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Prevalence of Malaria with Anaemia and HIV Status in Women of Reproductive Age in Onitsha, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors VCE, IBE and OO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author EIO managed the analyses of the study. Authors ACN and USO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The major health problems affecting pregnant women in sub-Saharan African are anaemia, malaria and HIV. A case-control study aimed at determining the prevalence of malaria, anaemia and HIV status among women of reproductive age between 20-49 years attending General Hospital, Onitsha was conducted. Blood samples of three hundred and sixty-two apparently healthy gravid women attending antenatal and 181 non-gravid apparently healthy women in Onitsha were recruited using random sampling method. A structured questionnaire was used for data collection. Haemoglobin was estimated using automated method, Malaria, diagnosed microscopically using gold standard staining method and HIV screened using qualitative immunochromatographic

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method, confirmed molecularly. Statistical analysis was done using SPSS version 21. Results revealed that out of the 362 pregnant test groups,125 (34.5%) had malaria parasite (mp) in their blood and were anaemic 53(37.6%) though fewer in control groups ;23,14 (12.7%; 16.1%) (p = 0.195; p =0.055). Out of 347 HIV sero-negative pregnant women, 110 (31.7%) tested HIV positive when confirmed by PCR molecular method while only 1(0.6%) sero-negative controls were confirmed positive with molecular method with statistical significance observed in the test and control groups (p = 0.000; p = 0.000). However, as pregnancy affects HIV serology testing, it is necessary to use molecular method to increase its sensitivity and more enlightment programmes on the importance of balanced diet, compliance to Malaria control and routine gynecological drugs intake in pregnant women.

Keywords: Malaria control; anaemia; reproductive age; pregnant women; HIV.

1. INTRODUCTION

Maternal mortality is a major health problem in developing countries [1]. Global Anaemia prevalence is estimated about 47% in children younger than 5 years, 42 % in pregnant women and 30% in non-pregnant women aged 15 to 49 years with African and Asia accounting for more than 85% of the absolute Anaemia burden in high risk groups [2]. In sub- Saharan Africa, the prevalence of Anaemia during pregnancy is estimated to be 35%-75% and is associated with risk of maternal mortality [3]. In Nigeria, malaria and HIV are serious life threatening problems besides non-communicable diseases that are on the rise among populations [4]. Malaria infection during pregnancy is a major public health problem in tropical and subtropical regions globally.

Anaemia is defined as a decrease in the concentration of haemoglobin below what is normal for a person's age, gender and environment, resulting in the reduction of oxygen carrying capacity of the blood Cheesbrough [5], or below 11.0 g/dl for pregnant and 12.0 g/dl for non-pregnant women [6]. Anaemia can develop following blood loss, when the red cells are destroyed (haemolysed) or when the normal production of red cells is reduced. Anaemia can be genetic, such as in haemoglobinopathies; caused by infectious diseases, such as malaria, intestinal helminthes and chronic infection or nutritional deficiency; which includes iron deficiency as well as deficiencies of other vitamins and minerals, It occurs at all stages of the life cycle but is more prevalent in pregnant women and young children (WHO, 2008).

The Human Immunodeficiency Virus (HIV) and malaria combined causes more than 2 million death each year [7]. The Human Immunodeficiency Virus and Malaria are among the causes of morbidity and mortality in sub-Saharan Africa where there is a resource-limited settings [8]. HIV is a member of the genus Lentivirus within the family Retroviridae. HIV causes progressive impairment of the body's cellular immune system, which leads to infections and tumors and the fatal condition AIDS (acquired immunodeficiency syndrome). In a survey done by UNAIDS in 2008, Nigeria is one of the countries in sub-Saharan with a high HIV/AIDS burden with estimated number of 1.6 million consisting of women aged 15 years and older ones living with HIV [9].

Malaria is caused by Plasmodium which is transmitted by the bite of a female anopheles mosquito. Four species of plasmodium cause malaria in humans: *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium malariae* and *Plasmodium ovale*. Malaria in pregnancy has been associated with increased risk of anaemia in pregnancy, increased risk of intra-uterine growth restriction, increased risk of low birth weight and preterm birth although some studies have published contradictory findings [10,11,12,13]. Young primigravidae and multigravidae females are at greater risk of being infected by malaria and its adverse effects than older primigravidae and multigravidae respectively because of continuous development of malaria immunity in older women [14]. In a previous study by Desaie et al. [15], Primigravidas and adolescents who are infested with malaria and those co-infected with HIV are at highest of morbidity. Co-infection in women is associated with higher levels of parasitaemia leading to a greater risk of severe anaemia and HIV viral load increase, creating opportunity for infection and more severe disease [16]. A previous study showed that HIV in pregnant women increases the chances of contracting prenatal malaria and developing anaemia, delivery a low birth weight infant and premature

delivery [17]. Anaemia increases plasma viral load and decreases CD4 count among HIV infected (Skinner *et al.,* 2008). Judging by the high mortality rate of Anaemia, Malaria and HIV in pregnancy and its effect on the unborn children, the need to carry out a study to determine the prevalence of co-infection of Malaria, Anaemia, and HIV status among pregnant women attending General Hospital, Onitsha is a necessity as data on the prevalence in the study area is scarce, this will help in combating their adverse effects.

The aim of the study was to determine prevalence of Malaria with anaemia and HIV status and analyze HIV testing methods in pregnant and non-pregnant visiting General Hospital Onitsha, Anambra State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Design

This is a four months case-control study carried out to compare the prevalence of Malaria with anaemia and HIV status and analyze HIV testing methods in pregnant and non - pregnant in General Hospital Onitsha, Anambra State, Nigeria.

2.2 Study Area

This research was carried out at General hospital Onitsha in Onitsha-North local government area, Anambra state, south-east, Nigeria. Majority of its populace are of Igbo ethnic group. It is located on 6.15^0N latitude and 6.79^0E. It is a secondary level state owned public hospital. It has about 300 bed spaces and runs its gynecology services twice a week. It started around 1957.

2.3 Study Population/Control

These consist of 513 women of age range 20-49 years. The test group consists of 362 gravid apparently healthy women who were registered attendants of antenatal clinic at General Hospital Onitsha as at the time of research. Subjects were recruited irrespective of town of residence while controls consisted of 181 confirmed non-gravid apparently healthy women of the same age range attending clinics in the hospital.

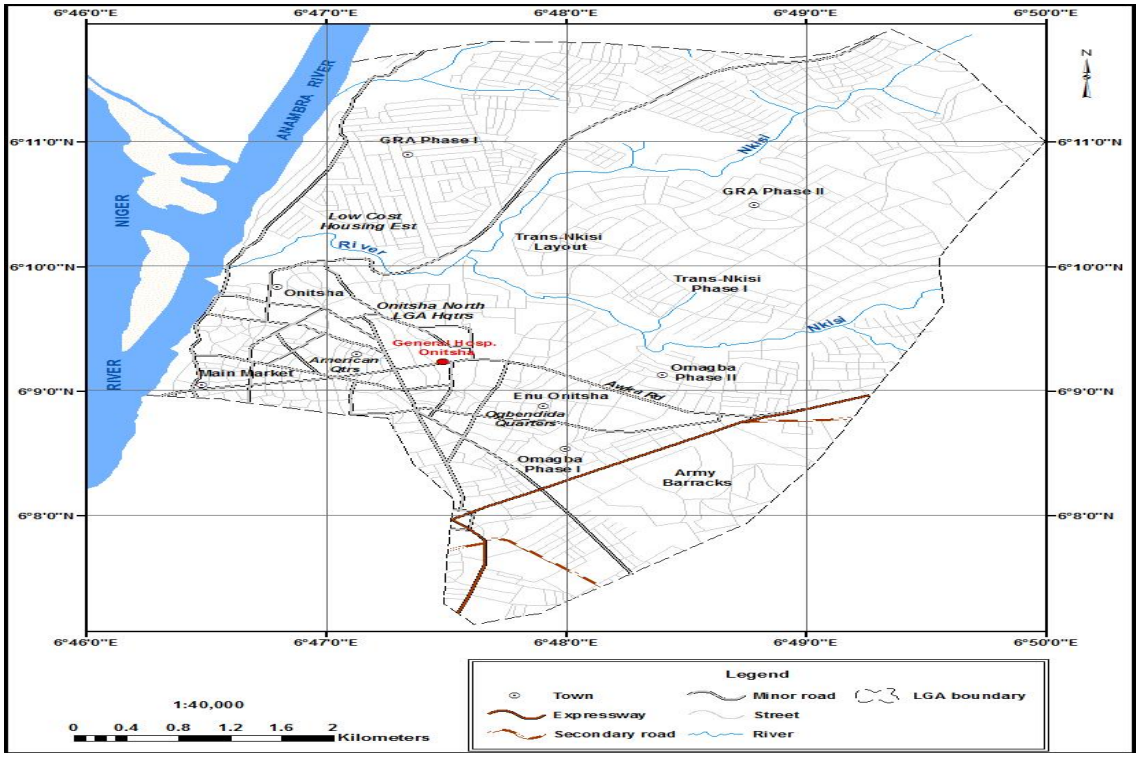

Fig. 1. Map of Onitsha urban with co-ordinates
https://www.goggle.com/earth/

2.4 Sample Collection

Five (5 ml) of blood were collected from each of the subjects using five(5) ml sterile, dry disposable plastic syringe (B.D., India) attach to a needle. Three (3) ml of blood was drawn into Ethylene diamine tetra acetic acid (EDTA) container (Stericon, Nigeria) according to standard methods, it was used for Malaria parasite, Haemoglobin and HIV serology screening. Two (2) ml of blood was collected into a plain container containing RNA shield for molecular PCR testing. Batch collection was done for MP, Haemoglobin and HIV while pool collection was done for PCR.

2.5 Sample Storage/Transport

Thick films were prepared from the three (3) ml of EDTA stored blood; smears where made on a clean free pre-treated slide (Ningbo, China). Three (3 ml) of blood was added in EDTA container and stored in the refrigerator at 4^0C. A cold chain flask was used to transport blood samples stored in a plain container containing RNA shield. The plain container was stored in the freezer at 4^0C.

2.6 Sample Size

The minimum sample size determination was obtained using formula for calculating sample size
by [18].

$$N = Z^2pq/d^2 \text{ [18].}$$

Where,

N = minimum sample size
Z= standard normal confidence interval, usually set at 1.96
P = Proportion in the target population.

Using prevalence of 62% from previous work by Dibua et al. [4] on prevalence of HIV and malaria co-infection in pregnant women in Anambra state.

Q = 1.0 – P (1-0.68) = 0.38
d = degree of accuracy usually set at 0.05

$$\text{Therefore: } n = [(1.96)\ 2\ x0.62x0.38] / (0.05)^2$$

Sample size = A sample size of 362 was calculated but 513 was used.

2.7 Sampling Technique

Simple random sampling technique was used for subject recruitment and sample collection.

2.8 Sample Processing

2.8.1 Haemoglobin estimation

Haemoglobin was estimated using Cyano-methaemoglobin method with an automated HB Sysmex kx-21N Haematology analyzer (Sysmex,Kobe Japan).

2.8.2 Blood film making and staining

This was assessed using slide and stain method as described by Cheesbrough [5].

2.8.3 Thick blood film making for malaria parasite

Twelve (12) micro liters (µl) of blood was placed on the slide to make a circle of 10mm using a clean smooth edged spreader (Thick film). The end of the spreader was wiped clean using cotton wool. The film was allowed to dry, a lead pencil was use to write number of the participant on the slide. When completely dried, the slide was placed into the shallow tray containing freshly prepared diluted Geimsa stain (Guangdona Guanghua, China) (1 in 10 dilution using buffered PH 7.2 water). It was stained for 25 minutes and after it was be brought out and washed off using cleaning water and the back of the slide was cleaned and was placed on a draining rack and allowed to air dry for 5 minutes.

2.8.4 Film reading

Immersion oil was placed on the blood film. The film was examined microscopically for malaria parasite using x 100. A well stained thick blood film was done to achieve a clear background, deep rich purple WBCs' nuclei and malaria parasites with deep red chromatin when viewed microscopically.

2.8.5 HIV screening test

Serum sample was used for screening for the presence or absence of HIV and 2 viruses in serum using the three panel testing method recommended by (CDC, 2014). Immunochromatographic method described by Ariah et al. (1999) was used. Determine kit (Determine Alere kit, Alere medical co.Ltd,

Chiba, Japan) Unigold (Trinity Biotech plc Ireland) and Chembio HIV1/2 Stat-pak: (Chembio Diagnostic system, inc NY, U.S.A) and for control, a procedural control in cooperated in the device was used.

2.8.6 Detection of viral nucleic acid

Plasma viral nucleic acid of HIV virus 1 or 2 were detected using gel electrophoresis according to methods described by Karim [19] with an RT-PCR machine (Perkin Elmer, USA). This involves five (5) processes; extraction, master mix preparation, PCR amplification, gel electrophoresis, detection and documentation.

2.8.7 Extraction

Viral nuclei acid was extracted from cells using methods described by Saeed and Ahmad-Saeed [20] with Quick-RNA miniprep kit (Epigenetic, USA). Rnase-free water was added directly to the column matrix and centrifuge to elute the RNA.

2.8.8 Polymerase chain reaction

Polymerase chain reaction of the nucleic acid was conducted described by Karim [19] using one Taq quick-load 2X master mix and a standard buffer (New England Biolabs Inc.). Forward (27F; 5 '-AGAGTTTGATCMTGGCTCAG -3') and reverse (5'- 1492R TACGGTTACCTTGTTACGACTT -3,) universal primers were used. The cocktail reaction was gently mixed, vortexed and transferred to a preheated thermal cycler (AB applied bio systems, Singapore).

2.8.9 PCR amplification

PCR amplification was conducted according to method described by Karim [19], on an Eppendorf nexus gradient master cycler (Epperdorf, Germany).

2.8.10 Agar-rose gel electrophoresis

Bands of HIV amplified protein were separated by agar gel electrophoresis method according to method described by Armstrong and Schulz [21]. Gel electrophoresis chamber and run at 90 Volts for 60 minutes then viewed under gel documentation system with UV trans illuminator (Deutschland and Osterreich, U.K).

2.8.10.1 Visualization

The picture of the gel documentation separation of DNA bands of the HIV proteins were distinguished by fluorescence illuminating method as described by Adkins and Burmeister [22] and stained gel viewed under UV light of (254-366 m) and recorded by using Polaroid photograph and a gel documentation system. Results of RT-PCR pol HIV gene was obtained, see plate 1 and 2.

2.8.10.2 Quality controls

Standard positive and negative controls were included in the entire test procedures as provided in the kit by manufacturers and standard procedures followed as instructed in the manual.

2.8.10.3 Statistical analysis

The results obtained were analyzed using a Statistical package for social sciences (SPSS) version 21. Simple prevalence percentage and chi-squares were used to access prevalence and association. Spearman's coefficient was used to compare variables between test and controls. Level of significance was set at 95%. Confidence interval with P values of 0.05 considered significant in all case.

3. RESULTS

In Table 1, out of the total number of 362 women of reproductive age attending General Hospital, Onitsha the prevalence of malarial parasites was 125(34.5%). In the control group, of 181 women who were non-gravid, the prevalence was 23 (12.7).Overall prevalence of the malaria parasite was 148 (27.3%) out of the total number of women tested (543).

In Table 2, the test group with mp positive was more anaemic 53(37.8%) than control groups 14(16.1%). Those with mild anaemia had more mp positive status among the anaemic status in both groups. Statistical significant was observed between test and controls among the anaemic status and malaria ($p = 0.000$; $p = 0.000$).

In Table 3 those with mp ++ were more anaemic in the study group. There was significant difference being observed among the anaemia status and malaria ($p = 0.000$).

In the test groups, 347 who tested negative using serology, 237 (68.3%) tested PCR negative while 110(31.7%) tested positive with PCR

confirmatory method. In control, out of the 176 who were serological negative, 175 (99.4%) were PCR negative while only 1(0.6%) was positive when confirmed with PCR confirmatory method. All those who were serological positive were also positive when confirmed with PCR method. A strong statistical difference was observed between in both groups (p = 0.000; p = 0.000).

In the study group, 523 who tested negative using serology, 412 (78.8%) tested PCR negative while 111 (21.2%) tested positive with PCR confirmatory method. A strong statistical difference was observed (p = 0.000).

Plate 1 and 2: Electrophoretic bands of RT-PCR pol HIV gene extracted from participant blood samples.

Table 1. Prevalence of malaria parasite in the study group

Group	Malaria positive(+)	Parasite negative(-)	Total
	n(%)	n(%)	n(%)
Test	125(34.5)	237(65.5)	362(100)
Control	23(12.7)	158(87.3)	181 (100)
Total	148(27.3)	395(72.7)	543(100)

Table 2. Prevalence of malaria in relation to different Anaemia status in test and control group

	Malaria parasite	Mild	Moderate	Severe	Total	X^2	P
		n(%)	n(%)	n(%)	n(%)		
Test	+	0(0.0)	0(0)	0(0)	0(0.0)	53.375	0.000
	++	40(100)	0(0)	0(0)	40(100)		
		0(0)					
	+++	0(0.0)	13(100)	0(0)	13(100)		
	Negative	42 (47.7)	42(47.7)	4(4.6)	88(100)		
Total	82(58.2)	82(58.2)	55(39.0)	4(2.8)	141(100)		
Control							
Group	+	0(0.0)	0(0)	0(0)	0.0(0.0)	5.996	0.050
	++	14(100)	0(0)	0(0)	14(100)		
	+++	0(0.0)	0(0.0)	0(0)	0(0.0)		
	Negative	50(68.5)	22(30.1)	1(1.4)	73(100)		
Total		64(73.6)	22(25.3)	1(1.1)	87(100)		

Table 3. Prevalence of malaria status in relation to different Anaemic status in the study group

	Malaria parasite	Mild	Moderate	Severe	Total	X^2	P
		n(%)	n(%)	n(%)	n(%)		
Study	+	0(0.0)	0(0.0)	0(0.0)	0(0.0)		
Group	++	54(100)	0(0.0)	0(0.0)	54(100)	59.33	0.000
	+++	0(0.0)	13(0.0)	0(0.0)	13(100)		
	Negative	92(57.1)	64(39.8)	5(3.1)	161(100)		
Total	146(64.0)	77(33.8)	5(2.2)	228(100)			

Table 4. Prevalence of HIV screening method with gold standard PCR method in diagnosis of HIV infection in both groups

Group	Determine	PCR		Total	X^2	P
		Negative(-)	Positive(+)			
		n(%)	n(%)			
Test	Non-reactive	237(68.3)	110(31.7)	347(100)	29.669	0.00
	Reactive	0.0(0.0)	15(100)	15(100)		
	Total	65.5	125(34.5)	362(100.0)		
Control	Non-reactive	175(99.4)	1(0.6)	176(100)		
	Reactive	0(0.0)	5(100)	5(100)	149.978	0.00
	Total	175(96.7)	6(3.3)	181(100)		

Table 5. Prevalence of HIV screening method with PCR confirmatory methods in the total group

Group	Determine	PCR		Total	X^2	P
		Negative(-)	Positive(+)			
		n(%)	n(%)			
Study group	Non-reactive	412(78.8)	111(21.2)	523(100)		
	Reactive	0(0.00)	20(100)	20(100)		
	Total	412(75.9)	131(24.1)	543(100)	65.306	0.00

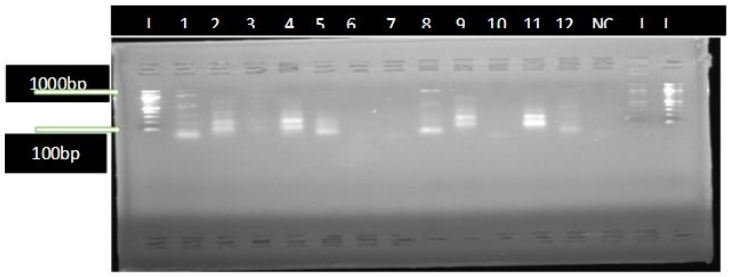

Plate 1: RT-PCR results for POL HIV genes analyzed on a 1.0 % agarose gel electrophoresis stained with ethidium bromide. L is a 100bp-1000bp DNA ladder (molecular marker). Samples 1, 2, 3, 4, 5, 8, 9, 11, 12 and 13 are positive bands for the expressed POL HIV genes at 100 and 200bp bp. Samples 6, 7 and 10 are negative bands. NC is a No template control.

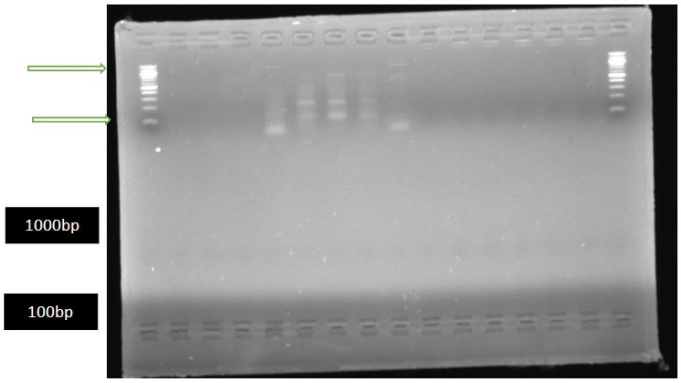

Plate 2: RT-PCR results for POL HIV genes analyzed on a 1.0 % agarose gel electrophoresis stained with ethidium bromide. L is a 100bp-1000bp DNA ladder (molecular marker). Samples 16, 17, 18, 19 and 20 are positive bands for the expressed POL HIV genes at 100 and 200 bp. Samples 13, 14, 15, 21, 22, 23 24 and 25 are negative bands. NC is a No template control

4. DISCUSSIONS

All malarial fever related deaths (90%) in 2015 and 2016 were reported from the WHO African Region. Of the 91 countries reporting indigenous malaria cases worldwide, around 80% of the total cases were from sub-Saharan African countries [7,23]. WHO [24] stated that in 2017, the Fragile States Index considered 15 countries (South Sudan, Somalia, Central African Republic, Yemen, Syria, Sudan, the Democratic Republic of the Congo, Chad, Afghanistan, Iraq, Haiti, Guinea, Zimbabwe, Nigeria and Ethiopia), to be "very high alert" or "high alert" or noted them as being a fragile state with a Maternal Mortality Rates (MMR) in 2017 ranging from 31 (Syria) to 1150 (South Sudan). In most cases, children, the immunosuppressed as well as pregnant women are most susceptible.

In this study, positive malaria status with anaemia was higher in pregnant women (test groups) (34.5%) than in control group (12.7%). This could be because in endemic areas,

substantial immunity acquired in adults to the disease and the more susceptibility rate to the infection in pregnancy makes parasites to sequester in the placenta, putting them at risk of severe anaemia and the risk of children being born with low birth weight. Again, their immune–compromised state, Haemoglobin level or lack of compliance with malaria prevention/control measures in pregnancy could be contributory factors. This finding is in support with that by Rogers et al. [25], who noted that pregnant women were more positive to malaria parasite than non-pregnant women and attributed their findings to reduction of immunity associated with pregnancy. The higher rate of anaemia in test groups (pregnant women) in this study could be attributed to iron deficiency resulting from prolonged negative iron balance which accounts for 20% of Anaemia in women worldwide, Stevens et al. [26] or due to malaria, intestinal parasite, poor feeding, or chronic illness [27]. Another reason could be poor level of preventive care of malaria adapted during pregnancy, poor compliance to preventive anti-malaria drug, non-compliance to iron supplement, supported by findings by [28]. Higher HIV status in these groups as observed in this research (34.5 %) could also increase their higher parasiteamia. HIV infection have been found to increase susceptibility in malaria, higher density infection with loss of gravidity-dependent-immunity (Ter kulie, 2004).The prevalence of malaria in pregnant women in this research conducted in 2019 in Onitsha, Anambra state, Nigeria was 34.5%, which is lower than what is recorded in Anambra (73.1%) by Ukibe et al. [29], Abia state (40.1%) by Ejike et al. [30], and Uyo (41.0%) by Opara et al., [31], but higher than what was recorded in Anambra state (16.7%) by Dibua *et al.*[4] and in Owerri (11.0%) by Igwe et al. [32]. The variance in the prevalence may due to geographical location and its environmental conditions, preventive compliance measures, as well as the diversity in season when study was carried.

In this study, total malaria status in relation to different Anaemia status in the total groups was (29.4%) with mild anaemia being having most prevalent malarial status 54 (100). Reasons for the higher prevalence of the mild Anaemia despite malaria endemicity in the area could because of a probable success rate of the roll-back-malaria program going on in the area as of the time of study, the geography of the study area, diet, life style, compliance to routine hematinic drugs and their levels and attitudes towards malaria preventive measures. The incidence of Anaemia dur ing pregnancy is aggravated in malaria high transmission settings (Afolabi and Adeola, 2018).

As regards to the methodological differences, out of the 347 who tested HIV negative using screening method, 110 (31.7%) tested HIV positive with PCR method in test group and 0.6% only in the control, out of the 176 who were non-reactive to screening method. Probable reason for the marked difference in false - negatives between the test group and controls could be associated with sensitivity of the test kits used, HIV antibody detectable alterations observed in pregnancy, and changes in window period . HIV tests herebeen found to be altered during pregnancy and postpartum Materno et al. [33]. Our finding is in agreement with findings by Matemo et al. [33]. The low detectable HIV antibody in pregnant women makes the antibody test to be negative and differing window period in pregnant and non –pregnancy women are also noted [34, 35].

5. CONCLUSION

Proper personal hygiene and keeping of our environment clean will aid in the reduction of prevalence associated with malaria parasite with anaemia and HIV. More awareness should be created on the importance of compliance to malaria and routine gynecological drugs intake in pregnant women

CONSENT

As per international standard or university standard, patients' written consent have been collected and preserved by the authors.

ETHICAL APPROVAL

Ethical approvals were obtained from the Ethical Committee of faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Awka, Nnamdi Azikiwe University Teaching hospital, Nnewi and Anambra State Management Board of Health, Awka.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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