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Research Article

Plasmodium falciparum Detection and Assessment of Utilization of Insecticide Treated Nets among Pregnant Women Attending Sir Muhammad Sanusi Specialist Hospital, Kano State

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ABSTRACT

Pregnancy associated with malaria (PAM) is a major cause of morbidity among pregnant women and their offspring in *Plasmodium falciparum* endemic areas. This study assessed the utilization of the insecticide-treated nets by *Plasmodium falciparum*-infected pregnant women attending Sir Muhammad Sunusi Specialist Hospital, Kano. Blood samples of 220 pregnant women were collected and analysed by Rapid diagnostic test, microscopy, Parasite Density estimation, Haematological parameters and molecular analysis of *Plasmodium falciparum*. Questionnaires were used to obtain information about occupation, age, trimesters (gestation of pregnancy), and use of mosquito nets. The results showed that the age range of 18-30 recorded the highest prevalence of 21.9%. Highest incidence was also recorded in the 3rd trimester, followed by the 2nd and 1st trimesters with percentage prevalences of 22.4%, 19.3% and 18.2% respectively**.** The parasite density was not significantly different among respondents of various trimesters and severe parasitaemia was observed to be higher in women in their first trimester. The haematological parameters did not differ significantly (P<0.05). Among the pregnant women. The proportion of pregnant women who used the net 201(91.4%) was significantly higher (P>0.05) than those who did not use the nets 19 (8.6%). Out of the 10 samples that had the highest parasite density, only 3 (30) were positive for *Plasmodium falciparum* when tested by molecular analysis. Despite the low prevalence, pregnant women should be encouraged about the need for adequate utilization of ITN to prevent maternal and infant morbidity and mortality due to malaria.

Keywords: Insecticide; Malaria; *Plasmodium falciparum*; Pregnant Women; Prevalence

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INTRODUCTION

Malaria is a serious and potentially deadly illness caused by protozoan parasites of the *Plasmodium* genus, affecting people of all ages (WHO, 2022). There are five main species responsible for the disease: *P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax* and *P. knowlesi*. Among these, *P. falciparum* and *P. vivax* are the most common, with *P. falciparum* being responsible for the most severe cases. The infection spreads through the bite of female *Anopheles* mosquitoes carrying the parasite. The parasites initially multiply in the hepatocytes (liver cells) and subsequently move to invade red blood cells. Symptoms which often appear about 9 to 14 days after the bite of an infected mosquitoes include headaches, fever, vomiting and other flu-like symptoms. Untreated malaria can lead to Coma, life threatening anaemia and death. (CDC, 2014).

Outside of sub-Saharan Africa, *P. vivax* is responsible for nearly half of all malaria cases and is particularly common in countries working toward malaria elimination. In areas reporting fewer than 5,000 annual cases, *P. vivax* contributes to over 70% of infections. Globally, malaria is responsible for approximately 2.6% of the total disease burden. Children under the age of five are particularly vulnerable to infection (Guyatt *et al*., 2001). Among infectious diseases, malaria is one of the deadliest, causing around 429,000 deaths in 2015 alone (WHO, 2021). In sub-Saharan Africa, *Plasmodium falciparum* accounts for nearly 99% of malaria- related deaths, with 70% of these fatalities occurring in children under five (WHO, 2022). Approximately 25 million pregnant women are at risk of contracting malaria and the World.

Health Organization reports that the disease is linked to over 10,000 maternal deaths and200,000 newborn deaths each year (WHO, 2021). In tropical Africa, *Plasmodium falciparum* is the most pathogenic species accounting for up to 99% of malaria death with 70% of this death occurring in children under the age of 5 years (WHO, 2022). Twenty- five million pregnant women are currently at risk for malaria and according to the World Health Organization (WHO), malaria accounts for over 10,000 maternal and 200,000 neonatal deaths per year (WHO, 2021).

Pregnant women are especially vulnerable to malaria in endemic regions, where *Plasmodium falciparum* infections are more prevalent. The parasite-infected red blood cells (RBCs) often develop knob-like structures on their surface, enabling them to adhere to the endothelial lining of blood vessels—a process known as cytoadherence (Gajida *et al*., 2010). This adherence encourages the clustering of infected and uninfected RBCs, leading to sequestration in small blood vessels (Ejike *et al*., 2017).

The adhesive nature of infected erythrocytes is largely due to the presence of a protein called *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1), which is encoded by the var gene family (Nash *et al*., 2018). PfEMP1 allows infected red blood cells to bind to specific receptors on the vascular endothelium, helping the parasite to evade destruction by the spleen through a mechanism known as sequestration (Zipser *et al*., 2002). In pregnancy, a similar process occurs when infected RBCs adhere to receptors on the placental lining, particularly the syncytiotrophoblast, leading to a condition known as pregnancy-associated malaria (Zhang *et al*., 2018).

MATERIALS AND METHODS

Study Area

The study was conducted in Sir Muhammad Sunusi Specialist Hospitals Kano Nigeria. It is a Secondary facility which serves as both specialist and a referral center to almost all Hospital under the state, neighboring states and even neighboring countries like Niger Republic, Chad, Cotonou etc.

The Hospital is located in Nassarawa Local Government Area of Kano State (Figure 1). The hospital is bordered from north by Sauna quarters, Mariri, Tokarawa and Hayed by South, East and West Respectively. Kano State is situated in North West geographical region South of Sahara. It is geographically located between latitude 10 30‘N and 13‘N and between longitude 7 40 and 1035‘E. The state is situated in Nigeria’s North-Western region, sharing boundaries with Katsina to the northwest, Jigawa to the northeast, Bauchi to the southeast and Kaduna to the southwest. Its population was estimated at 14.3 million in 2019 and increased to 15.8 million by 2022. The state has two primary seasons: a dry season from November to April and a rainy season from May to October, with an average annual rainfall of approximately 813.5 mm.

Study population

This study was restricted to the pregnant women attending Sir Muhammad Sanusi Specialist Hospital for antenatal services only.

Inclusion and Exclusion Criteria

Pregnant women that attended ANC, gave consent, donated blood and filled questionnaire in Sir Muhammad Sanusi Specialist Hospital during the study only were included. Those that did not attend the ANC Clinic and did not fulfill the above activities were excluded.

Sample size Determination

The sample size was calculated using the prevalence rate of 17.3% reported by (Dantata et el.2017) using the sample size formula below:

$$n = Z^2 P \frac{(1-P)}{d^2}$$

Where n = minimum sample size

P= prevalence obtained from previous report 17.3%= 0.173

Z= the standard normal distribution at 95% confidence interval D= absolute desired precision of 5%= 0.05
Therefore:

$$n = 1.962X0.173\ \frac{(1-0.173)}{0.05^2}$$

Therefore, the minimum sample size =220

Figure 1: Map of Nassarawa Local Government Area showing the Study Area

Sample Collection

About five milliliter (5.0ml) of whole blood was collected using Vacutainer containers according to the method of (Rogerson *et al.*, 2018). Each sample was labeled appropriately. Questionnaire was administered during sample collection to obtain required information of each of the participant such as age, occupation, duration of pregnancy and knowledge of Insecticide treated net usage.

Sample Analysis

Malaria detection using rapid diagnostic test (rdt)

Malaria diagnosis was conducted using the SD Bioline Malaria Antigen (Ag) *P. falciparum*/Pan Test Kit, produced by Standard Diagnostics, Inc. (2013). This rapid diagnostic test (RDT) provides a qualitative and differential detection of the histidine-rich protein 2 (HRP-2) specific to *Plasmodium falciparum*, as well as the common *Plasmodium* lactate dehydrogenase (pLDH) enzyme found in multiple *Plasmodium* species, using human whole blood samples. Prior to testing, all components of the kit were brought to room temperature. The kit was positioned on a clean, flat surface. A 5 µL pipette included in the kit was used to collect the blood sample up to the black line and the sample was then dispensed into the circular sample well. Four drops of assay diluent were then carefully added to the square well. The result was observed between 15 and 30 minutes after sample application. A single-colored band appearing at the control line (C) indicates a negative result. Two colored bands—one at the test line (T or pan) and another at the control line—indicate a positive result. If the control line does not appear, the test is considered invalid (CDC, 2014).

Microscopic detection and identification of malaria parasite

Microscopy remains the gold standard for the parasitological diagnosis of malaria. Thick blood films are preferred for detecting and quantifying parasites, whereas thin films are used to identify the *Plasmodium* species present (Rogerson *et al*., 2008). Separate slides were used to prepare thick and thin blood smears. On a clean, grease-free frosted slide, 7 µL of blood was placed at the center to create a thick film. On another grease-free slide, 2 µL of blood was placed approximately 15 mm from one end to prepare the thin smear. A smooth-edged slide spreader was

used immediately to spread the thin film. Without delay, the larger drop was spread into a circular thick smear roughly 15 mm in diameter.

Each slide was labelled with a sample identification number using a permanent marker. The prepared films were air-dried in a horizontal position and stored in a protected area until staining. Both films were stained using a 3% Giemsa solution following the method outlined by Rogerson *et al*. (2008). A fresh 3% Giemsa dilution was prepared and both films were allowed to dry thoroughly. The thin smear was fixed in methanol for two minutes before staining. The diluted Giemsa stain was gently poured over the smears and left to act for 30 minutes. After staining, the slides were rinsed using clean buffered water. The reverse side of each slide was wiped clean and the slides were placed on a drying rack. After air drying, the stained blood films were examined under a 100x oil immersion objective lens for malaria parasite detection and species identification (Rogerson *et al*., 2008).

Parasite density estimation

Parasite density per microliter of blood was determined by counting the number of malaria parasites observed relative to 200 white blood cells (WBCs) on a Giemsa-stained thick smear and the result was calculated using the following formula:

No. of parasite /ul of blood =No of malaria parasite counted x Total WBC (8000) x 200

Samples with high parasite density was spotted on filter paper for molecular detection of *Plasmodium falciparum*.

Determination of Haematological parameters

Haematological parameters of each sample were determined using automated blood analyzer System KX-21N (2006) hematology analyzer. The System KX-21N is an automated

hematology analyzer for a clinic satellite laboratory or research testing. It provides a CBC with 17 reportable parameters including histogram for WBC, RBC and PLT. Specific amount of sample corresponding to EDTA ratio was collected and Sample Identification number was imputed, Sample was mixed sufficiently, plug of sample tube was removed with caution and the sample tube set to sample probe and start bottom was pressed. The sample tube removed when the buzzer beeps 2 times and LCD screen displays ―analyzing‖ Results for each sample collected/analyzed was recorded.

DNA Extraction

Extraction of DNA from blood spotted on filter papers was carried out using Kit (Accu prep Genomic DNA extraction kit, Bioneer, USA, 2018). The Kit was used based on the manufacturers operating procedures where the Filter paper spotted with positive sample was inserted GB buffer in a 1.5 ml tube to dissolve, using a heating block, overnight. 20 ul of proteinase K was added these were mixed by vortexing. It was then spun down briefly to remove drops from the tube after incubation at 60oC for 10 minutes. 400 ul of ethanol was added and mixed by vortexing. It was then centrifuged for 8000 rpm for 1 minute. The tube containing the filter was then discarded. DNA adhered to the membrane within the spin columns, which were then placed into clean, sterilized 2 mL collection tubes. A volume of 500 µL buffer was added carefully, avoiding contact with the tube rim. The cap was secured and the column was centrifuged at 8000 rpm for 1 minute. After centrifugation, the column was transferred to a fresh 2 mL collection tube and the flow-through was discarded into a waste container. Next, 500 µL of Buffer AW2 was gently added without touching the rim, followed by centrifugation at 8000 rpm for 1 minute. To ensure complete removal of residual ethanol, a second spin was performed at 12,000 rpm for 1 minute. The spin column was then placed into a clean 1.5 mL microcentrifuge tube for the elution step. A total of 200 µL of elution buffer (EL) was added directly onto the membrane and allowed to sit for 1 minute to fully absorb. Finally, the column was centrifuged at 8000 rpm for 1 minute to elute the DNA before storage (Gore *et al*., 2020).

Polymerase chain reaction (PCR) to confirm the presence of *Plasmodium falciparum*

Polymerase chain reaction (PCR) was conducted in a total reaction volume of 20 µL using the Start Premix Kit, as described by Rogerson *et al*. (2018). A two-step nested PCR protocol was employed for the detection and species-level identification of *Plasmodium*. In the first round, the reaction mixture included 16 µL of nuclease-free water, 2 µL of primer mix and 2 µL of DNA template. The second round comprised 17 µL of nuclease-free water, 2 µL of primers and 1 µL of the primary PCR product. Thermal cycling conditions consisted of an initial denaturation at 95°C for 5 minutes, followed by 40 seconds of denaturation at 94°C, annealing at 54°C for 40 seconds, extension at

72°C for 40 seconds and a final extension at 72°C for 5 minutes (Smith *et al*., 2011). PCR products were separated on a 2% agarose gel, stained with ethidium bromide for 15 minutes and visualized under UV illumination. Primers used are as presented in Table 1.

Data Analysis

Data generated from this study was analysed using Statistical Package for Social Science. Chi- square test was used to determine the association between *Plasmodium falciparum* infection and level of use of long-lasting insecticide treated net, utilization of net, convenience in usage of net, age, Occupation and stages of pregnancy.

Table 1. Primer Sequences for the Amplified genes

Primer	Sequence of Primers	Expected band Size (Amplicons)
PF1	AAT GAA GAG CTG TGT ATC	200-400bp
PF2	GGA ATC TTA TTG CTA ACA	200-400bp

RESULTS

Demographic Data of the Respondents

A total of two hundred and twenty pregnant women were involved in the study. Out of which one hundred and thirty 130 (59.1) were between the age of 18-30 years, 81(36.8) were within age range of 31-40 and 9(4.1) were 41 or older. Based on employment status, the self-employed had the highest proportion of 133(60.5) followed by unemployed with 81(36.8) and the Employed ones 6(2.7). 12(5.5) were in their first Trimester, 56 (25.5) were in the second Trimester and 152(69.1) were in the 3rd Trimester (Table 2).

Level of Use of Long- lasting Insecticide Treated Nets

Out of 220 participants that attended SMSSH for ANC, 201 (91.4%) were found to have possessed the Long-lasting Insecticide Treated net, while 19(8.6) were admitted not having the nets. Based on this finding It was gathered that the proportion of the respondents that possessed the nets are significantly higher (P<0.05) than those that do not have the nets (Table 3).

Utilization of Insecticide Treated Nets among the Participants

Out of 220 participants that use the LLIN, 153(69.5%) Use the nets daily while 48(21.8%) use it occasionally (Table 4). Results indicated that there was no significantly difference (P>0.05) in the number of pregnant women that do not use the nets daily compared to those that use the nets daily.

Convenience in Usage of Long -Lasting Insecticide Treated Nets

There was no significant difference in convenience in use of the net and the Pregnant women that attended the facility for antenatal care as 183(82.3) did not report any inconvenience in using the net Only 39(17.7%) reported inconveniency in using the nets (Table 5). The difference between those that do not utilize the nets daily was not significantly (P>0.05) lower than those that utilize it daily.

Detection of *Plasmodium falciparum* in Pregnant Women Attending Sir Muhammad Sunusi Specialist Hospital by Microscopic Method

The findings revealed a statistically significant difference (p < 0.05) in the prevalence of *Plasmodium falciparum* malaria among pregnant women attending Sir Muhammad Sanusi Specialist Hospital. The microscopy conducted shows that out of 220 pregnant women that were involved in the study, 47(21.4%) were positive of *Plasmodium falciparum* while 173 (78.6%) were found to be negative The Result is presented in Table 6. The result shows that there was no significant difference (P<0.05) in the occurrence of *Plasmodium falciparum* malaria in pregnant women attending Sir Muhammad Sanusi Specialist Hospital.

Prevalence of *Plasmodium falciparum* Based on Age

Age range of 18-30 with the number of pregnant women 196 had the highest prevalence of (21.9%), this was preceded by 31-40 age groups with 23 pregnant women and (17.4%) and 41 and above age group with 0% prevalence (Table 7). No statistically significant difference was observed in the prevalence of *Plasmodium falciparum* among pregnant women across age groups (P > 0.05).

Table 2. Demographic Data of the Respondents

Parameters	Number Examined	Number Infected (%)
Age (Years)		
18 - 30	196	43 (21.9)
31 - 40	23	4 (17.4)
41 & above	1	0 (0.0)
Total	**220**	**47 (21.4)**
Occupation		
Employed	6	1 (16.7)
Unemployed	81	20 (24.7)
Self-employed	133	24 (18.0)
Total	**220**	**45 (20.4)**
Trimester		
First	11	2 (18.2)
Second	57	11 (19.3)
Third	152	34 (22.4)
Total	220	47 (21.4)

Table 3. Level of Use of Long- lasting Insecticide Treated Nets

Respondents	Frequency	Percentage %
In Possession of LLIN	201	91.4
Not having LLIN	19	8.6
Total	220	100

$X^2(x)=19.225$; $p=0.003$ ($p<0.05$)

Table 4. Utilization of Insecticide Treated Nets among the Participants

Respondents	Frequency	Percentage
Everyday	153	6.1
Occasionally	48	23.9
Total	220	100

$X^2(x)=2.498$; $p=0.460$ ($p>0.05$)

Table 5. Convenience in Usage of Long -Lasting Insecticide Treated Nets

Respondents	Frequency	Percentage (%)
Convenient in Use Of LLIN	183	82.3
Not Convenient in Use of LLIN	39	17.7
Total	220	100

$X^2(x) = 3.438$; $p= 1.021$($p>0.05$)

Table 6. Detection of *Plasmodium falciparum* in Pregnant Women Attending Sir Muhammad Sanusi Specialist Hospital by Microscopic Method

Respondents	Frequency	Percentage (%)
No. Positive	47	21.4
No. Negative	173	78.6
Total	220	100

$X^2(2) = 13.289$; $p=0.003$ ($p<0.05$)

Table 7. Prevalence of *Plasmodium falciparum* Based on Age

Age (Years)	Number Examine	Number Infected	Percentage Infected
18-30	196	43	21.9
31-40	23	4	17.4
41 and above	1	0	0
Total	220	47	21.4

$X^2(x) =3.193$: $p=0.312$ ($p>0.05$)

Prevalence of *Plasmodium falciparum* Based on Occupation

Among the 6 pregnant women that were employed, 1 was infected with *Plasmodium falciparum* which recorded (16.7%) prevalence. Of the 81 pregnant women that were Identified as unemployed, 20(24.7%) were presented with the infection. Whereas 24(18.0%) of the Self- employed were also infected with the parasite. Analysis revealed a significant difference (P<0.05) in the prevalence of *Plasmodium falciparum* as presented in Table 8.

Malaria *Parasitaemia* in Relation to Trimester (Stages of Pregnancy)

Pregnant women of Third trimester stage recorded the highest prevalence of *Plasmodiun falciparum* of (22.4%) as parasite was detected in 34 of 152 pregnant women that were in their Third Trimester, followed by those in their second Trimester with (19.3%) prevalence as parasite was detected in 11 out of 57 women tested, With the least recorded in the pregnant women of their first Trimester that had prevalence of (18.2%) percentage prevalence as parasite was detected in 2 out of 11 pregnant women that were tested for *Plasmodium falciparum* infection. There was a notable difference, which was statistically significant (P<0.005) in malaria parasitaemia between the different stages of pregnancies (Table 9).

Distribution of Parasite Density Among Pregnant Women According to Trimester

Out of 47 pregnant women that were tested positive for *Plasmodium falciparum*, 10 (21.3%) had parasite density greater than 3000 parasites per µl of blood while 37 (78.7) had parasite density 3000 parasite/µl (Table 10). Among the 2 Pregnant women of their first Trimester that also presented with *Plasmodium falcifarum*, 0(0%) had parasite density less than 3000 parasite /µl of blood while 2 (100%) had parasite density greater than 3000 parasite /µl. Out of 11 pregnant women of their second Trimester, 8 (72.7%) had parasite density less than 3000 parasite/µl while 3(27.7%) had parasite density greater than 3000 parasite/µl of blood, 29(85.3%) of 34 Pregnant women of third Trimester, had parasite density less than 3000 parasite/µl while 5(14.7%) had parasite density less than 3000 parasite /µl of blood. The difference in the distribution of parasite density among the pregnant women was not significant (p>0.05).

Haematological Parameters of *Plasmodium falciparum* Infected and Uninfected Pregnant Women

Table 11 Shows that the difference between some haematological parameters and level of Pregnancy was found to be statistically insignificant (P>0.05). This shows that some haematological parameters are not affected by the level of pregnancy (Trimesters). Level of monocytes increases in the first trimester then fall as pregnancy advances. While Eosinophil and basophil counts remain unchanged during Pregnancy (Table 11).

Molecular Analysis of *Plasmodium falciparum*

Among the 10 samples that had severe malaria (Parasite Density > 3000) During microscopy, 6(60%) were positive for the DNA of *Plasmodium falciparum*. And the parasites were detected at 400bp band sizes (Plate1).

T**able 8. Prevalence of *Plasmodium falciparum* Based on Occupation**

Occupation	Number Examined	Number Infected	Percentage Infected (%)
Employed	6	1	16.7
Unemployed	81	20	24.7
Self employed	133	24	18.0
Total	220	45	20.5

X^2 (2) = 227.91, P=0.000 (P<0.05)

Table 9. Malaria *Parasitaemia* in Relation to Trimester (Stages of Pregnancy)

Trimester	Number Examined	Number Infected	Percentage Infected
1st Trimester	11	2	18.2
2nd Trimester	57	11	19.3
3rd Trimester	152	34	22.4
Total	220	47	21.4

X^2 (2) = 221.00, P= 0.00, (P<0.05)

Table 10. Distribution of Parasite Density Among Pregnant Women According to Trimester

Trimester	Number Examined	Parasite Edensity per microlitre of blood	
		Mild	Severe
1st Trimester	2	0(0)	2(100)
2nd Trimester	11	8(72.7)	3(27.3)
3rd Trimester	34	29(85.3)	5(14.7)
Total	47	37(78.7)	10(21.3)

$X^2(x)=2.241$; p=0.326 (p>0.05)

Table 11: Haematological Parameters of *Plasmodium falciparum* Infected and Uninfected Women

Parameters	1st Trimester	2nd Trimester	3rd Trimester	P Value
WBC counts	8.22+-1.54	7.53+-2.23	7.55+-1.96	0.55
Haemoglobin GLDL	7.43+-0.10	8.53+-0.92	8.82+-6.65	0.71
Platelets X203/YL	68.25+-113.95	174.14+-111.46	1658.51+-18	0.81
Lymphocytes (%)	30.57+-4.95	28.85+-7.16	27.84+-8.11	0.31
Neutrophil (%)	63.97+-10.32	64.13+-10.06	62.65+-8.11	0.53

Note* Difference is not Significant (P>0.05)

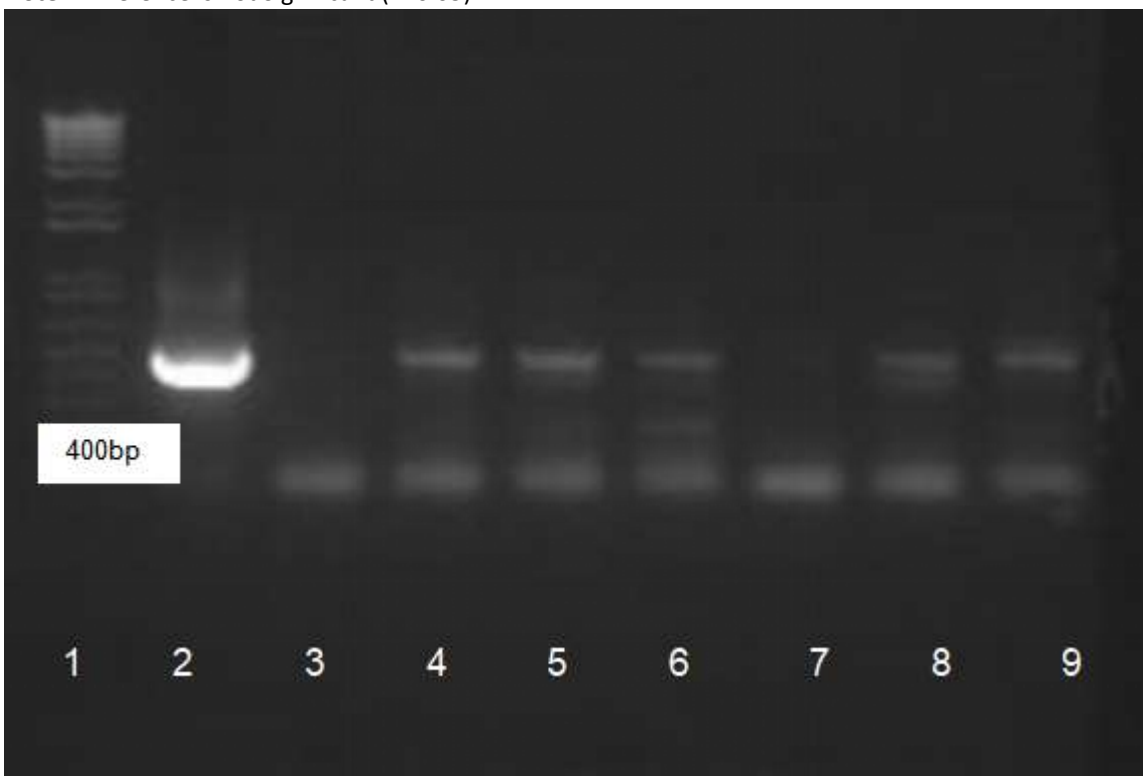

Plate 1: Amplified *Plasmodium falciparum* DNA

Lane 1 Molecular Ladder 1000bp, Lane 2, 4, 5, 6, 8 and 9: positive samples (Approx 400bp) Lanes 3, 7 Samples from other individuals

DISCUSSION

In this study the significantly high ownership of long-lasting Insecticide Treated nets 201 (91.4%) could be attributed to the high proportion of the respondents that received free ITNs from Government Health facilities and some NGOs, Health education of mothers on malaria and effective utilization of Insecticide

Treated nets, Abaje *et al.* (2014) had reported a much higher proportion of respondents in their study where about (77%) Owned Long-lasting Insecticide Treated nets. Similar finding was also reported by Abaje *et al.* (2021) where the possession of the Long-lasting Insecticide was about (97%). Yakasai *et al.* (2017) reported a contrary finding in which Ownership of long-lasting Insecticide treated net was (27.5%). The 55% of the daily utilization of the nets could be as result of convenience in usage of the nets according to the reports of the studied participants. This is in line with the finding of Idris *et al. (*2023) similar finding was also found in Oladele, *et al.* (2018). The prevalence of *Plasmodium falciparum* among the pregnant women that utilize the net could be as a result of their stay outside the net before bed time and this definitely predispose them to vector. Sleeping inside torn mosquito n*et al*so serve as predisposing factor While the prevalence found among the respondents that do not have or not use the net frequently was due to total exposure to the insect vector as a result of not possessing or not frequently using the nets.

The significantly low prevalence (21.4%) of *Plasmodium falciparum* detected among Pregnant women attending Sir Muhammad Sanusi Specialist Hospital by Microscopy could be due to

regular ANC attendance, health education acquired, good adoption of malaria prevention and control strategies, better socio-economic Condition of most of the participants as most of them are self-employed. This finding was in Agreement with work of Oboh *et al. (*2022) who reported a 16.1% rate in their investigation into malaria prevalence and associated risk factors among initial antenatal attendees in rural Burkina Faso. It also Correspond to the finding of Idris *et al. (*2023) that reported low prevalence of (8.70%) in their cross-sectional study on Pregnant women attending University College Hospital Ibadan.

This study was however in contrast with the study of some authors who reported higher prevalence of malaria infection among pregnant women. (Muhamed *et al.*, 2021) recorded (95%) prevalence among in Lagos South-west Nigeria. Similarly, Nkoka *et al.* (2018) reported a prevalence of 66.7% of pregnant women that attended Health Centers in Ideato South Local Government of Akure, Ondo State.

The occurrence of *Plasmodium falciparum* infection was found to be higher among younger pregnant women. This may be attributed to the lack of acquired immunity against placental malaria, which typically develops following repeated encounter to malaria infection during the gestational period (Yakasai *et al*., 2017). Younger women, particularly those experiencing their first pregnancy, are less likely to have developed this protective immunity. In contrast, older women, who are more likely to be multigravidae, tend to have greater resistance to malaria infection due to prior exposure. This shows that as parity increases with age, repeated exposure to *Plasmodium falciparum* during pregnancy induces the acquisition of this immunity against *Plasmodium falciparum* malaria (Scott *et al.*, 2021). The lower prevalence of malaria among older women on the other hand, might be due better exposure to health services, awareness about the disease, its effects and the ways of its prevention among this age category (Avatta *et al.*, 2014. The work is in line with the finding of Simon *et al. (*2019), Avatta *et al. (*2023) who reported higher prevalence of *Plasmodium falciparum* in pregnant women of lower age category, contrary to this finding was that of Zango (2022), who reported higher prevalence of malaria among older age group of pregnant women.

The higher incidence of *P. falciparum* recorded among the third trimester in this study could be as a result of delay in registration with the ANC Clinic until in the third trimester due to personal, financial and religious reasons (Muhammad *et al.*, 2023). There was the likelihood that some participants might be asymptomatic hence were not tested and treated in the previous trimesters. These therefore carried the parasites into the following trimesters (Muhammad *et al.*, 2023). Higher prevalence of malaria at latter trimesters might be attributed to postponement of malaria treatment, especially in the first trimester of pregnancy for fear of the outcome of the therapy. (Fatima *et al.*, 2023). This was consistent with the result of Nkoka *et al. (*2018) and Adedokun *et al. (*2020) who reported higher prevalence of *P. falciparum* among pregnant women in their third trimesters. It was believed that Immuno- suppression, high adrenal steroid levels, chronic gonadotrophin and fetoprotein in the blood together with the possibility of the depression of the lymphocyte's activity may count for the higher susceptibility to malaria by women in their third trimester of pregnancy, (Scott *et al.*, 2021). Contrary to this was finding of Oladele *et al. (*2018) and Yakasai *et al. (*2017) who observed that

higher prevalence of *Plasmodium falciparum* was associated with the first trimester of pregnancy in their finding. However, (Dikwa *et al.*, 2023), Ajegena (2020) and Ankumah (2014) recorded higher prevalences of *P. falciparum* malaria among women in their second trimester of pregnancy. Based on the above findings, it can be concluded that pregnant women are susceptible to *P. falciparum* infection irrespective of their trimester level of pregnancy. Therefore, routine screening of pregnant women for malaria parasites and treatment for malaria is essential during all trimesters (Dikwa *et al.*, 2023).

The relatively low parasite density in this study was observed in the second and third trimesters of the studied participants and this could be as a results of law endemicity, adoption of control measures or exposure to malarial drugs (Fatima, 2023) This finding is in conformity with the finding of Jegede *et al. (*2020) who reported that the proportion of pregnant women with law parasite density was higher than the proportion of pregnant women with severe malaria. The relatively high parasite density in this study was noted among women in their first trimester than in women in their second and third trimesters. This could be as a result of sudden decrease in immunity of pregnant women in their first trimester than in other trimesters where their body had already adapted to the changes due to pregnancy (Dikwa, 2023). Furthermore, women in their second and third trimesters must have been exposed to malaria antigens as such are aware of their susceptibility to malaria and must have taken medication or adapted certain control measures against the parasites. (Adedokun *et al*., 2019).

The relatively high incidence (24.7%) of *Plasmodium falciparum* infection observed among unemployed pregnant women in this study could be as a result of poor standard of livings which pave ways to mosquito breeding sites, inability to purchase/afford the long-lasting Insecticide treated nets. This corroborated with the work of Adedokun *et al.* (2019) and Oladele *et al.* (2018) both reported higher incidence of *P. falciparum* amongst pregnant women that were unemployed. However, this was contrary to the finding of Fatima, (2023) who noted higher incidence of *P. falciparum* in Self-employed pregnant women and based her reason on the fact that the self-employed stay outdoors at late hours of the night conducting businesses and consequently got bitten repeatedly by mosquitoes.

Out of the 10 Samples that had the highest parasite density, only 3 (30%) were confirmed as true positive using molecular analysis. Molecular analysis is a standard method in all laboratories that requires DNA replication via polymerase chain reaction (PCR) technique (Abaje *et al.*, 2014). The polymerase chain reaction is a rapid technique with high sensitivity and is valued as confirmatory test for micro-organisms detection including the parasites (Scott *et al.,* 2021). This finding corresponds with the finding of Dikwa *et al. (*2023) who reported that only 5 (41.7%) samples out of 10 samples were confirmed positive using molecular analysis. Contrary to this finding was that of Oladele *et al. (*2019) whose samples were all confirmed positive using molecular analysis.

CONCLUSION

It can be concluded that there is an Incidence of *Plasmodium falciparum* in women receiving antenatal care at Sir Muhammad Sanusi Specialist Hospital. Although, the incidence is low compared to other studies but the Incidence varied with the age, Trimester of pregnancy and occupation of the participants. Highest prevalence was observed among pregnant women of young age group (21.9%), in their second Trimesters (19.3%) and in Unemployed participants (24.7%).

The proportion of the participants who own LLINs are significantly higher than those who do not. Of those who own LLINs, 76.1% use the nets daily while 23.9% use the nets occasionally and the use of LLINs was not associated with any form of Inconvenience among a significant fraction (82.3%) of the study Population.

Some haematological parameters did not differ ($p>0.05$) significantly between Plasmodium falciparum of infected and uninfected Individuals. Malaria parasite did not significantly ($p>0.05$) affect some haematological parameters.

Authors Contribution: All authors were involved fully during the conceptualization and design stages of the study. They also participated in data collection, data analysis, interpretation, preparation, and review of the draft manuscript.

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