to NC group. The beneficial and toxic activities of tannins have also been investigated and documented[47]. The presence of tannins in both extracts of study may be implicated in the residual hepatotoxic changes observed in the result.

Furthermore, as revealed by the periodic acid Schiff staining, glycogen depletion in the liver was observed in all treatment groups besides the normal control and positive control (ACT-treated). Glycogen is a stored form of glucose, the

primary source of human energy received from carbohydrates absorbed through meals. The skeletal muscles and liver are the two main storage facilities for glycogen. Due to its pivotal role in metabolic fuel production, depletion of glycogen in the liver, the metabolism hub of the body could be linked to reduction in body weight as observed in the *M. indica* and *A. indica* extract-treated groups. Though the mechanism by which the extracts may likely induce glycogen depletion in the experimental groups remains unclear, following this storage depletion, there may be a probable cellular competition for available glucose after food intake. Therefore, insufficient glucose or a lack thereof may hinder several cellular activities including repairs and regeneration, which will negatively impact on the overall body weights of the experimental animals. This is unison with the report of Lacombe et al[48] who reported that after glycogen-depleting exercise, significant decreased in body weights were recorded before incremental exercise test in a horse model.

Glycogen storage has been a documented function of the liver[49]. The presence of these stored molecules in the liver has been studied applying the periodic acid Schiff technique[50]. As a histochemical technique, it is utilized to assist in the examination of tissues. The liver section stained for glycogen will display magenta staining, but with the PAS samples (depleted sample cells) will have a profound loss/absence of the magenta staining[51]. This study demonstrated a high expression of glycogen stain in the liver tissue of the normal control group and the standard drug (AL) treated group. The profound absence of glycogen expression in the PNT group and the extract treatment groups may be due to competitive activities of the parasites for systemic glucose to be utilized and stored by the hepatocytes, which is distinct from the presence of glycogen in the hepatic tissue of the AL (standard drug) treated group may be due to the drug toxicities to the parasites, causing their destruction hence lessening the competitive tendencies of the parasites for liver glucose, and hindering of glycogen storage within tissue.

CONCLUSION

Alkaloids are abundantly present (+++) in *Azadirachta indica* leaf extract but totally absent (-) in *Mangifera indica* bark extract. Notably, the median lethal dose (LD_{50}) demonstrated that *Azadirachta indica* (3240.37 mgkg) comapred to *Mangifera indica* (2738.61 mg/kg). Interestingly, *A. indica* single doses outperformed *M. indica* in reversing *P. berghei*-induced hepatotoxicity through mitigating parasite progression and associated elevated liver enzymes. Dose-dependent concentrations of *M. indica* severely altered hepatic collagen and glycogen storage compared to *A. indica.*

Author Contributions

MIU, IAE, AIP, and JAU conceptualized and designed and supervised the study, AJP, IUU and MAA performed the experiments, analyzed the data, and co-drafted the manuscript with MIU, IAE, AIP, and JAU.

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Conflicts of Interest

There are no conflicts of interest.

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Pattern of Female Genital Tract Malignancies at Delta State University Teaching Hospital, Oghara, Nigeria

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Abstract

Introduction: Cancers of the female genital tract contribute significantly to cancer-related morbidity and mortality globally. The pattern and distribution of these malignancies vary from region to regions.

The objective of this study was to determine the incidence, pattern of presentation and distribution of the different types of genital tract malignancies in Delta State.

Materials and Methods: It was a cross sectional descriptive study of gynaecological malignancies managed at the Delta State University Teaching Hospital, Oghara from January 2013 to December 2015. Case notes of all patients seen with gynaecological malignancies confirmed histo-pathologically during the studied period were retrieved. The required information was extracted from the case notes.

Results: A total of 3964 patients were seen at the gynaecological clinics during the period and 137 of these patients had gynaecological malignancies, with incidence of 3.5% of total gynaecological patients. Cervical cancer accounted for 68.42% of the cases. This was followed by ovarian cancer, comprising 15.79% and endometrial cancer, 12.78%. Vulvar cancer and choriocarcinoma were least occurring, accounting for 1.5% each. The mean age of all patients with genital tract malignancies was 51.26 ± 13.2years. The mean ages of patients with cervical, ovarian and endometrial cancers were 42.70 ± 3.15, 61.52 ± 6.82 and 58.76±5.57years respectively. The mean parity was 5.62 ±1.74.

Conclusion: Cervical cancer was the most common female genital malignancy and a higher proportion of the patients were grandmultiparous women with postcoital bleeding being the most common clinical presentation.

Keywords: Pattern, Female genital tract, Malignancy, Oghara, Nigeria

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INTRODUCTION

Malignancies of the female genital tract are major public health issues and contribute significantly to cancer-related morbidity and mortalityglobally.[1]The burden of gynaecological malignancies in developing countries including Nigeria is enormous and it poses a significant challenge to the health systems where poor awareness and late presentation prevail.[2]

Gynaecological malignancies account for about 10% of all newly diagnosed female malignancies and 12% of all female cancer deaths worldwide.[2] Most female genital tract malignancies have

worldwide distribution, but the distribution and frequency vary from one region to the other.[3] These variations may be attributed to different environment, life style, genetic and socioeconomic backgroung.[3] The proportion of cancers in the females which are of genital tract origin range from as high as 31.6% to 35.0% in sub-Saharan Africa and as low as 12.7% to 13.4% in North America and other developed nations where health-seeking and organized screenings methods have greatly improved.[4]

Cervical cancer is the most common female genital cancer in the developing countries including Nigeria.[5] In Nigeria, it is the most common cancer of the female genital tract, accounting for 74.4%,73.2%, 73.1% and 73.6% of female cancers in Benin City, Ibadan, Ilorin and Port Harcourt respectively.[6,7] It is the second most female common malignancy after breast cancer.[8] Chronic infection with human papilloma virus (HPV) is known to induce pre-malignant change in the cervical cells and ultimately cervical cancer in a few years, with a host of multiple risk factors.[9,10]

Ovarian cancer is one of the female genital tract malignancies that is associated with high mortality.[8] About 75% of the patients with ovarian cancer present with advanced stages due to non-specific symptoms of the disease and failure to detect the tumour early, poor accessibility due to the peculiar position of the ovary and absence of definitive screening tools.[8] Endometrial cancer is the third commonest gynaecological cancer in the developing countries, though it is becoming the most common of the gynaecological malignancies in the developed world.[11,12] It is commoner in the post menopausal women, with peak incidence being 58-60 age group.[6]

Vulva, vaginal and Primary Fallopian tube cancer are rare cancers accounting for less than 5% of gynaecological malignancies. [13,14,15]. However, histologic, molecular, and genetic evidence shows that as many as 80% of tumors that were classified as high-grade serous carcinomas of the ovary or peritoneum may have originated in the fimbrial end of the fallopian tube.[16]

This study is a retrospective review of the female genital tract malignancies that were diagnosed, histo-pathologically confirmed and managed at the Delta State University Teaching Hospital, Oghara (DELSUTH) over a 3 year period. The study therefore aims to determine the incidence, clinical pattern of presentation and the distribution of female genital tract malignancies in DELSUTH, Oghara, Nigeria. It therefore sorts to add to already existing knowledge on the subject matter in the region.

MATERIALS AND METHODS

This was a cross sectional descriptive study of female genital tract malignancies at the department of Obstetrics and Gynaecology of Delta State University Teaching Hospital, Oghara, Delta State, and covered a period of 3 years from January 1,2013 to December 31, 2015. The case notes of patients managed for female genital tract malignancies were retrieved from the medical records department and relevant data were extracted. The search spanned records from the gynaecological clinics, gynaecological wards and theatre registers. All cases of female genital tract malignancies diagnosed clinically, radiologically or surgically and confirmed by histo-pathological examinations were included in this study.

Permission to access hospital records and data for this study was obtained from the hospital management and ethical approval was obtained from the hospital's Health Research and Ethics

Committee.

The data extracted included; socio-demographic characteristics (age, parity, marital status and socio-economic status), presenting complaints, diagnosis, stage of the disease and the management offered. The patients socio-economic status classification was done using the scheme proposed by Oyedeji, based on the educational attainment and occupation.[17] Data collected were coded and analysed by simple descriptive statistics using statistical package for social science (SPSS) version 22, IBM Company. The results were expressed in simple descriptive statistics using mean, standard deviations, frequency and percentages, in tables and compared to other reports where appropriate.

RESULTS

There were 3964 patients seen at the gynaecological clinics during the period, 623 of these patients were admitted into the gynaecological ward and 137 patients had histo-pathologically diagnosed female genital tract malignancies during the period, accounting for 3.5% of total gynaecological patients seen in the hospital clinics and 22.0% of patients admitted into the wards respectively. One hundred and thirty three (133) case folders of 137 cases were available with complete information data extraction and analysis, given a retrieval rate of 96.35% and these formed the basis for further analysis.

The age range of the patients that had genital tract malignancies was between 29 to 84 years with a mean age of 51.26 ± 13.2years. The mean age of patients that had cervical cancer was 42.70 ± 3.15 years. Forty eight (36.36%) of these patients were in the age group of 40-49 followed by 32 (24.24%) each in 30-39 and 50-59 age groups respectively. The mean age of patients with ovarian cancer was 61.52 ± 6.82 years and 11 (52.38%) of these patients were aged 60 years and above. Majority of the patients with endometrial cancer were post menopausal with a mean age of 58.76 ±5.57 years. Eleven (64.71%) of patients with endometrial cancer were between 50-59 years and 5 (29.41%) were more than 60 years old.

The mean parity for patients with genital tract malignancies was 5.62 ±1.74. Majorities of patients with ovarian cancer, 9 (42.86%) were nulliparous. Most of the patients with cervical cancer were of low socio-economic class (classes 4 and 5). Thirty-nine (42.85%) were social class 5 and 28 (30.77%) were in class 4. Eight (38.10%) of patients with ovarian cancer were of socioeconomic class 5, 4(19.05%) each were class 1 (upper class) and 3 respectively. Almost half of the patients with endometrial cancer, 8(47.06 %) were in class 2 The patients with vulva cancer were in socioeconomic class 4 and 5 while the 2 patients with choriocarcinoma were in socioeconomic class 2 and 4.(table 1)

The study showed that the commonest genital tract malignancy was cervical carcinoma and it comprised of 91 (68.42%) of the cases. This was followed by ovarian cancer, 21 (15.79%) and endometrial cancer 17 (12.78%). Vulvar cancers and choriocarcinoma accounted for 2(1.50%) each of the cases (figure 1). There were no cases of uterine sarcomas, vagina or fallopian tube cancers during the period.

Thirty-five (38.46%) patients with cervical cancer presented with post coital bleeding while majority of patients with ovarian cancer, 13 (61.91%) presented with abdominal swelling and abdominal pain. The most common presenting complaint in patients with endometrial cancer was post-menopausal bleeding as demonstrated in 12 (70.59%) of the patients. All the patients with vulva cancers and choriocarcinoma

presented with vulva ulceration and recurrent bleeding per vaginum respectively. (table 2)

Majority of the patients with cervical cancer, 61(67.03%) presented with advanced disease; 11 (12.09%) presented with stage II disease, 31 (34.07) presented with stage III and 19 (20.88%) with stage IV. Eleven (52.38%) of patients with ovarian malignancy presented with stage IV disease, while 7(33.33%) and 3 (14.29%) presented with stage III and stage II disease respectively. 7 (41.18%) of patients with endometrial cancer presented with Stage II disease while 8 (47.06%) were seen at Stage III. One (50.0 %) each of patients with vulva cancer presented with stages II and III disease respectively/(Table 3)

Table 1: Socio-demographic Characteristics of Patients with Genital Tract Malignancies.

VARIABLE	TYPES OF CANCER (Frequency(%)				
AGE	CERVICAL CANCER (n=91)	OVARIAN CANCER (n=21)	ENDOMETRIAL CANCER (n=17)	VULVAL CANCER (n=2)	CHORIOCARCINOMA (n=2)
20-29	2(2.20%)	1(4.76%)	-	-	-
30-39	23(25.27%)	-	-	-	2(100%)
40-49	31(34.07%)	2(9.53%)	1(5.88%)	-	-
50-59	23(25.27%)	7(33.33%)	11(64.71%)	-	-
≥60	12(13.19%)	11(52.38%)	5(29.41%)	2(100%)	-
PARITY					
0	1(1.10%)	9(42.86%)	3(17.64%)	-	-
1	4(4.40%)	4(19.05%)	-	-	-
2	3(3.30%)	1(4.76%)	-	2(100%)	-
3	6(6.59%)	-	-	-	1(50.00%)
4	17(18.68%)	-	1(5.88%)	-	1(50.00%)
≥5	60(65.93%)	7(33.33%)	14(82.35%)	-	-
SOCIO-ECONOMIC STATUS					
CLASS 1	4(4.40%)	4(19.05%)	1(5.9%)	-	-
CLASS 2	8(8.79%)	3(14.29%)	8(47.06%)	-	1(50.00%)
CLASS 3	12(13.19%)	4(19.05%)	3(17.65%)	-	-
CLASS 4	28(30.77%)	2(9.52%)	3(17.65%)	1(50.00%)	1(50.00%)
CLASS 5	39(42.85%)	8(38.10%)	2(11.76%)	1(50.00%)	-

Figure 1: Relative Frequencies of Genital Tract Malignancies.

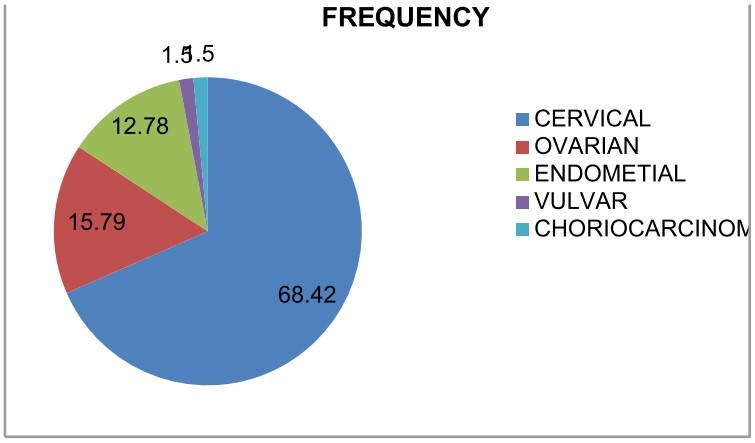

Table 2: Clinical Presentation Of Different Genital Tract Malignancies

PRESENTING SYMPTOMS	CERVICAL CANCER	OVARIAN CANCER	ENDOMETRIAL CANCER	VULVAL CANCER	CHORIOCARCINOMA
POSTCOITAL BLEEDING	35(38.46%)	-	-	-	-
ABNORMAL OFFENSIVE VAGINAL DISCHARGE	21(23.08%)	-	-	-	-
POSTMENOPAUSAL BLEEDING	23(25.27%)	-	12(70.59%)	-	-
IRREGULAR/RECURRENT VAGINAL BLEEDING	-	-	-	-	2(100.0%)
ABDOMINAL PAIN ALONE	12(13.19%)	-	5(29.41%)	-	-
ABDOMINAL SWELLING + PAIN	-	13(61.91%)	-	-	-
ABDOMINAL SWELLING		8(38.57%)			
TOTAL	91(100.0%)	21(100.0%)	17(100.0%)	2(100.0%)	2(100.0%)

Table 3: Stage at Presentation

STAGE AT PRESENTATION	CERVICAL CANCER	OVARIAN CANCER	ENDOMETRIAL CANCER	VULVAL CANCER	CHORIOCARCINOMA
STAGE I	-	-	2(11.76%)	-	1(50.0%)
STAGE II	11(12.09%)	3(14.29%)	7(41.18%)	1(50.0%)	1(50.0%)
STAGE III	**61(67.03%)**	7(33.33%)	8(47.06%)	1(50.0%)	-
STAGE IV	19(20.88%)	11(52.38%)	-	-	-
TOTAL	91(100.0%)	21(100.0%)	17(100.0%)	2(100.0%)	2(100.0%)

FIGURE 1: RELATIVE FREQUENCIES OF GENITAL TRACT MALIGNANCIES.

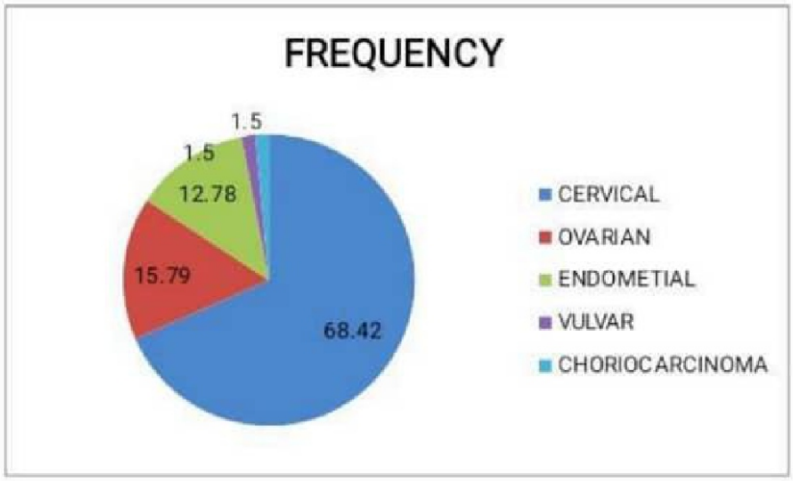

TABLE 1: SOCIO-DEMOGRAPHIC CHARACTERISTICS OF PATIENTS WITH GENITAL TRACT MALIGNANCIES.

VARIABLE	TYPES OF CANCER (Frequency(%)				
AGE	CERVICAL CANCER (n=91)	OVARIAN CANCER (n=21)	ENDOMETRIAL CANCER (n=17)	VULVAL CANCER (n=2)	CHORIOCARCINOMA (n=2)
20-29	2(2.20%)	1(4.76%)			
30-39	23(25.27%)				2(100%)
40-49	31(34.07%)	2(9.53%)	1(5.88%)		
50-59	23(25.27%)	7(33.33%)	11(64.71%)		
≥60	12(13.19%)	11(52.38%)	5(29.41%)	2(100%)	
PARITY					
0	1(1.10%)	9(42.86%)	3(17.64%)		
1	4(4.40%)	4(19.05%)			
2	3(3.30%)	1(4.76%)		2(100%)	
3	6(6.59%)				1(50.00%)
4	17(18.68%)		1(5.88%)		1(50.00%)
≥5	60(65.93%)	7(33.33%)	14(82.35%)		
SOCIO-ECONOMIC STATUS					
CLASS 1	4(4.40%)	4(19.05%)	1(5.9%)		
CLASS 2	8(8.79%)	3(14.29%)	8(47.06%)		1(50.00%)
CLASS 3	12(13.19%)	4(19.05%)	3(17.65%)		
CLASS 4	28(30.77%)	2(9.52%)	3(17.65%)	1(50.00%)	1(50.00%)
CLASS 5	39(42.85%)	8(38.10%)	2(11.76%)	1(50.00%)	

TABLE 2: CLINICAL PRESENTATION OF DIFFERENT GENITAL TRACT MALIGNANCIES.

PRESENTING SYMPTOMS	CERVICAL CANCER	OVARIAN CANCER	ENDOMETRIAL CANCER	VULVAL CANCER	CHORIOCARCINOMA
POSTCOITAL BLEEDING	35(38.46%)				
ABNORMAL OFFENSIVE VAGINAL DISCHARGE	21(23.08%)				
POSTMENOPAUSAL BLEEDING	23(25.27%)		12(70.59%)		
IRREGULAR/RECURRENT VAGINAL BLEEDING					2(100.0%)
ABDOMINAL PAIN ALONE	12(13.19%)		5(29.41%)		
ABDOMINAL SWELLING + PAIN		13(61.91%)			
ABDOMINAL SWELLING		8(38.57%)			
TOTAL	91(100.0%)	21(100.0%)	17(100.0%)	2(100.0%)	2(100.0%)

TABLE 3: STAGE AT PRESENTATION

STAGE AT PRESENTATION	CERVICAL CANCER	OVARIAN CANCER	ENDOMETRIAL CANCER	VULVAL CANCER	CHORIOCARCINOMA
STAGE I			2(11.76%)		1(50.0%)
STAGE II	11(12.09%)	3(14.29%)	7(41.18%)	1(50.0%)	1(50.0%)
STAGE III	61(67.03%)	7(33.33%)	8(47.06%)	1(50.0%)	
STAGE IV	19(20.88%)	11(52.38%)			
TOTAL	91(100.0%)	21(100.0%)	17(100.0%)	2(100.0%)	2(100.0%)

DISCUSSION

Patients with female genital tract malignancies constituted 3.5% of all patients seen at the gynaecological clinics in DELSUTH, South-South Nigeria during the period under review. This finding is similar to 4.6% reported by Ijaiya and Ibrahim in Ilorin.[17] However, this finding is lower than 11.5% reported by Yakasai et al in Kano.[14]

It was reported that the most important aetiological factors contributing to cervical cancer in these women were early marriages, and early age of initiating coitus which were more common in the Northern part of the country like Kano.The study revealed that cervical cancer was the most common gynaecological cancer accounting for 68.42% of cases. This was followed by Ovarian 15.79% and endometrial 12.78%. Vulva and choriocarcinoma accounted for 1.5% each of the patients. This is similar to the findings reported by Yakasai et al in Kano, Udigwe et al in Nnewi, Okeke et al in Enugu.[14,15,18]

This is also comparable with findings by Nkyekyer in Ghana and Taulo in Malawi.[19,20] Kyari et al. in a study conducted in Maiduguri, northern Nigeria, reported a slightly higher incidence of 70.5% of cervical cancer.[21] However, our finding is in contrast to the findings of Sobia et al in Pakistan where the most common gynaecological malignancy was ovarian cancer, followed by endometrial and then cervical cancer.[22] The low prevalence of cervical cancer in the study by Sobia et al in Parkistan may be due to less smoking in ladies, adherence to social norms, religious practices and male circumcision.

The prevalence of cervical cancer in developing world is in contrast to what obtains in developed countries where endometrial cancer is more common. This is thought to be due to well established screening services for pre-malignant lesions of cervical cancer in the developed countries.[7] The leading position of carcinoma of the cervix in this study indicates that the uptake of cervical cancer screening and treatment of

premalignant lesions of the cervix is far below optimal level in Nigeria. This is quite understandable with the absence of a national cervical cancer screening program. Furthermore, opportunistic screening for cervical cancer as practiced in many Centres including ours, has been shown not to impact on the population incidence of cervical cancer.[23] Opportunistic screening as practiced in our centre involves screening all women who come for gynaecological consultation in the hospital after proper counselling and obtaining informed consent. There were two cases of vulva cancer during the period under review. This is a rare cancer and it contributed 1.5% of genital tract cancers similar to 1.8% reported by Udigwe et al in Nnewi and Yakasai et al in Kano.[14,15]

The mean age for gynaecological malignancies was 51.26 ± 13.2 years and the mean parity was 5.62 ±1.74. This was similar to the finding by Udigwe et al in Nnewi and Ijaiya and Ibrahim in Ilorin.[15,17] Most of the patients with cervical cancer (34.07%), were in the age group 40-49 while 25.27% were in 30-39 and 50-59 age group respectively, thus further reaffirming the existing knowledge that cervical cancer is a disease of sexually active women with bimodal peak ages (4th and 6th decades).[15] Sixty (65.93%) of patients with cervical cancer were 5 and above. This is similar to findings by Yakasai in Kano and Nkyekyer in Ghana.[14,19]. Eleven (52.38%) of the patients with ovarian cancer were 60 years and above. This is in-keeping with established knowledge that ovarian cancer is a disease of older woman with peak incidence at 67years.[24] Most of the patients with endometrial cancer were in the age group 50-59 and 82.35% were Para 5 and above. This is similar to findings by Udigwe et al in Nnewi and Nkyekyer in Ghana.[15,19] This contrasts the findings of Yakasai in Kano where mean age of the patients with endometrial cancer was 62 and mean parity was 4 and also current belief that endometrial cancer is a disease of the 7th decade and nulliparous women.[14,15]

The majority of patients with cervical cancer, 42.85% (39) were of the low socioeconomic status. This agrees with general knowledge that low socioeconomic factor is a risk factor for cervical cancer.[25] The most common presenting complaint from patients with cervical cancer was post coital bleeding and offensive vaginal discharge. This is similar to findings of Gaya et al in Kano.[25] Most of the patients with ovarian cancer presented with abdominal distension and pain. Most of the patients with ovarian and cervical cancer presented with advanced diseases. Eleven (52.38%) of patients with ovarian cancer presented with stage IV disease. It is a recognized fact worldwide that women with ovarian cancer present with advanced disease and this may be attributed to nonspecific symptoms and signs of early disease and this has an effect on survival. 87.91% of patients with cervical cancer presented with Stage III and IV disease and none presented with stage I disease. This is similar to findings of Yakasai et al in Kano.[14] Late presentation by patients may be attributed to absence of established national cervical cancer screening programs, lack of awareness and a penchant to patronize unorthodox health practitioners.[26] The low socioeconomic status with associated low financial power may in turn delay presentation as health insurance is not widely available in developing countries.[24] This study shows consistency with general observation that patients with endometrial cancer present at an early stage; 52.94% in this study. This is similar to findings of Nkyekyer in Ghana.[19]

In conclusion, cervical cancer is the most common female genital tract malignancy in DELSUTH, Oghara followed by ovarian cancer, endometrial cancer and least common were

vulvar cancer and choriocarcinoma. Most of the patients with cervical cancer were in the 4th decade of life, with most patients presenting with advanced disease. The reduction of morbidity and mortality from cervical cancer involves early detection, treatment, and palliative care to facilitate comfort, dignity, autonomy, and personal rehabilitation and development, especially in the face of an incurable disease. Fortunately, this is possible because cervical cancer is a preventable disease.

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Distribution and frequency of blood groups and haemoglobin genotype pattern among blood donors in a tertiary hospital in southern Nigeria

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Abstract

Introduction: The ABO is a blood group system that is responsible for most blood transfusion reactions, transplant rejections and determining some forensic cases. The ABO and Rh blood group systems have been shown to show variations in different parts of the world and race. Haemoglobin is an intracellular protein found in red blood cells. Qualitative and quantitative abnormalities in this protein manifest as haemoglobinopathy.

The study is to show the frequency and distribution of ABO, Rh blood groups and haemoglobin phenotype of eligible blood donors in Delta state University Teaching Hospital (DELSUTH), Nigeria

Materials and Methods: This is a cross-sectional study of all blood donors attending the blood bank in DELSUTH from November 2022 to April 2003. Consecutive sampling technique was used and samples for blood group and haemoglobin genotype was collected from eligible donors. Data from the blood ban analysed using SPSS version 23.

Results: A total of 95 donors were involved in the study. Analysis of the ABO blood group showed that the frequency blood group O,A,B and AB, were 83.2%,7.4%,7.4% and 2.2% respectively. RhD positive donors accounted for 93.7% of donors and RhD negative were 6.3%. 83.2% of donors had genotype AA while16.8% were genotype AS.

Conclusion: Blood group O and RhD positive were the commonest blood group while genotype AA was the commonest genotype among blood donors in the facility.

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Introduction

Human red blood cells contain a series of glycoproteins and glycolipids on their surfaces which constitute blood group antigens.[1] These antigens are genetically controlled and are inherited in the Mendelian manner. Approximately 400 red blood cell group antigens have been described in literature.[1] These different blood group antigens vary greatly in their clinical significance, with ABO and Rh groups being the most important in view of the safety of blood or blood products transfusion till date.[2]

The ABO blood group system was the first human blood group system to be discovered by Landsteiner in 1901 and later on, Landsteiner and Wiener defined the Rh blood group in 1941. The protein that defines the ABO antigens is a glycosyl transferase that is encoded from a single gene for which there are three major alleles A, B, O.[1] The A and B alleles catalyze the addition of different carbohydrates residues (N-acetyl galactosamine for group A and galactose for group B) to a basic antigenic glycoprotein or glycolipid with a terminal sugar on the red cell, known as the H substance.[1] The O allele is non-

functional and so does not modify the H substance. Apart from their expression on the red blood cells (RBC), ABO antigens are also highly expressed on the surface of a variety of human cells and tissues, including the epithelium, sensory neurons, platelets and the vascular endothelium.[3] Antibodies of the ABO system are naturally occurring antibodies as they arise without immune stimulation by relevant blood group antigens.[1] They are not detectable in the blood until 3 to 6 months of life.

The Rhesus (Rh) blood group locus is composed of two related structural genes, RhD and RhCE which encode the membrane protein that carry D, Cc and Ee antigens. The RhD gene may be either present or absent, giving the RhD+ or RhD- phenotype respectively. Rh antigens are expressed only on red cells and are fully expressed before birth. Rh antibodies are generally immune antibodies and can cause hemolytic transfusion reaction.

Distribution of ABO and Rh D blood groups varies between populations and races reflecting the underlying genetic and ethnic diversity of human populations.[3] The studies on blood groups are important parameters in various genetic studies for reliable geographical information, researching population migration patterns and in blood transfusion process. The knowledge of the distribution of ABO and Rh blood groups is essential for effective management of blood banks inventory, be it a facility of a smaller local transfusion service or a regional or national transfusion service. Apart from the above, these blood groups can be used in resolving certain medico-legal issues, particularly of disputed parentage. In addition, there are accumulating evidence that the ABO blood group plays a key role in various human diseases such as diabetes, cardiovascular, neoplastic, carcinoma and infectious disorders.

The intracellular protein found in RBC called haemoglobin (Hb) is made up of four polypeptide globin chains that are folded around heme molecules. The globin chains are known to have many alleles and are encoded by the relevant genes on chromosomes 11 and 16. Normal adult blood contains three types of haemoglobin ; Hb A (96-98%), Hb F (0.5-0.8%) and Hb A_2 (1.5-3.2%).Haemoglobin abnormalities could result from synthesis of abnormal Hb with altered amino acid sequence of from reduced synthesis of normal alpha or beta globin chains.

Nigeria is a large nation with diverse ethnic groups and a population of about 200million. As with many other genetic traits, haemoglobin genotype and the gene frequency of ABO and rhesus blood group varies significantly within the six geopolitical zones in Nigeria. Despite the numerous studies carried out on the distribution of haemoglobin genotypes, ABO and rhesus blood groups in Nigeria, to the best of my knowledge, no study has been carried out among residents blood donors in Oghara, Delta state, South-South Nigeria.

This study was therefore carried out to determine the distribution of haemoglobin genotypes, ABO and rhesus blood groups among eligible blood donors in Delta state university teaching hospital (DELSUTH), Oghara, Delta state.

MATERIALS AND METHODS

The study was a cross-sectional study.

Samples were collected at the donor Clinic of the Delta state University Teaching Hospital (DELSUTH), Oghara, Delta state, Nigeria. DELSUTH is a state government owned teaching hospital with over 300 bed capacity, located in Ethiope-west local government area of Delta state. It is affiliated to Delta state university,

Abraka and it boast of over 20 different medical disciplines.

Study Population
The study population comprised of apparently healthy voluntary and eligible blood donors who would gave written consent to participate in the study. We excluded blood eligible donors that were not willing to participate in the study.

Sample Size Estimation
Minimum sample size was determined using the formula: n = N/1+N (e)2 with 95% confidence interval level.

Where:
n= minimum required sample size
N = number of blood donors per month (estimated to be 126)
e = allowable error (%) which was set as 0.05.
Substituting in the formula:
n = 126/1+126(0.05)2 = 95.8.

Thus, a minimum of 96 blood donors were be required to be enrolled in the study.

Sampling Technique
A consecutive sampling procedure was used in this study.

Approximately 2milliliters (mls) of venous blood was drawn aseptically from the antecubital vein of each subject with minimal stasis and dispensed into commercially prepared ethylene di-amine tetra-acetic acid (EDTA) bottle for blood group and genotype.. The sample was mixed gently but thoroughly to prevent cell lysis and ensure adequate anticoagulation. All specimens was labelled with personally generated identification numbers and analysed within one hour of collection. The ABO and Rh blood group were determined using tile method and the haemoglobin genotype was determined using haemoglobin electrophoresis. Blood group was done using the cell (forward) grouping method using monoclonal Anti-A, Anti-B and Anti-D sera with LOT number-224037. Reagent storage and labeling of samples were managed properly according to the kit manufacturer (Immucor, Inc, Germany) standards.

Daily quality control of selected red blood cells and antiserum was performed to confirm the reactivity and specificity of the reagent. These reagents were tested with the corresponding antigen-positive and antigen-negative red blood cells. The reagents were considered appropriate for use if only antigen-positive red blood cells demonstrate a positive result.

Study Duration
The study was carried out within a period of six months (November 2022- April 2003)

Data Analysis
Data obtained was analysed using Statistical Package for the social sciences (SPSS) version 23. Probability values less than 0.05 ($p < 0.05$) were considered as significant. Results were presented in tables and percentages.

RESULTS

Sociodemographic Distribution
A total of 95 blood donors were recruited in this study comprising of 10 voluntary donors, 16 family replacement donors and 69 professional donors.

Most donors were below 30 years old (70.5%) and the fewest were above 40years of age (3.2%). Most donors below 30 years and between 30- 39 were professional donors (84.1% and 11% respectively). The only group of donors above 40 years were family replacement donors (3 donors).

The difference in age group amongst the three groups of patient was statistically significant ($p < 0.001$).

Eight- nine (93.7%) donors were males and six (6.7%) were females. All professional donors (100%) were males, while 12 males and 8 males were family replacement and voluntary donors respectively. There was a significant difference between the sex groups in the study ($p < 0.001$). Thirty-eight (40%) of the donors had tertiary level of education, thirty-two (33.7%) had secondary levels and twenty- five (26.3%) had primary education. Neither voluntary nor family replacement donor had primary level of education but 2(20%) and 3(18.8%) reached secondary level of education respectively. Most professional donors (39.1%) had secondary level of education, closely followed by (36.2%) and (24.6%) with primary and tertiary education respectively. The difference in level of education reached statistical significance ($p<0.001$).

Seventy- four (77.8%) of donors were single and twenty-one (22.1%) were married. Most family replacement donors were married (62.5%) while most of the voluntary and professional donors were single (70% and 88.4%) respectively. The difference in marital status was statistically significant ($p<0.001$).

Table 1: Demographic pattern of blood donors

	FR n = 16	**PD n = 69**	**VD n = 10**	**Total n = 95**	**P-value**
Age group					
<30	4 (25.0)	58 (84.1)	5 (50.0)	67 (70.5)	
30 – 39	9 (56.3)	11 (15.9)	5 (50.0)	25 (26.3)	<0.001
40 – 49	3 (18.8)	0 (0.0)	0 (0.0)	3 (3.2)	
Sex					
Male	12(75.0)	69(100)	8(80)	89(93.7)	<0.001
Female	4(25.0)	0(0.0)	2(20)	6(6.3)	
Education					
Pry	0 (0.0)	25(36.2)	0(0.0)	25(26.3)	
Sec	3 (18.8)	27(39.1)	2(20)	32(33.7)	<0.001
Tertiary	13 (81.2)	17(24.6)	8(80)	38(40)	
Marital status					
Single	6 (37.5)	61 (88.4)	7 (70.0)	74(77.8)	,<0.001
Married	10 (62.5)	8 (11.6)	3 (30.0)	21(22.1)	
Religion					
Christians	16(100.0)	69(100.0)	10(100.0)	95(100.0)	

FR (Family replacement), VD (Voluntary donor and PD (Professional donor)

Haemoglobin genotype and blood group of blood donors

Seventy-nine (83.2 %.) donors were genotype AA and sixteen (16.8%) had genotype AS. Amongst the genotype AA donors, majority were professional donors (56 donors) while voluntary donors accounted for the fewest number (9

donors). A similar trend was noticed in donors with haemoglobin genotype AS.

Seventy-nine (83.2%) donors had blood group O, seven each had blood group A and B while two had AB. Fifty-seven professional donors had blood group O, while family replacement and voluntary donors had fourteen and eight persons with blood group O respectively. Seven donors (7.4%) had blood group A and six of them were professional donors. Seven donors (7.4%) had blood group B, five of them were professional donors and the remaining were voluntary donors. Two persons (2.2%) had blood group AB and were family replacement and professional donors.

Eighty-nine (93.7%) donors were Rh D positive and six negative (6.3%). Sixty-five of those who were Rh D positive were professional donors, while fifteen and nine of them were family replacement and voluntary donors respectively. Four donors with Rh D negative were professional donors.

Table 2: Haemoglobin genotype and blood group pattern amongst donors

Genotype	FR	PD	VD		
AA	14 (87.5)	56 (81.2)	9 (90.0)	79 (83.2)	0.001
AS	2 (12.5)	13 (18.8)	1 (10.0)	16 (16.8)	
Blood group					
O	14(87.5)	57(82.6)	8 (80.0)	79 (83.2)	
A	1(6.3)	6(8.7)	0(0.0)	7 (7.4)	0.928
B	0 (0.0)	5 (7.2)	2(20.0)	7 (7.4)	
AB	1(6.3)	1 (1.4)	0 (0.0)	2 (2.2)	
Rhesus					
Pos.	15 (93.8)	65(67.7)	9(90.0)	89 (93.7)	0.793
Neg.	1 (6.3)	4 (5.8)	1(10.0)	6 (6.3)	

Key: FR (Family replacement), VD (Voluntary donor and PD (Professional donor)

DISCUSSION

In studies of the human population, helpful genetic markers such as the ABO and rhesus blood groups are important.[4] They are the most often used and important blood types as regards blood transfusion. Haemoglobin (Hb) genotype is an important blood component that determines haemoglobinopathies. Haemoglobinopathies are among the most frequently inherited genetic disorders in the world and they are inherited from healthy carrier/disease parents.

In this study, the distribution of the ABO blood phenotype showed that blood group O was the most common blood group (83.2%), followed by blood group A and B with 7.4% each and the least blood group was AB (2.2%). The result of this study on the ABO blood group phenotype frequency distribution is similar with the pattern seen in previous studies that reported high frequencies of group O and low prevalence of AB.

Similar distribution of the ABO blood group amongst donors and residents were reported by *Faduyile et al.*, *Enosolease et al.* and *Onuoha et al.* in

Lagos, Adamawa, Edo and Bayelsa states of Nigeria respectively.[2,5,6] Although the studies had similar pattern, the prevalence of each blood group varied from region to region. The slight discrepancy between our findings and that reported in other studies may be attributed to the ethnic difference among the population of Nigeria or it could be due to the smaller sample size in the present study.

The index study showed that professional donors had the highest prevalence for each blood group type and the trend is depicted as 82.6% for O, 8.7% for A, 7.2% for B and 1.4% for AB (O>A>B>AB). This is in concordance to a previous study that have reported the high prevalence of professional donors in Nigeria and in the locality.[7]

The high predominance of blood group O individuals in Nigeria, as seen in this study and previous studies, is advantageous since it indicates that there will always be blood available in an emergency situation.

In this study the distribution of rhesus positive (Rh+) blood group (93.7%) was more predominant than rhesus negative (Rh-) blood group (6.3%). While we reported a value of 6.3% prevalence for Rh- in our study, other studies reported values as low as 1.2% in Gusau and 2.9% in Yola, Nigeria.[8,9] A similar prevalence of 6.03% was reported in Benin by *Enosolease et al.*[2]

The low frequency of the Rh- blood type in the study are advantageous for blood banking and the prevalence of diseases like hemolytic disease of the newborn (HDN). It reduces the need for Rh-blood for transfusions, which is good news for blood bank management and vendors who often have a monumental task in procuring suitable donors. In terms of Rhesus alloimmunization and attendant HDN, it also gives the population certain obstetric advantages as HDN contributes to perinatal morbidity and mortality.

We identified two haemoglobin combinations among our donors (AA and AS). The result of this study showed that 83.2% of blood donors had haemoglobin phenotype AA and 16.8% AS. The prevalence of haemoglobin AA was similar to studies by *Damulak et al.* in Jos (77.7%), *Jeremiah et al.* in Port-Harcourt (80.32%) and *Umoh et al.* in Uyo (78%).[10-12] No sickle cell disease electrophoretic pattern was reported in this study. This was possible due to deferral during screening and also the small sample size.

In conclusion, the prevalent blood group in the study area is blood group O and the least is AB. RhD positive antigen and haemoglobin phenotype AA had the highest prevalence.

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Socio-clinical and Immuno-inflammatory-related differences between early-onset and late-onset colorectal cancer

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Abstract

Introduction: This study aimed to identify socio-clinical and immuno-inflammatory markers of early-onset colorectal cancer (CRC) for early detection of aggressive cancer in Southern Nigeria

Material and Methods: This study included 89 patients with CRC diagnosed from Jan. 2016 to Dec. 2022. The patients were sub-grouped based on age and chemotherapy response. The neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio (PLR), platelets-neutrophils-to-lymphocytes ratio (PNLR), and neutrophils-to-lymphocytes platelets ratio (NLPR) were assessed and analyzed accordingly. Significance was set at $p< 0.05$.

Results: Metastatic and stage III/IV CRCs were prevalent among patients older than 50 years compared with patients aged 50 years or less ($p< 0.05$). Among patients aged > 50 years, the pre-treatment (pre-T) to post-treatment (post-T) total white blood cell count (TWBC), neutrophils, monocytes, and NLPR significantly increased whereas the post-T lymphocyte count and LMR significantly declined ($p< 0.05$). The Post-T TWBC count was significantly higher among patients aged > 50 years ($14.20 \pm 4.50\ 10^{\wedge 9}$/L) compared with patients 50 years old or younger ($9.19 \pm 1.50\ 10^{\wedge 9}$/L) whereas the Post-T monocyte count and LMR were lower among the former (9.12 ± 2.33 and 2.87 ± 0.60) than the post-T values of patients who were ≤ 50 years old (11.81 ± 3.57 and 6.76 ± 3.92) at $p= 0.033$, 0.026, and 0.001, respectively.

Conclusion: This study revealed a higher frequency of CRC and mortality risk among patients older than 50 years. It suggests that SIII could be used as a prognostic tool for CRC.

Keywords: Haematological indices, gastrointestinal cancer, Immune cells, Chemotherapy, Southern Nigeria

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INTRODUCTION

According to the GLOBACAN 2020 estimates, colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related deaths in the world.[1] The average age-standardized incident rate per 100,000 (ASIR) of colon cancer (CC) in Europe, America, Asia, and Africa were 22.4, 13.0, 10.3, and 5.1, respectively.[1] Comparison of incidence-to-mortality revealed lower case fatalities in countries with a high/very high human development index (HDI) compared with countries with a low/medium HDI; 43.6% vs 62.3%.[1,2] Reasons for the difference between the two HDIs may be due to differences in the stage at presentation, access to healthcare facilities, and affordability of chemotherapy.[3] Another reason of note is that CRCs in Africa are very aggressive

and unusually metastatic.[4,5] In Nigeria, for instance, evidence shows that 34%, 70%, and 96%, of CRCs are poorly differentiated, right-sided (RCC), and invasive, respectively.[3,6] The RCCs are larger, more advanced, and poorly differentiated compared with the left CCs, and patients with RCCs are older.[7-9] RCC patients have poorer overall survival (OS) and disease-free survival (DFS) rates compared with LCC patients.[9] These factors reveal the importance of identifying affordable procedures and biomarkers for CRC that are alternatives to the expensive repeated imaging for high-mortality risk patients who are in low-resource settings. One such approach is precision medicine, in which the assessment of systemic immune-inflammatory (SIII) biomarkers has emerged as a promising option.[10] High pretreatment inflammatory indices have been associated with both a greater risk of cancer relapse in radically resected tumours and shorter survival for cancer patients with metastatic disease.[11] The circulating high neutrophil and low lymphocyte counts, especially post-chemotherapy, are independently associated with poor OS and progression-free survival (PFS), especially in metastatic CRC patients.[12] A low lymphocyte–monocyte ratio (LMR < 2.82), can reflect an active inflammation status and has been associated with high-grade tumours and worse OS and DFS and is more likely to be left-sided.[12] This study assessed the clinical utility and prognostic value of inflammation-related markers for better stratification of CRC patients in Southern Nigeria.

METHODS

Study Population and Ethics

From January 2016 to December 2022, 98 patients with gastrointestinal diseases presented at the Department of Gastroenterology, Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nigeria. Patients with inadequate records, especially haematological parameters (n=9) were excluded from the study. Finally, this study included a total of 89 patients diagnosed with CRC who were living in Anambra State. In addition to some antibiotics and surgeries, some patients received capecitabine and oxaliplatin as platinum chemotherapy. This retrospective study was approved by the NAUTH ethics committee (NAUTH/CS/66/VOL.15/VER.3/107/2022/081). The medical records of the patients were accessed for socio-clinical demographics such as age, gender, comorbidities, time of presentation, time of death, and contact for follow-up. All analyses were performed by the ethical standards laid down in the Declaration of Helsinki.

Sample collection and handling

Peripheral whole blood samples (5 ml each) were collected into EDTA containers one week before the first chemotherapy and a week before discharge. Full blood counts were carried out on the whole blood samples using a Haemo-autoanalyzer. Following ultrasound investigations, sections from biopsies or resected tissues were reviewed for evidence of malignancy and metastasis. The neutrophil, lymphocyte, platelet, monocyte, and total white cell counts ($10^{\wedge 9}$/L) were assessed. Overall survival was calculated from the date of presentation or diagnosis to the date of death or last follow-up. The blood neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio (PLR), platelets-neutrophils to lymphocytes ratio (PNLR; [Platelet count x Neutrophil count]/Lymphocyte count), and neutrophils-to-lymphocytes-platelets ratio (NLPR; [Neutrophil count x 100]/Lymphocyte count x platelet count]).

Study design

The cases of CRC were categorized based on the following: 1. Age (≤ 50 years or > 50 years), 2.

Chemo-sensitive and chemo-resistance. The assessment of features such as the alleviation of symptoms, liver and renal function tests, tumour response or degree of tumour shrinkage, and the need for second-line chemotherapy determined chemoresistance.[13]

Statistical analysis

Chi-square/Fisher was used to determine the association between age and socio-clinical demographics of the patients. Pearson's correlation was used to determine the relationship between the variables (NLR, PLR, PNLR, NLPR, and LMR) before and after the last treatment. A T-test was used to compare data of 1. patients aged ≤ 50 years and > 50 years, 2. chemotherapy naïve and experienced patients. The overall survival of patients was analyzed using the Kaplan-Meier method. The survival probabilities between the subgroups were compared using the log-rank test.

RESULT

The mean age, median age, and age range of the participants were 56.40 ± 13.58 years, 58.0 years, and 25 to 92 years, respectively.

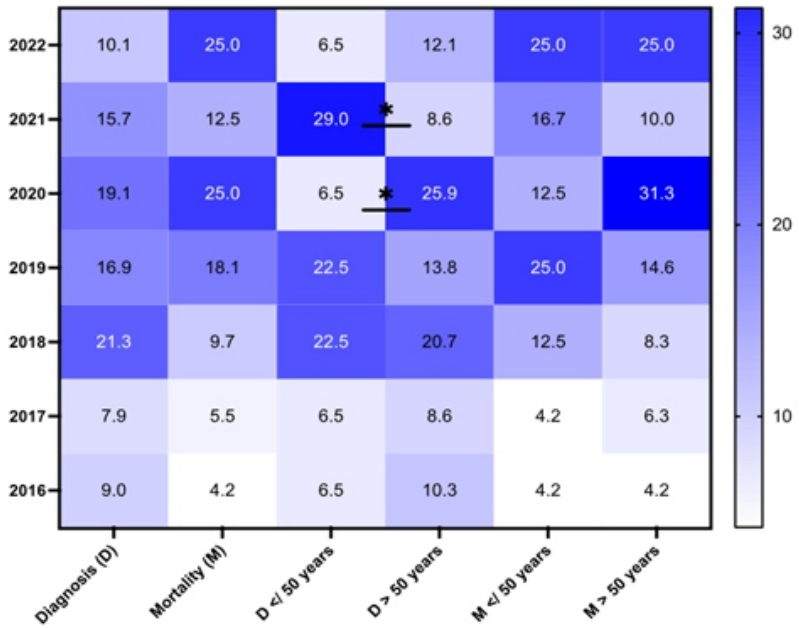

Figure 1: Heatmap of the percentage diagnosis (D) and mortality (M) rate among CRC patients aged ≤ 50 years and > 50 years

There was a high number of CRC diagnoses in 2018 (21.3%) compared with other years while a high rate of mortality was observed in 2020 (25.0%) and 2022 (25.0%) compared with other years. The prevalent features presented by the patients were: Weight loss, constipation, intermittent diarrhoea, low abdominal pain, and anorexia (see supplementary file). In 2020, the CRC diagnosis and mortality rates were approximately 4 times and 2.5 times higher among patients aged > 50 years compared with patients whose ages were less than or equal to (</;

≤) 50 years at p= 0.045 and 0.147, respectively. In 2021, the rate of CRC diagnosis was 3.4 times higher among patients who were ≤ 50 years old compared with those aged > 50 years at p= 0.016 (figure 1).

Table 1: Socio-clinical characteristics of CRC patients in NAUTH

Variables	**No. (%) N= 89**	**≤ 50 years n= 31 (%)**	**>50 years n= 58 (%)**	**p- value**
Sex				0.266
Male	43 (48.3)	12 (38.7)	31 (53.4)	
Female	46 (51.7)	19 (61.3)	27 (46.6)	
Employment status:				0.325
Civil servant	22 (24.7)	7 (22.6)	15 (25.9)	
Dependant	15 (14.6)	3 (9.7)	12 (20.7)	
Self employed	52 (58.4)	21 (67.7)	31 (53.4)	
Level of Education:				0.034*
No formal education	5 (5.6)	0 (0.0)	5 (8.6)	
Basic education	52 (58.4)	15 (45.5)	37 (63.8)	
Tertiary education	32 (36.0)	16 (50.0)	16 (27.6)	
Alcohol consumption:				0.658
No	44 (49.4)	14 (45.2)	30 (51.7)	
Yes	45 (51.6)	17 (54.8)	28 (48.3)	
Tobacco Use:				0.746
No	77 (86.5)	26 (83.9)	51 (87.9)	
Yes	12 (13.5)	5 (16.1)	7 (12.1)	
History of Hypertension:				0.500
No	56 (62.9)	18 (58.1)	38 (65.5)	
Yes	33 (37.1)	13 (41.9)	20 (34.5)	
History of Herbal therapy:				0.999
No	77 (86.5)	27 (87.1)	50 (86.2)	
Yes	12 (13.5)	4 (12.9)	8 (13.8)	

TSMP:				0.077
> 12 months	17 (19.1)	9 (29.1)	8 (13.8)	
7-12 months	33 (37.1)	13 (41.9)	20 (34.5)	
≤ 6 months	39 (43.8)	9 (29.0)	30 (51.7)	
Tumour site:				0.698
Right-sided Colon	25 (28.1)	7 (22.6)	18 (31.0)	
Left-sided Colon	27 (30.3)	10 (32.3)	17 (29.3)	
Rectum/Recto-sigmoid	37 (41.6)	14 (45.1)	23 (39.7)	
Metastasis				0.002*
No	59 (66.3)	27 (87.1)	32 (55.2)	
Yes	30 (33.7)	4 (12.9)	26 (44.8)	
Chemotherapy experience:				0.026*
No	52 (58.4)	13 (41.9)	39 (67.2)	
Yes	37(41.6)	18 (58.1)	19 (32.8)	
Tumour grade				0.052*
Well differentiated	37 (41.6)	9 (29.0)	28 (48.3)	
Moderately differentiated	43 (48.3)	16 (51.6)	27 (46.5)	
Poorly differentiated	9 (10.1)	6 (19.4)	3 (5.2)	
Histologic type				0.023*
Adenocarcinoma	66 (74.2)	19 (61.3)	47 (81.0)	
Squamous cell carcinoma	17 (19.1)	7 (22.6)	10 (17.2)	
Others	6 (6.7)	5 (16.1)	1 (1.8)	
Disease Stage				0.006*
Stage 1	34	15 (48.4)	19 (32.8)	
Stage 2	25	12 (38.7)	13 (22.4)	
Stage 3	13	4 (12.9)	9 (15.5)	
Stage 4	17	0 (0.0)	17 (29.3)	

TSMP: Time of symptom manifestation to presentation. Descriptive analysis and Chi-square/Fisher's exact test. *Significance was set at $p < 0.05$.

Age-related differences among CRC patients

The prevalence of CRC was slightly higher among females compared with men, especially among those who were under 50 years of age (Table 1). RCCs and metastasis were prevalent among patients who were over 50 years old whereas rectal tumours were prevalent in patients who were ≤ 50 years (p> 0.05). Less than 50% of the patients received chemotherapy and a higher percentage of those patients were under 50 years (p= 0.05). No significant difference was observed between the two age groups in terms of the history of herbal therapy use. The level of tertiary education was higher among patients who were ≤ 50 years compared with their over 50 years counterparts (p< 0.05). The history of tobacco use, alcohol consumption, and hypertension was prevalent among patients who were aged ≤ 50 years compared with their > 50 years counterparts (p> 0.05). Patients who were aged > 50 years old were approximately 1.8 times more likely to present at the clinic in ≤ 6 months of symptom development compared with their 50-year counterparts (p= 0.046). The rate of unemployment was lower among patients who were older than 50 years compared with their 50-year counterparts (p> 0.05). The patients who were 50 years old and under had higher tumours grade than patients who were older than 50 years (p< 0.05). Based on histology, the prevalence of adenocarcinoma was higher among patients aged > 50 years compared with patients aged ≤ 50 years (p< 0.05). The rate of surgical resection uptake was higher among patients aged > 50 years (58.6%) compared with their ≤ 50 counterparts (54.8%) at p= 0.823. Post-T TWBC count was 1.5 times higher among patients who were above 50 years compared with their 50-year counterparts (p< 0.05) while the post-T monocyte and LMR were also lower among the former than the latter at p< 0.05 (table 2). Among patients aged ≤ 50 years, only the post-T monocyte significantly increased compared with the pre-T values at p< 0.05 (table 2). Among patients aged > 50 years, the post-T TWBC, neutrophils, monocytes, and NLPR values significantly increased whereas the post-T lymphocyte and LMR significantly reduced compared with the pre-T values (p< 0.05).

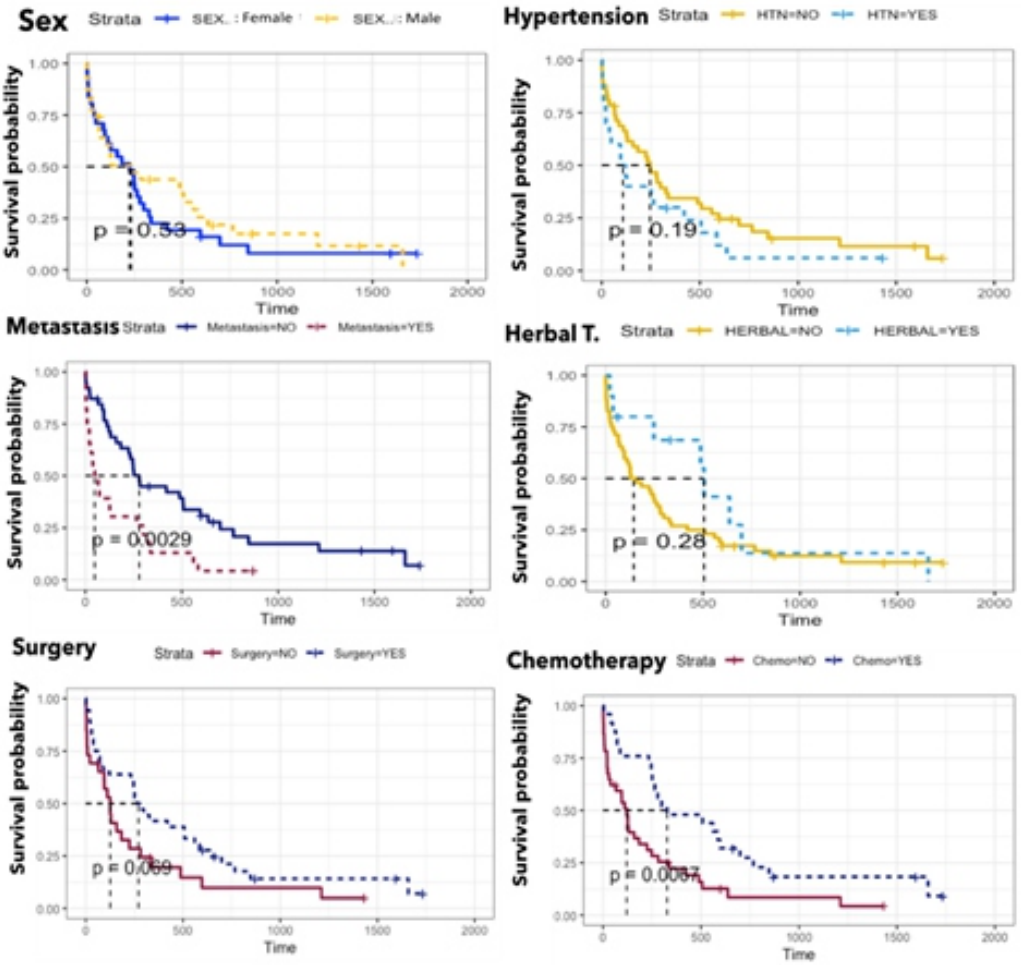

Figure 2: Survival analysis of CRC patients based on sex, history of hypertension and herbal therapy, uptake of surgery, chemotherapy, and occurrence of metastasis (Kaplan–Meier curve)

Figure 2 shows that patients with non-metastatic CRC lived longer than their counterparts (mean = 594.6 days vs 268.2 days, respectively; $p< 0.05$). Those who were chemo-experienced also lived longer than their chemo-naïve counterparts (mean = 524.3 days vs 205.2 days, respectively; $p< 0.05$). Patients with surgical resections lived longer than their surgery-naïve counterparts (mean = 487.8 days vs 280.7 days, respectively; $p> 0.05$). More so, normotensive patients lived longer compared with patients with a history of hypertension (mean = 454.1 days vs 288.0 days, respectively; $p> 0.05$). The figure also shows that males lived longer than their female counterparts (mean/median= 462.2/233 days vs 344.8/225 days, respectively; $p> 0.05$). Interestingly, patients with a history of herbal therapy lived longer compared with herbal therapy-naïve patients (mean/median= 581.3/506.0 days vs 374.2/145 days, respectively; $p> 0.05$). Patients who presented at the clinic between 7 to 12 months of symptom development had a higher mean survival rate (207.0 ± 55.3/192.2 days) compared with those presented at > 12 (197.5 ± 34.9/172.6 days) and ≤ 6 months (182.6 ± 35.1/45.3 days) at $p= 0.820$. Patients without a history of tobacco use and alcohol consumption lived longer (mean/median= 162.8 ± 37.2/39 days and 159.5 ± 50.0/35 days) than those with a history of the lifestyles (98.2 ± 38.3/15 days and 135.8 ± 34.3/43 days) at $p= 0.200$ and 0.580, respectively. Surprisingly, the mean/median survival rate among patients with DM (n= 12) was higher (629.0 ± 185.7/506.0 days) compared with those without the disease (373.2 ± 71.2/145 days, respectively) at $p= 0.190$ (see supplementary file).

Chemotherapy-associated Survival analysis

The overall mortality rate was 91.8%. Based on follow-up, the two-year and four-year survival rates of the patients were 5.2% and 3.9%, respectively. Chemo-experienced patients and patients who had non-metastatic tumours lived longer than chemo-naïve and patients with metastatic tumours (figure 2). A significant inverse relationship was observed between metastasis and survival (p= 0.001). The in-hospital mortality rate was higher among cases with metastasis (46.2%) compared with non-metastatic cases (13.7%). Based on the survival rate, no significant difference was observed between chemo-experienced and herbal-experienced patients at p= 0.263. Patients without a history of tobacco use, and alcohol consumption lived longer (162.7 days and 159.5 days) compared with the history (mean = 98.2 days and 135.8 days, respectively; p> 0.05). Patients who were both herbal and chemo-experienced had a higher mean survival rate (n= 2; 613 ± 76.50 days) compared with herbal/chemo-naïve patients (n= 42; 51.91 ± 14.61 days) at p< 0.001. Only 54.1% (n= 20) of the chemotherapy-experienced patients had 6 courses of chemotherapy; chemo-resistant patients (Chemo-R.; 30%) = 6 and chemo-sensitive (Chemo-S.; 70%)= 14.

Table 2: Comparative analysis of haematological indices between two age groups

Variable		≤ 50 years n= 31	> 50 years n= 58	*P* -value
		Mean ± SD	**Mean** ± SD	
WBC (10^9/L) BT		8.38 ± 0.90	8.37 ± 1.09	0.580
WBC (10^9/L) AT		9.19 ± 1.50	14.20 ± 4.50	0.033*
	P- value	0.560	0.017*	
Lymphocyte (%) BT		36.40 ± 3.60	34.12 ± 3.60	0.203
Lymphocyte (%) AT		31.03 ± 4.11	27.05 ± 7.40	0.056
	P- value	0.240	0.031*	
Neutrophil (%) BT		52.38 ± 4.09	51.70 ± 5.05	0.783
Neutrophil (%) AT		46.14 ± 7.54	57.33 ± 10.26	0.138
	P- value	0.287	0.016*	
Platelet (10^9/L) BT		322.03 ± 32.11	405.08 ± 34.57	0.979
Platelet (10^9/L) AT		376.42 ± 46.84	330.44 ± 33.62	0.316
	P- value	0.820	0.546	

Monocytes (%) BT		7.23 ± 0.77	3.33 ± 0.60	0.138
Monocytes (%) AT		11.81 ± 3.57	9.12 ± 2.33	0.026*
	P- value	0.026*	0.018*	
NLR BT		2.12 ± 0.36	2.50 ± 0.56	0.260
NLR AT		2.83 ± 0.74	3.23 ± 1.07	0.831
	P- value	0.197	0.236	
PLR BT		173.5 ± 23.83	204.4 ± 37.98	0.621
PLR AT		193.0 ± 55.00	219.5 ± 45.51	0.849
	P- value	0.754	0.802	
PNLR BT		830.63 ± 212.89	1225.08 ± 232.1	0.316
PNLR AT		953.90 ± 325.32	1385.67 ± 211.3	0.102
	P- value	0.901	0.518	
NLPR BT		0.84 ± 0.15	0.57 ± 0.12	0.307
NLPR AT		1.12 ± 0.32	1.08 ± 0.33	0.736
	P- value	0.154	0.007*	
LMR BT		15.93 ± 4.01	8.73 ± 2.23	0.397
LMR AT		6.76 ± 3.92	2.87 ± 0.60	0.001*
	P-value	0.388	< 0.001*	

Keys: BT; before treatment, AT; after treatment. Statistics: T-test. *Significance was set at $p < 0.05$.

DISCUSSION

In this study, we analyzed the levels of systemic immune-inflammatory indices as an alternative and cost-effective tool for monitoring CRC patients at high mortality risk, especially those in low/medium HDI countries. First, this study revealed that the frequency of the diagnosis and mortality rate significantly increased among the former at the peak of the COVID-19 pandemic, 2020, possibly due to limited access to health facilities at the time. This study also revealed that the disease was more prevalent among patients

above the age of 50 years (58%) than in patients who were 50 years and below. This aligns with the study carried out by Alatise *et al.* and by Saluja *et al.* who investigated 347 and 160 cases of CRC in Western Nigeria and observed that the disease was dominant among patients aged > 50 years (62.8% and >50%, respectively).[3,14] The findings of this study are at variance with two other studies carried out in Northern Nigeria, one of 50 cases and one of 605 cases, reported 72% and 62.6% disease dominance among patients aged 50 years.[15,16]

Regarding tumour sites, this study revealed a lower frequency of RCCs (48.1%) compared to LCCs (51.9%). This is discordant with the findings of Edino *et al.* and Theyra-Enias *et al.* who reported a high frequency of RCC (77.8% and 55.6%) compared with LCC (22.2% and 44.4%) in Northern Nigeria from 1999 to 2015.[15,17] This study is also at variance with the findings of Alatise *et al.* who observed a high frequency of RCC (80%) in Western Nigeria compared with LCC (20%).[3] The findings of this study in terms of tumour sites align with the findings of Saluja *et al.* who reported a lower frequency of RCC (47.6%) compared with LCC (52.4%) in Western Nigeria.[14] This suggests that the CRCs in Northern Nigeria are more lethal than those observed in Southern or Western Nigeria. The reason for the variation and similarities between the regions is unknown but it could be related to diet, lifestyle, or rate of genetic mutations.[18]

The findings of Irabor *et al.* are very similar to the findings of this study in terms of sex and age-related differences in tumour site and grade.[6] They reported a lower RCC frequency but higher frequencies of poorly differentiated adenocarcinoma and rectal tumours among patients aged 50 years (15%, 43%, and 72%) compared with their > 50 years counterparts (32%, 27%, and 59%, respectively). Additionally, Irabor *et al.* reported a higher frequency of the disease among females and males who were aged ≤ 50 years (62%) and > 50 years (56%), respectively.[6] The prevalent tumour grades in the study carried out by Edino *et al.* and Alatise *et al.* were poorly differentiated adenocarcinoma (34%) and moderately differentiated adenocarcinoma (55.3%), respectively whereas well-differentiated adenocarcinoma was prevalent among the patients of this study.[3,15] The high frequency of rectal tumours and poorly differentiated adenocarcinoma among patients aged 50 years could be due to a high frequency of history of tobacco use, and alcohol consumption. This may affect the clinical outcomes of the patients. Previous studies show that RCC is associated with lower survival compared with LCC due to its metastatic potential, microbiome changes, and high microsatellite instability; MSI-high.[6,19] This might be the reason for the higher rate of metastasis among patients older than 50 years in this study.

The number of patients who received chemotherapy in this study is lower than the frequency recorded by Edino *et al.* and Theyra-Enias *et al.* in Northern Nigeria (94% and 73%, respectively).[15,17] and the frequency of 67.5% and 50.5% recorded by Saluja *et al.* and Sharma *et al.* in Western Nigeria.[14,20] The reasons for low chemotherapy uptake are due to patients' reasons, side effects, and lack of funds.[17] Even though our findings and that of Edino *et al.* are fourteen years apart, both studies revealed that most of the CRC patients in Nigeria present after 6 months of symptom development.[15] This might be the explanation for the higher frequency of metastasis among our cohort and high mortality among Nigerian patients. This suggests that the level of awareness and knowledge of the disease is quite low.

Based on the time of presentation at the clinic, the NLR value shows that patients who presented after 6 months of symptom manifestation were at a higher risk of mortality at presentation than patients who presented within 6 months of symptom manifestation. Even though most of the patients aged > 50 years presented at the clinic within 6 months of symptom development, they had a higher frequency of metastasis and stage III/IV CRC compared with their ≤ 50-year-old counterparts. Despite surgical resections in this group, the three-fold reduction in post-T NLPR was mitigated by the four-fold decrease in post-T LMR. The significant increase in post-T total WBC, neutrophil, monocytes, NLPR, and a significant decrease in lymphocyte, and LMR suggests that patients aged > 50 years responded poorly to treatment due to age-related physiologic limitations. In China, patients with NLR>2.72, PLR>219.00, and LMR≤ 2.83 were significantly associated with decreased OS and DFS.[21] In this study, these features were seen among patients aged > 50 years. Thus, the low uptake of chemotherapy by patients aged > 50 years may be responsible for their high mortality rate in 2020. More so, the high NLR and low LMR among our cohort could be the reason for the high in-hospital death in this study.

CONCLUSION

This study shows that age is a strong driver of disease aggressiveness among patients in Southern Nigeria. It revealed that NLR, PLR, LMR, and PNLR values provide affordable insights into treatment outcomes, especially among elderly patients with late-stage diseases. There is a need for increased awareness and closer follow-up among patients aged 50 years and above. Due to the high prevalence of early-onset CRC in West Africa, this study suggests that screening for CRC should begin at 40 years.

Conflict of Interest

The authors have declared no competing interests.

Contributions to joint publication

Author JOO conceptualized and designed the study. Authors JOO, CME, MFE, IEC, and NR collected, collated, and interpreted the data. Authors JOO and JO analyzed the study. All authors have read and approved the final version.

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Platelet Count Variability in Breast Cancer Patients Undergoing Chemotherapy: Implication for Haematopoietic System Health

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Abstract

Introduction: Platelet count variability and indices are critical markers in assessing haematopoietic system health during chemotherapy in breast cancer patients. Chemotherapy-induced thrombocytopenia (CIT) poses a risk of bleeding and delays treatment. This study evaluated platelet count changes during chemotherapy and their implications for haematopoietic health.

To analyze platelet count variability in breast cancer patients undergoing chemotherapy and identify factors influencing haematopoietic system health.

Materials and Methods: This prospective study recruited 100 female breast cancer patients aged 21–60 years undergoing chemotherapy at RSUTH. Participant's demographics were recorded. Platelet parameters, including platelet counts (PLT), mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT), were measured pre-chemotherapy (control and baseline) and after the 1st, 2nd, and 3rd chemotherapy cycles. Statistical significance was set at $p < 0.05$

Results: Most participants (48%) were aged 31-40 years, and 69% were at stage III cancer. Chemotherapy significantly altered platelet indices. PLT increased from the control (179 ± 75.58 x 10^9/L) to baseline (264 ± 103.4 x 10^9/L) and showed variability across cycles (1st: 272.0 ± 142.6 x 10^9/L, 2nd: 247 ± 142.6 x10^9/L, 3rd:259.1 ± 109.3 x 10^9/L; p = 0.001). MPV declined steadily (control: 9.5 ± 1.0 fL to 8.1 ± 0.6 fL by the 3rd cycle; p=0.032). PDW increased significantly (control: 16.3 ± 2.0% to 19.4 ± 3.5% by the 3rd cycle; p=0.022). PCT showed a consistent decline (control: 0.30 ± 0.05% to 0.20 ± 0.03% by the 3rd cycle; p=0.034).

Conclusion: Chemotherapy significantly affects platelet parameters in breast cancer patients, potentially indicating altered haematopoietic function. Monitoring these indices is vital for optimizing patient care and mitigating risks associated with treatment.

Keywords: *Platelet count, Breast cancer, Chemotherapy, Haematopoiesis*

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INTRODUCTION

Platelets are essential components of the circulatory system, derived from megakaryocytes in the bone marrow, and are critical for haemostasis, thrombosis, and immune regulation. Their levels and functional activity are tightly regulated under normal physiological conditions. In the context of malignancies such as breast cancer, platelet dynamics can be significantly altered, reflecting the interplay between tumor biology, systemic inflammation, and therapeutic interventions (1).

Breast cancer remains a leading cause of cancer-related morbidity and mortality among women globally, and chemotherapy is a cornerstone of its management (2). However, chemotherapy, while effective against tumor cells, often induces

profound haematopoietic suppression, leading to thrombocytopenia or, in some cases, thrombocytosis (3). Chemotherapy-induced thrombocytopenia (CIT) is a common and serious complication in breast cancer treatment, increasing the risk of bleeding and necessitating dose reductions or delays in treatment, potentially compromising therapeutic outcomes (4). Conversely, thrombocytosis has been linked to tumor progression, angiogenesis, and metastasis, driven by tumor-derived cytokines and systemic inflammation (5).

These alterations in platelet count and function underscore the importance of platelets as both biomarkers and mediators of disease progression and treatment response in breast cancer patients. Emerging evidence highlights the prognostic and predictive value of platelet variability in cancer management. Elevated platelet counts have been associated with poor overall survival in breast cancer patients, while chemotherapy-induced thrombocytopenia serves as a marker of hematopoietic stress and bone marrow suppression (6; 7). Despite these findings, data on platelet dynamics in breast cancer patients undergoing chemotherapy remain limited, particularly in sub-Saharan Africa. The unique genetic, environmental, and healthcare factors in this region may influence hematological outcomes, necessitating localized studies to inform clinical practice.

This study aimed to evaluate platelet count variability in breast cancer patients undergoing chemotherapy and its implications for Haematopoietic system health. By analyzing patterns of platelet count changes and their clinical significance, this research seeks to contribute to improved patient management strategies, minimize treatment-related complications, and optimize therapeutic outcomes.

MATERIALS AND METHODS

This was a longitudinal, prospective observational study carried out on 100 female breast cancer patients, undergoing chemotherapy at the Rivers State University Teaching Hospital (RSUTH).

The study was conducted at the Oncology department of RSUTH, Port Harcourt with a focus on breast cancer management, leveraging oncology wards and haematology laboratories for patient recruitment and data collection.

The study recruited female breast cancer patients undergoing chemotherapy at RSUTH

Adult female patients aged 18–60 years, diagnosed with histologically confirmed breast cancer, who were scheduled to receive chemotherapy (either neoadjuvant, adjuvant, or palliative). Patients with baseline platelet counts within the normal range (150,000–450,000/μL) were included.

Female Cancer patients with pre-existing haematological disorders (thrombocytopenia or thrombocytosis), those on concurrent use of anticoagulant therapy or antiplatelet medications, with metastatic bone marrow involvement, and those pregnant or breastfeeding women were excluded.

Comprehensive patient history and clinical examination, blood sample collection for baseline haematological parameters, including platelet count, hemoglobin, and white blood cell count, demographic and clinical data (age, cancer stage, chemotherapy regimen) were all recorded.

3mL of venous blood was aseptically collected at baseline and during chemotherapy cycles using standard venipuncture procedures to ensure accuracy and prevent contamination. Samples

were stored in K2EDTA bottles to maintain stability and prevent clotting. The analysis of platelet parameters, platelet count, mean platelet volume, platelet width distribution, and plateletcrit was performed using a Sysmex XN-330 analyzer, ensuring high precision in results.

Data was analyzed using SPSS software Version 13. Descriptive statistics was used to summarize the patient demographics and One-Way ANOVA test was applied to demonstrate the effect of chemotherapy on platelet indices.

Ethical approval was obtained from the Ethics and Research Committee of the Rivers State University Teaching Hospital. A written informed consent was obtained from all participants and confidentiality was maintained through anonymized data handling.

RESULTS

Table 1.Demography of the Study Population

Variable	Frequency	Percentage (%)
Age		
21-30	2	2
31-40	48	48
41-50	27	27
51-60	13	13
Total	**100**	**100**
Sex		
Male	0	0
Female	100	100
Total	**100**	**100**
Marital Status		
Single	13	13
Married	86	86
Divorced	3	3
Total	**100**	**100**
Cancer Stage		
Stage I	10	10
Stage II	13	13
Stage III	69	69
Stage IV	8	8
Total	**100**	**100**

Table 2 Mean ± Standard Deviation on the effect of chemotherapy on platelet indices of the study population

PARAMETER	CONTROL (N=100)	BASELINE (N=100)	1ST CYCLE (N=100)	2ND CYCLE (N=100)	3RD CYCLE (N=100)	P-VALUE	REMARK
PLT(10^9/L)	.....	264 ± 103.4	272.0±142.6	247±142.6	259.1±109.3	0.001	Significant
	9.5 ± 1.0	9.0 ± 0.9	8.7 ± 0.8	8.4 ± 0.7	8.1 ± 0.6	0.032	Significant
MPV (fL)							
PDW (%)	16.3 ± 2.0	17.0 ± 2.5	18.1 ± 3.0	18.7 ± 3.2	19.4 ± 3.5	0.022	Significant
PCT (%)	0.30 ± 0.05	0.28 ± 0.04	0.25 ± 0.04	0.22 ± 0.03	0.20 ± 0.03	0.034	Significant

DISCUSSION

The majority of participants in this study were aged 31–40 years (48%), aligning with the peak age range for breast cancer incidence globally. This trend corroborates findings by (8), which reported that breast cancer is most prevalent in women aged 30–50 years. The relatively low representation of younger (2%) and older age groups (13%) may reflect both the population demographics and the increased breast cancer screening awareness in middle-aged women. The study's 100% female population aligns with the well-established fact that breast cancer predominantly affects women. While male breast cancer constitutes less than 1% of all cases, its absence in this study emphasizes the rarity of male breast cancer (9). The predominance of married participants (86%) is consistent with studies suggesting that marital status influences health-seeking behavior and outcomes in breast cancer patients. For instance, Study by (10) highlighted that married individuals often have better support systems, which can enhance treatment adherence and prognosis. The data reveal that the majority of participants were diagnosed at Stage III (69%), indicating delayed presentation, a common issue in resource-limited settings like Nigeria. This finding is supported by (11), who reported late-stage diagnoses in 70% of breast cancer cases in sub-Saharan Africa. This delay underscores the need for improved cancer awareness and early screening programs. Table 4.2 highlights significant changes in platelet indices, reflecting the haematopoietic impacts of chemotherapy on breast cancer patients. A marked increase in PLT count was observed from the control (179 ± 75.58 × 10/L) to baseline (264 ± 103.4 × 10/L). The elevation at baseline may be attributed to systemic inflammation triggered by the malignancy itself, as noted by (12). During chemotherapy, PLT count showed fluctuations, with a significant rise during the 1st cycle (272.0 ± 142.6 × 10/L) followed by declines in subsequent cycles. This biphasic pattern likely reflects the interplay between chemotherapy-induced bone marrow suppression and reactive thrombocytosis due to inflammatory cytokines (13). MPV declined significantly from 9.5 ± 1.0 fL in the control to 8.1 ± 0.6 fL by the 3rd cycle ($p = 0.032$). A lower MPV suggests suppressed megakaryocyte activity, consistent with chemotherapy-induced bone marrow suppression. This observation aligns with findings by (14), who reported a similar decline in MPV among breast cancer patients receiving anthracycline-based chemotherapy. PDW showed a significant increase, rising from 16.3 ± 2.0% in the control group to 19.4 ± 3.5% by the 3rd cycle ($p = 0.022$). An elevated PDW reflects heightened platelet anisocytosis, possibly due to the release of immature platelets during bone

marrow recovery phases (15). PCT consistently declined from 0.30 ± 0.05% in the control to 0.20 ± 0.03% in the 3rd cycle ($p = 0.034$). This reduction highlights the combined effects of chemotherapy-induced thrombocytopenia and reduced platelet production, findings supported by (16). The significant alterations in platelet indices observed in this study underscore the haematological toxicity associated with chemotherapy. The variability in PLT count, MPV, PDW, and PCT suggests that platelet indices could serve as valuable biomarkers for monitoring chemotherapy-induced myelosuppression. Regular assessment of these indices could aid in early identification of haematological complications, facilitating timely interventions to minimize treatment interruptions and enhance patient outcomes.

Limitations and Future Directions

The study's limitations include its relatively small sample size and its focus on a single-center cohort, which may limit the generalizability of the findings. Additionally, the absence of male participants precludes analysis of gender-specific differences in chemotherapy-induced platelet variability. Future studies should include larger, multi-center cohorts and explore the prognostic significance of platelet indices in predicting chemotherapy outcomes.

CONCLUSION

This study highlights significant platelet variability in breast cancer patients undergoing chemotherapy, reflecting the impact of the treatment on haematopoietic health. These findings underscore the need for routine monitoring of platelet indices to mitigate treatment-related complications and improve clinical outcomes.

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Determination of Neurophysiological P300 and P50 in Patients with Schizophrenia at a Tertiary Hospital in Sokoto, Nigeria

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Abstract

Introduction: Schizophrenia is a severe and complex mental disorder. It currently lacks an objective biological diagnostic test. Neurophysiological markers like P300 and P50 event-related potentials have shown some diagnostic usefulness in previous studies. However, very few of these studies have been done among Africans. The objective of the study was to evaluate neurophysiological P300 and P50 event-related potentials in 70 schizophrenia patients and 70 healthy controls at a tertiary psychiatric hospital in Sokoto, Nigeria.

Materials and Methods: The instruments used were the Electroencephalogram (EEG) machine, laptop with installed auditory P300 and P50 tones, headphones, Positive and Negative Syndrome Scale (PANSS).

Results: Schizophrenia patients were significantly associated with higher amplitude of P300 and prolonged P300 peak latency auditory event-related potential compared to healthy controls (U= 1077.000, P=<0.001 and U= 1191.000, P=<0.001) respectively. Schizophrenia patients were also significantly more associated with higher P50 amplitude ratio (U= 1342.500, P=<0.001). The P300 event-related amplitude had the highest area under the curve of 0.78. P300 peak latency was the most specific (Specificity=0.93) while P50 ratio was most sensitive (Sensitivity=0.76). The determined cut-off points for P300 amplitude, P300 peak latency, and P50 ratio were 6.84μv, 445ms, and 0.89 respectively. There was a significant positive correlation between P50 ratio and age of participants among schizophrenia patients (rs=0.29, P=0.02).

Conclusion: The study has determined the cut-off points for P300 and P50 neurophysiological markers in patients with schizophrenia. These will serve as an adjunct for diagnosis and for forensic purposes in these patients. Further studies including neuroimaging, biochemical, and genetic aspects are recommended in African countries.

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INTRODUCTION

Schizophrenia is a chronic, severe and debilitating mental disorder characterized by disordered thought, abnormal perceptions and behaviours.[1] The symptoms usually emerge in late teens and early 30's[1]. Early detection is therefore crucial in the management of the disorder. Assessment of neurophysiological markers in schizophrenia patients are current methods that will serve as an adjunct in early diagnosis.[2] This will also help in predicting the risk and outcome of the disorder.

The Neurophysiological markers are laboratory-based measurements and intermediates between phenotypes and genetic predisposition.[3] The polygenic nature of schizophrenia and the current lack of a candidate gene for the disorder necessitates the assessment of these intermediates or allied phenotype markers. This

will conceptualize the complex genetic predisposition into quantitative measurements.[4] Cortical evoked-response potentials can be averaged into event-related potentials (ERPs) recorded using encephalography.[5] These waveforms have been reported by several studies to be genetically-linked markers.[6] The P300 and P50 event-related potentials in schizophrenia have been extensively studied and all previous studies were carried out predominantly among Caucasians.[7-9] The generalization of the findings of these studies poses a challenge among Africans. It is therefore, important to consider how these markers are expressed in the African population considering the varied genetic make-up between Africans and Caucasians. This will provide a more robust evidence-based data and a broader view of these neurophysiological markers and their diagnostic accuracy across races.

The determination of a cut-off point for these neurophysiological markers will also be necessary considering the fact that similar findings on P300 and P50 event-related potential have been reported in other disorders like Alzheimer's disease, bipolar disorder and panic disorder apart from schizophrenia.[10-12] There is a dearth of studies that have determined a cut-off point for P300 and P50 event-related potential measurements. This present study has provided a comprehensive evidenced-based assessment of the pattern and utility of P300 and P50 event-related potential among schizophrenia patients in Nigeria. The findings have implications as regards the genetic underpinnings of schizophrenia in this region.

METHODOLOGY

The study was conducted at the Federal Neuropsychiatric Hospital, Kware (FNPHK), Sokoto State. The hospital is a tertiary specialist health care facility situated in Kware Local Government, Sokoto Nigeria.

This is a cross-sectional, descriptive study.

(1). Schizophrenia patients attending follow-up clinic at the out-patient department of Federal Neuropsychiatric Hospital Kware, Sokoto.

(2). Non psychiatric healthy controls were staff of Federal Neuropsychiatric Hospital Kware, Sokoto.

Materials

(A**) Electroencephalography (EEG) machine** (Model: NIHON KOHDEN 1200K). The digital EEG machine was used to monitor and record the event related potentials of the brain. It was non-invasive, with the electrodes placed along the scalp. The electrode locations and names were as specified by the International 10–20 system which ensures that the naming of electrodes is consistent across laboratories for clinical and research applications.

The digital EEG signal was kept electronically and filtered for display. The high-pass filter was set at 150Hz and the low-pass filter filters at 15Hz.The impedance threshold was set at 10kΩ. The measurement of the amplitude of the evoked potential was done manually based on the scale ratio of the EEG tracing.[13]

(B) **A Laptop** with installed oddball (target) tone that is randomly interspersed within an ongoing train of a standard (non-target) tone, presented every 2 seconds for the P300 assessment. with installed tone of clicks at 500ms interval and frequency of 1500Hz for P50 assessment

(C) **A headphone stereo sound Headset** was used to transmit the sounds to participant ears from the laptop. This was to reduce the external sound interference and to enhance the concentration of participants when listening to the tones from the laptop.

(D) **ICD-10 Diagnostic Criteria for Research**

This diagnostic instrument was used to diagnose schizophrenia based on patients recall of morbid symptoms and documented symptoms in the case notes.

(E) **Positive and Negative Syndrome Scale** (PANSS)
It is a 30-item rating scale specifically developed for assessing individuals with schizophrenia especially in research settings. It is an adaptation from earlier psychopathology scales such as the Brief Psychiatric Rating Scale (BPRS). It is based on the grounds that schizophrenia has two distinct syndromes, a positive and a negative syndrome. The positive symptoms refer to an excess or distortion of normal functions (e.g., delusions and hallucinations) while the negative syndrome represent a diminution or loss of normal functions and comprise things like social withdrawal and flattened or blunted affect.

PANSS is applicable only to schizophrenia patients and takes 45 to 50 minutes to administer. The main indication for its use is to determine the presence of symptoms, their influence on activities, functions of the individual and the frequency of symptoms. The patient is rated from 1 to 7 on 3 scales consisting of 30 different symptoms.[14]

(F) **A proforma questionnaire** designed by the authours was used to record socio-demographic data and other relevant clinical variables of participants. reference

Sample size
This was calculated using the lower effect size in an Event-related Potential study for P300 latency which was 0.48 in a previous study.[15]The P50 event -related potential studies generally had higher effect sizes. The effect size of the P50 event-related study was selected to achieve sufficient sample size.
Formula for sample size using effect size for two independent samples=$2(Z_{1-\alpha/2}+Z_{1-\beta})^2/ES^2$. [16]
$Z_{1-\alpha/2} = 1.96$.
$Z_{1-\beta}$-=0.84
ES = Effect size
Sample size approximately =70
That is, 70 Schizophrenia patients were recruited for the study and also 70 Healthy controls.

Inclusion criteria
- Participants must be 18 years and above
- Schizophrenia patient must fulfill the ICD-10 diagnostic criteria for research and collaborated with diagnosis of the psychiatrist.
- Schizophrenia patients must be clinically stable.
- Healthy controls having no family history of mental disorder.

Exclusion criteria
- Those with co-morbid medical/physical illness.
- Those with co-morbid substance use.
- Individuals with hearing difficulties.

Ethical considerations
The ethical approval was obtained from the health research ethics committee of Federal Neuropsychiatric Hospital Kware, Sokoto with reference code was FNPHK/ADM/SUB/809. Informed consent for the research was obtained from all participants.

Procedure
The 10-20 system electrode placement was applied on grease free scalp and all jewelries removed while sitting down.

The Headset was fitted without interfering with the electrodes. P50 tones and P300 auditory tones were presented to the ears through the headset from the laptop installed with these tones. There were 150 presentations per participant for P300 at ratio of 1:2 (i.e., 50 oddball tones to 100 standard

tones).

The 50 paired auditory clicks presented 500ms apart were used to elicit the P50 evoked potentials. The inter-trial interval for the paired clicks was 7seconds.

The P300 standard and oddball tones were set at 1000Hz and 1500Hz respectively. The P50 tone was set at 1500Hz. P300 evoked potential was identified in the EEG tracing on the Pz electrode and defined as the largest positive-going peak occurring within 300-800ms.The P50 evoked potentials were identified on the Cz electrode and defined as the most prominent peak in the 40-80msec post-stimulus window.[7]

The amplitude of the evoked potentials was measured manually from the peak to trough (Peak-to peak amplitude) while the peak latency was measured from the time of stimulus onset.

The amplitude of the evoked potentials was measured manually from the peak to trough (peak to peak amplitude) while the peak latency was measured from the time of stimulus onset.

Data Analysis

Data was analyzed using the Statistical Package for Social Sciences version 20(SPSS v20)
Continuous variables were presented as means with standard deviation and the categorical variables as proportions.

Unpaired t-test or Mann Whitney U test was used to compare continuous variables while categorical variables were compared using Chi-square test. Logistic regression analysis was done to control for possible confounders and Receiver Operating Characteristic (ROC) Curve analyses was used for diagnostic accuracy determination. Significant P value was set at < 0.05.

RESULTS

1.The schizophrenia patients were significantly more associated with no formal education and being unmarried than the healthy controls as shown in Table 1.

2. Schizophrenia patients were significantly more associated with higher mean rank conditioning and test amplitudes compared to their conditioning P50 amplitude. Also, Schizophrenia patients were statistically more associated with higher mean rank P50 amplitude ratio compared to healthy controls. Schizophrenia patients were significantly associated with higher amplitude of P300 auditory event-related potential and delayed P300 Peak Latency compared to healthy controls. These are as shown in Table 2.

3. The P300 Event-related Amplitude Potential, P300 Peak Latency and P50 Amplitude ratio had an Area Under the Curve (AUC) of 0.78, 0.76 and 0.73 respectively. These are as shown in Table 3 and Figure 1-3.

4. The P300 Peak latency was the most specific (Specificity=0.93) while P50 Amplitude ratio was the most sensitive (Sensitivity=0.76). The determined Cutoff point for the P300 Event-related Amplitude Potential, P300 Peak Latency and P50 Amplitude ratio were 6.84μv, 445ms and 0.89 respectively. These are as shown in Table 4.

5. Among Schizophrenia patients, the Spearman's correlation coefficient was used to determine the socio-demographic and clinical variables correlation with each neurophysiological marker. There was no statistically significant correlation between Duration of illness, Total PANSS score, Positive PANSS score, Negative PASS score, General Psychopathology scale score, Age of onset of illness, Duration of treatment, Chlorpromazine equivalent dose and each neurophysiological marker. There was a significant positive correlation between age and

The P50 amplitude ratio measurements among patients with Schizophrenia. There was however, no significant correlation P300 event-related amplitude, P300 peak latency and age. These are as shown in Table 5

6. The P300 event-related potential amplitude, P300 peak latency and P50 amplitude ratio were also significant predictors of Schizophrenia after the regression analysis was carried out. These are as shown in Table 6.

Table 1: Comparison of Sociodemographic characteristics of schizophrenia patients and healthy controls

Variable	Schizophrenia	Healthy Control	X2/Fisher's exact/T/	p-value
Age (years) Distribution (n/%)				
18 – 28	23(32.9)	26(37.1)		
29 – 39	27(38.6)	17(24.3)		
40 – 50	15(21.4)	16(22.9)	5.23	0.25
51 – 61	4(5.7)	10(14.3)		
62 – 72	1(1.4)	1(1.4)		
Age (mean)	34.31(10.48)	35.46(12.49)	-0.59	0.56
Range in years	18 – 65	18 – 65		
Sex				
male (n/%)	52(74.3)	47(67.1)	0.86	0.35
Female (n/%)	18(25.7)	23(32.9)		
Employment Status				
Employed	39(55.7)	50(71.4)	3.73	0.05
Unemployed	31(44.3)	20(28.6)		
Education				
Formal	31(44.3)	57(81.4)	20.68	<0.001*
Informal	39(55.7)	13(18.6)		
Marital Status				
Married	31(44.3)	53(75.7)	14.41	<0.001*
Unmarried	39(55.7)	17(24.3)		
Tribe				
Hausa	68(97.1)	68(97.1)		
Non-Hausa	2(2.9)	2(2.9)	0.00	1.00

Table 2: Comparison of Neurophysiological Markers between Schizophrenia Patients and Healthy Controls

Variable	Schizophrenia (Mean Rank)	Healthy Control (Mean Rank)	Mann-Whitney U	Z	p-value
P50 amplitude (Conditioning) R1	80.59	60.41	1744.000	-2.95	<0.001*
(Test) R2	89.58	51.42	1114.500	-5.57	<0.001*
P50ratio ($R2/R1$)	86.32	54.68	1342.500	-4.62	<0.001*
P300 Amplitude	90.11	50.89	1077.000	-5.76	<0.001*
P300 Latency	88.49	52.51	1191.000	-5.26	<0.001*

Table 3: Receiver Operating Characteristic Curve Analysis of P300 Auditory event-related Potential Amplitude, P300 Peak Latency and P50 Amplitude ratio of Participants.

	Variable	Area Under Curve	Std Error	p-value	95% Confidence Interval	
					Lower band	Upper band
1.	**P300 auditory event-related potential amplitude**	0.78	0.039	<0.001*	0.70	0.86
2.	**P300 Peak Latency**	0.76	0.042	<0.001*	0.67	0.84
3	**P50 amplitude ratio ($R2/R1$)**	0.73	0.043	<0.001*	0.64	0.81

Table 4: Sensitivity, Specificity, Likelihood Ratios and Cut-off point of the Neurophysiological markers

Variable	Youden Index	Sensitivity	Specificity	Positive likelihood ratio	Negative likelihood ratio	Cutoff point
P300 event-related potential amplitude	0.43	0.53	0.90	5.29	0.52	6.84μv
P300 Peak Latency	0.44	0.51	0.93	7.24	0.52	445ms
P50 amplitude ratio	0.39	0.76	0.63	2.04	0.39	0.89

Table 5: Sociodemographic and Clinical variables associated with P50 Amplitude ratio in Schizophrenia patients (continuous variables).

Variable	Spearman's(rho) correlation coefficient	p-value
Age (years)	0.29	0.02*
Duration of illness	0.17	0.17
Duration of Treatment	0.16	0.20
Chlorpromazine equivalent dose	-0.14	0.26
Positive PANSS Scale score	-0.02	0.90
Negative PANSS Scale Score	-0.11	0.37
General Psychopathology Scale Score	-0.03	0.82
Total PANSS Score	-0.05	0.7
P300 Amplitude	0.14	0.24
P300 Latency	-0.16	0.18
Age of Onset of illness	0.2	0.09

n=70

Table 6: Logistic regression analysis of independent variables associated with Schizophrenia

	Variable	B	Std Error	p-value	Exp(B)	95% CI Exp (B)	
						Lower band	Upper band
1.	**Education (1)**	1.36	0.50	0.01*	3.90	1.451	10.468
2.	**P300 amplitude**	0.17	0.07	0.01*	1.18	1.037	1.343
3	**P300Peak Latency**	0.01	0.00	0.00*	1.01	1.004	1.016
4.	**P50 amplitude ratio**	2.38	0.72	0.00*	10.81	2.661	43.895
5.	**Marital Status (I)**	1.42	0.49	0.00*	4.14	1.598	10.733
6.	**Constant**	-8.39	1.60	0.00	0.00		

Education (I) – Informal; Marital Status (I) – Not married; Occupation (1) – Unemployed
B-Unstandardized beta

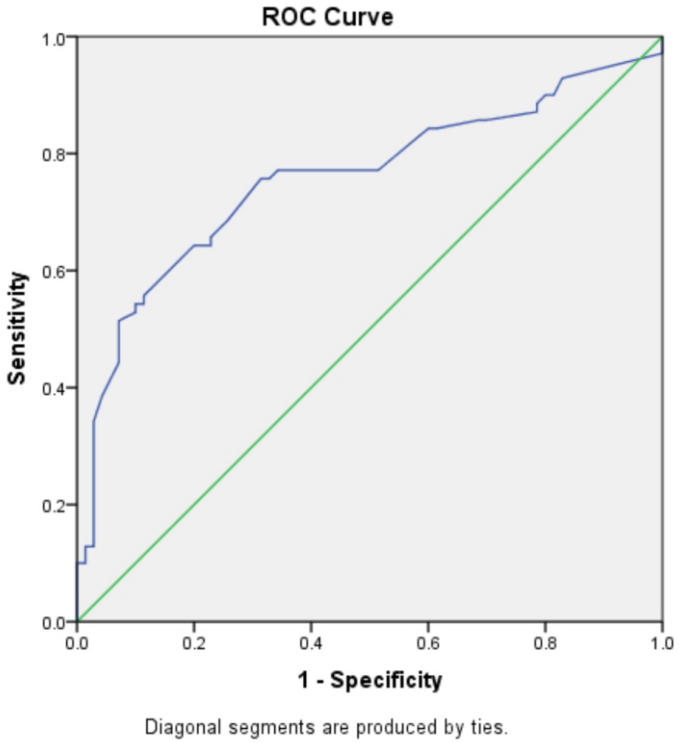

Figure 1: Receiver Operating Characteristic Curve of P300 Auditory Event-related Potential Amplitude of Participants

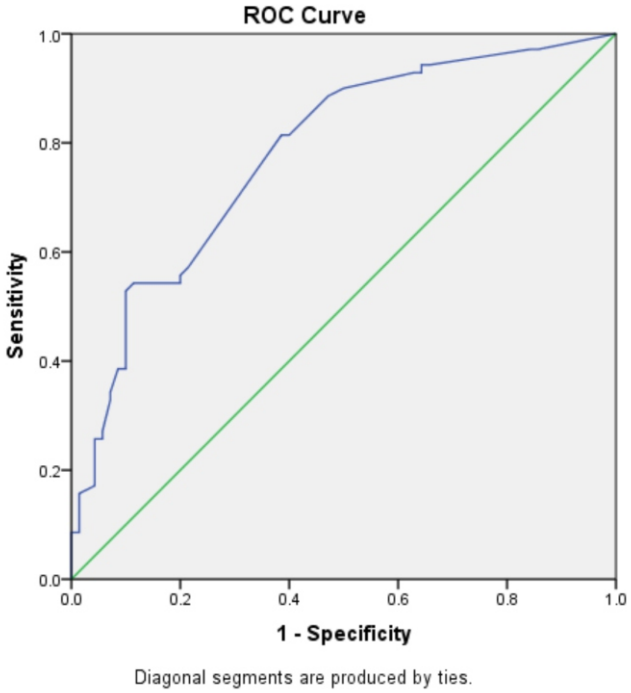

Figure 2: Receiver Operating Characteristic Curve for P300 Peak Latency of Participants

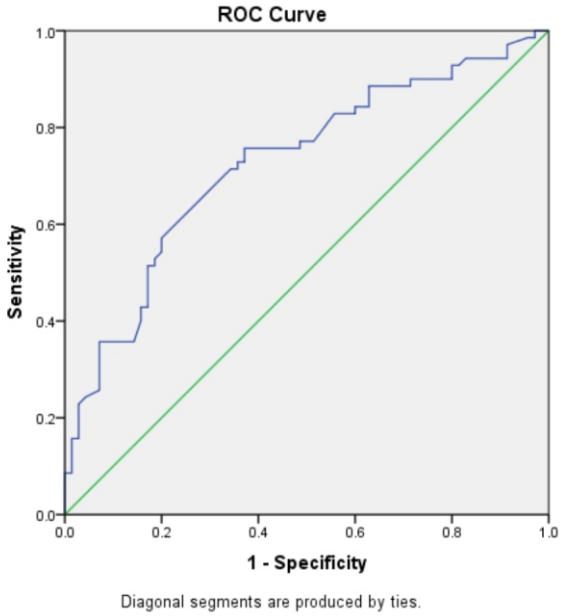

Figure 3. Receiver Operating Characteristic Curve for P50 Amplitude Ratio(R2/R1) of Participants

DISCUSSION

The socio-demographic pattern of participants showed that the healthy controls differ significantly in terms of educational status compared to schizophrenia patients in having higher proportion of participants with formal education in that group. This is supported by a previous study which reports that there is increased risk of no secondary or higher education among schizophrenia patients compared to healthy controls.[17] Cognitive deficits which are usually present before onset of psychiatric symptoms,[18] low socio-economic background and possible family history of mental disorder may contribute to higher risk of lack of formal education.

This study showed that schizophrenia patients were significantly more associated with being unmarried compared to healthy controls. Several studies have reported significant difference in terms of marital status between schizophrenia patients and healthy controls. Adewuya, [19] reported that patients with schizophrenia were more likely to be unmarried in Nigeria while Gupta *et al*.,[20] in Canada reported the same thing. This has been attributed to divorce or marital separation sequel to disability in marital relationship. Also, the earlier onset of the disorder may lead to socio-occupational decline that will make marriage impossible.

In P50 Auditory event-related potential, there were higher test amplitudes in schizophrenia patients that were significant compared to their conditioning P50 amplitude. This is consistent with previous studies among Caucasians.[21-22] where schizophrenia patients exhibited significant P50 non-suppression more than the controls and higher mean P50 ratio (That is, ratio of test amplitude to conditioning amplitude).

Abnormal sensory gating has been postulated to be the reason for some of the symptoms seen in schizophrenia and the P50 amplitude non-suppression. This is said to occur following sensory overload leading to impaired concentration in schizophrenia patients.[23] Furthermore, a link has been reported between abnormal Alpha-7 nicotinic receptors and abnormal P50 response.[24] The Alpha-7 nicotinic receptor is a type of nicotinic acetylcholine receptor (nAchRs) implicated in long term memory and positive effects on neurocognition in schizophrenia patients .[25]They are also widely distributed in different brain regions. These findings need further investigation to authenticate the validity of P50 non-suppression in schizophrenia diagnosis and to serve as a new pathophysiologic target for drug discovery.

Auditory P300 evoked potential has been used as an evaluation of cognitive processes, like attention allocation, activation of immediate memory, updating of task-relevant information in the working memory in many studies.[26-27] In this study, schizophrenia patients had significantly larger amplitude and increased (delayed) latency of P300 auditory evoked potential compared to healthy controls. Most of the previous studies reported lower amplitude among schizophrenia patients.[15, 28-29]while other studies have found no significant difference in the P300 amplitudes of schizophrenia patients compared to controls.[30] The finding on P300 amplitude in this study is unlike most of the previous studies that were carried out among Caucasians. A study however, reported a differential impact of race on the association between schizophrenia and P300.[31] It initially found amplitude reduction among African-American controls, rather than the patients. This difference was lost when use of psychoactive substances was controlled for, in the study. However, this finding was not observed among

Caucasians in the same study. Another study found schizophrenia patients having significant reduction in temporo-parietal P300 amplitude and higher frontal P300 amplitude than controls.[32] This may be due to increased eye movements among schizophrenia patients.[33] Another possible explanation could be that higher P300 amplitude among schizophrenia patients could reflect higher engagement of the temporoparietal cortex believed to have maximal P300 amplitude at the Pz location on the scalp.[34] This may be a way of compensating for a cortical defect either in that region or elsewhere like in the frontal lobe. Similarly, previous studies have reported greater cognitive demands among patients with schizophrenia when involved in tasking procedures.[35-36] Another study reported that schizophrenia patients are capable of producing larger P300 amplitude under certain conditions and that this may indicate increased effort to compensate for cognitive deficits.[37]Abnormalities in the brain neurotransmitters that differ among races may also explain this increased significant difference. The P300 event-related potential may be caused by a direct excitatory postsynaptic effect of glutamergic neurotransmission with cholinergic, noradrenergic and GABAergic neurotransmitters being neuromodulatory while dopaminergic and serotonergic influences are minor in its generation.[38-39] Acetylcholine specifically enhances P300 amplitude while GABA reduces P300 amplitude. A number of studies have reported better prognosis among Africans with Schizophrenia compared to Caucasians with Schizophrenia.[40-42]Although, this has been attributed to environmental factors, it is possible that this may be also due to differing neuropathological and biochemical influences on the brain of schizophrenia patients across races. Several studies, have reported delayed latency to P300 peak amplitude similar to the finding in this study.[15, 43] P300 latency is considered to be a measure of stimulus classification speed. It is unrelated to response selection processes and independent of behavioural response time as seen in P300 event-related potential. The P300 amplitude and latency have been hypothesized to follow a maturation path from childhood to adolescence, resulting in a period that marks a plateau, after which degenerative effects begin.[27] This point of deflection between maturation and degeneration stages are said to occur at different ages for P300 amplitude and latency. The findings from the study by Van Dinteren *et al*,[27] revealed that P300 latency possibly indicates neural speed or brain efficiency while P300 amplitude might indicate neural power or cognitive resources which increase with maturation. This suggests that latency and amplitude reflect different aspects of brain maturation. Precisely, P300 amplitude might be an index for the number of cognitive resources being used, increasing in the first year of life and decreasing with further aging beyond adolescence.[27] Higher amplitudes are related to higher proportion of allocated cognitive resources and intra-subject re-routing of neural pathways to improve cognitive performance in a background cognitive decline. P300 latency may be a more direct index of information-processing speed and indirectly cognitive performance.[27]

The Area Under the Curve (AUC) of P300 amplitude, P300 Peak latency and P50 Amplitude ratio as a test variable was acceptable in the ability to predict schizophrenia diagnosis based on the current diagnostic guidelines.[44] The P50 amplitude ratio was least in discriminating ability between schizophrenia and healthy controls.

The finding on the diagnostic accuracy of P300 auditory event-related potential and P50 amplitude ratio is partly similar to findings in other studies. A study that utilized a three-factor solution comprising P200 and P300 amplitudes,

P50 ratio and differences scores including P300 latency resulted in an AUC of 0.793 with sensitivity of 0.829 and specificity of 0.703.[45] A similar study on P50 and P300 event-related potential also classified schizophrenia and healthy controls with 71% accuracy with a combined sensitivity of 0.70 and specificity of 0.72.[46] Another study utilized visual event-related potential to demonstrate P300 amplitude and latency and reported that they were able to classify schizophrenia by 61% and healthy control by 80% accuracy.[47] Although the present study did not set out to develop a combined ROC analysis, the average AUC, sensitivity and specificity were close to these previous studies. However, the specificity of the markers in our study are higher than their sensitivity except for the P50 amplitude ratio. The inflection point of the sensitivity and specificity of each marker in this study was generally fairly acceptable based on the principles of high-quality testing.[48] These inflection points show that the P50 ratio may be more useful as a screening test while P300 auditory event-related potential may be more relevant as a diagnostic test. These markers may be useful as an adjunct to the syndromic diagnosis that currently exist for schizophrenia. The determined Youden Index and cut-off point in this study for P300 event-related amplitude, P300 Peak latency and P50 Amplitude ratio have not been done in all known previous study on these markers. This will ultimately serve as a template for future studies on the diagnostic accuracy of these markers.

Previous studies have reported weaker P50 gating with increased age.[49-50] It was however, also reported in these studies that age seem to account for only a minimal part of the changes in P50 gating. Increase in age has been reported to have a significant influence also on P300 latency prolongation among schizophrenia patients.[51-52] while younger people have been reported to demonstrate increased P300 amplitudes.[53] The fact that P300 is affected by age-related changes which may actually be related to changes in cognitive capacities, suggests that P300 is a sensitive metric for cognitive performance.[27] It was also reported that developmental trajectories of the P300 amplitude across the lifespan exist for frontal and parietal electrode sites. The parietal P300 increased in childhood to reach its peak in adolescence, then declined for the rest of the lifespan. In contrast, the frontal P300 reached its peak at a mid-older age. This is about 46years after which it remains constant for the rest of the lifespan. This has been said to reflect compensatory activity within the brain which has been highlighted earlier.[27] The preponderance of young adults among the participants and the lower mean age of schizophrenia patients may partly contribute to the differences observed in this study.

The total PANSS score has been reported to have a negative correlation with P300 amplitude and latency in medicated schizophrenia patients.[43] Another study among unmedicated schizophrenia patients found similar inverse relationship between total PANSS score and P300 amplitude.[54] This present study found no relationship between PANSS score and any of the neurophysiological markers investigated. Some studies have also reported no significant correlation between PANSS positive/negative symptoms and P50 amplitude ratio .[55-57] The schizophrenia patients in this study were on medication for the psychotic symptoms. Therefore, the PANSS scores were likely to be significantly lower than at the onset of the illness prior to the use of medication. This may influence the relationship between PANSS score and these neurophysiological measures that are believed to be independent of the clinical state of the patients.

CONCLUSION

The study has determined cut-off point for the neurophysiological measures that are absent in previous studies. This will serve as an additional useful criterion in improving the diagnostic accuracy of schizophrenia and this could also be useful in medico-legal purposes.

LIMITATIONS

The manual method of measurement of the evoked potential parameters rather than the computerized method may limit the level of preciseness in measurements.

The inability to achieve a fully noise-proof setting for the neurophysiological procedures might have distracted some of the participants and affected their performance during the procedures.

It was a cross-sectional study and may not be sensitive to some changes that may occur over time.

RECOMMENDATIONS

(a) This study is paving a way for further studies on investigating neurophysiological makers among patients with schizophrenia in Nigeria. It will therefore be necessary to explore further, the diagnostic possibilities in P300 auditory event-related potential in future studies in this region.

(b) Neuropathological studies involving neuroimaging and genetic studies should be encouraged to validate the likely aetiology of the findings in this study.

(c) The investigation of these neurophysiological markers should be conducted among relatives of schizophrenia patients in order to assess the possible heritability of these measures and their genetic underpinnings.

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AUTHOURS CONTRIBUTION

Adebayo Sunday Adebisi, Chinedu Onwuchekwa, Umar Zayyanu Usman and Bello Sirajo Shiitu-Study Design

Adebayo Sunday Adebisi -Data collection and Literature review

Chinedu Onwuchekwa, Umar Zayyanu Usman and Bello Sirajo Shiitu-Literature review proofreading and corrections.

Adebayo Sunday Adebisi, Chinedu Onwuchekwa, Umar Zayyanu Usman and Bello Sirajo Shiitu--Data analyses.

CONFLICT OF INTEREST

There is no conflict of interest to disclose.

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