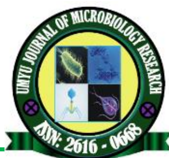
UJMR, Vol. 8 No. 2, December, 2023, pp. 227 - 235 *E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668*

https://doi.org/10.47430/ujmr.2382.026

Received: 09 November 2023 **Accepted:** 27 December 2023



Prevalence and Molecular Detection of *Plasmodium falciparum* among Pregnant Women Attending Selected Hospitals in Kaduna North Local Government Area, Kaduna State

[1]Dikwa, K. B., *[2]Baban Takko, F. S., [3]Vantsawa, P. A.,[4] Abdulsalami, M. S., [5]Muhammed, M.

[1]Depatrment of Biological Sciences, Nigerian Defence Academy, Kaduna, Nigeria.
[2]Department of Biology, Nigerian Army University Biu. Borno, Nigeria.
[3]Depatrment of Biological Sciences, Nigerian Defence Academy, Kaduna, Nigeria
[4]Department of Biotechnology, Nigerian Defence Academy, Kaduna, Nigeria
[5]Pharmacology Laboratory, College of Health Sciences, Kogi State University, Anyigba, Nigeria.
*Correspondence: fatimahsbtakko@gmail.com; 08030436606

Abstract

Pregnancy-related malaria is a serious public health issue that significantly raises the risk of death for both mothers and babies in malaria-endemic nations. This study was carried out to determine the prevalence of malaria and molecularly detect *Plasmodium falciparum*among pregnant women attending selected hospitals in Kaduna North Local Government Area, Kaduna State.Blood samples were collected from 309 pregnant women attending BarauDikko Teaching Hospital Kaduna, General Hospital Kawo and Nigerian Defence Academy Hospital Kaduna. The samples were analysed microscopically and molecularly for *Plasmodium falciparum*. A self-administered questionnaire was administered to each pregnant woman to obtain information such as occupation, age and gestation period. Results show a prevalence of 65.0% for*Plasmodium falciparum* malaria among the pregnant women with significant ($p < 0.05$) difference between malaria and sampling location. The highest prevalence was recorded in pregnant women attending General Hospital Kawo with a prevalence of 76.9%, followed by women attending NDA hospital with a prevalence of 65.0% and those attending Barau Dikko Teaching Hospital had 53.4%. %). Prevalence of *Plasmodium falciparum* was seen higher in women in their second trimester of pregnancy with 68.9% prevalence, younger aged pregnant women with 66.7% prevalence and self-employed pregnant women with 66.2% prevalence. Results further showed that out of 12 samples with high parasite density subjected to molecular analysis, only 5 (41.7%) were positive for *Plasmodium falciparum*. The study revealed the prevalence of 65.0% of *P falciparum* infection among pregnant women attending selected hospitals in the studied area. Adequate utilization of insecticide treated net is essential to prevent exposure to mosquito bites leading to reduction in maternal mortality.

Keywords:, Malaria, Microscopy, Molecular, *Plasmodium falciparum*, Pregnant women.

INTRODUCTION

The infectious disease malaria is spread by mosquitoes and affects both people and other animals. It is brought on by single-celled microorganisms belonging to the Plasmodium genus (O'Kane, 2015 and Dahalan *et al.*, 2019). It is spread exclusively through bites of infected female *Anopheles* mosquitoes. Upon inoculation into susceptible host, parasite travel to the liver, mature and reproduce. Five species of *Plasmodium* cause human malaria, namely; *Plasmodium vivax*, *P. ovale*, *P. falciparum*, *P. malariae* and *P. knowlesi* (Shigeharu, 2021). Most deaths are caused by *P. falciparum*, whereas *P. vivax*, *P. ovale*, and *P. malariae* generally cause a milder form of malaria (Caraballo and King, 2014).

An estimated 409,000 people died from malaria in 2019, with 229 million cases reported globally. Sub-Saharan Africa accounted for almost 94% of the cases and fatalities.A decrease in malaria morbidity was observed in 2010 to 2014 but gradually increased from 2015 to 2019 (WHO, 2020). An estimated 241 million clinical episodes and 627,000 fatalities were attributed to malaria in 2020. According to WHO estimates, the WHO African Region accounted for 95% of all deaths in 2020. In locations where malaria is endemic, it is a leading cause of maternal morbidity and mortality. There are an estimated 125 million pregnant women who are at risk of developing malaria, which is a major cause of maternal morbidity and mortality in endemic areas (Bauserman *et al.*, 2019).

Prompt malaria diagnosis was recommended by WHO to distinguish between malarial and non-malarial fevers (WHO, 2022). Diagnostic methods currently in use include microscopy, rapid diagnostic tests (RDT), and polymerase chain reaction (PCR). PCR is used widely to confirm reports obtained from microscopy since the specificity and sensitivity for PCR are the highest(Samina *et al*., 2017). The full capacity of RDTs and PCR techniques are not being utilized due to cost and other limitations that involves shortage of trained personnel, lack of access to equipment, and poor power supply. Despite all the limitations,PCR has been recommended to be a standard confirmatory and research technique due to the advantage of higher sensitivity and specificity compared to RDTs and microscopy (Mbanefo and Kumar, 2020).

Some of the antimalarial drugs used for the treatment of malaria are Quinine, Chloroquine andArtemisinin and its derivatives which includes artemether, artesunate and arteether. However, cases of antimalarial drugs resistance have been reported. Several other newer antimalarial drugs with different modes of action which are at different stages of development to curb the rising menace of antimalarial drugs resistance are also being reported (Tse *et al*., 2019).

Malaria can be easily prevented through the government's approved intermittent preventive treatment of malaria in pregnancy (IPTp) and controlled by keeping the environment safe, effectively utilizing the long lasting Insecticide-treated bed Nets in addition to chemotherapeutic intervention through early treatment of all infected persons (Fikrie *et al*., 2020).

Pregnancy is a state of having implanted products of conception located in the uterus resulting to changes in the mother's body to sustain the growing foetus which ends through either spontaneous or elective abortion or delivery (Pascual and Langaker, 2023).

Pregnant women are reported to be vulnerable to malaria infections and malaria infections expose the foetuses to greater risk. (Bauserman *et al*., 2019).

The study was aimed at evaluating the prevalence and molecular detection of *P. falciparum* among pregnant women attending some selected hospitals in Kaduna North Local Government Area, Kaduna State.

MATERIALS AND METHODS

The study was carried out in Kaduna State's Kaduna Metropolis's Kaduna North Local Government Area. The northern section of Nigeria's high plains is home to the city of Kaduna. With its sporadic short trees, shrubs, and grasses, the vegetation cover is primarily of the Guinea Savannah type. By total land area, the state came in at number four, while by population, it came in at number three. A tributary of the River Niger, the Kaduna River traverses the state (Yusuf, 2015).

Study Population

The studied population comprised of pregnant women attending antenatal care in BarauDikko Hospital Kaduna, General Hospital Kawo and Nigerian Defence Academy Hospital Kaduna, which are all within Kaduna North Local Government Area.

Ethical Clearance

EthicalClearancewas obtained from Kaduna State Ministry of Health (MOH/ADM/744/VOL.1/929 before commencement of the work. Approval was also granted by the Nigerian Defence Academy Hospital and BarauDikko General Hospital (BDTH/2022/028/VOL/1) respectively.

Inclusion and Exclusion criteria

Pregnant women were selected based on gestation, age, residents of Kaduna metropolis, those that gave consent, donated blood sample and completed questionnaire.All participants that failed to fulfil the inclusion criteria were excluded from the study.

Sample Size Determination

Sample size was determined using the formula for calculating sample size (Frankline, 2021) as follows:

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

Where:

n=sample size

z=statistics for a level of 95% confidence interval (1.96)

p=prevalence rate of (72% from Abdullahi*et al*, 2020) 0. 72

d=precision (allowable error) = 5% (0.05)

$$n = \frac{1.96^2 * 0.72(1-0.72)}{(0.05)^2}$$

$$= 309.78$$

Based on the above calculation, 309 samples were collected from pregnant women with the inclusion criteria.

Sample Collection

The study was conducted between May to August, 2022. Pregnant women attending Ante-natal Routine Check-up at the hospitals were randomly selected to participate in the study. About 2mL of venous blood was collected from women attending ante-natal sessions from the selected hospitals.

The samples were labelled and transported to the laboratory of the Nigerian Defence Academy Hospital for analysis. A self-administered questionnaire was administered to the

participants to obtain information about their occupation, age, gestation period.

Sample Analysis

Thick and Thin blood smear preparations

A thick smear can be used to identify mixed illnesses and to screen for parasites. A clean slide was centred with a drop of blood, which was then dispersed over a 15 mm area with a spreader. After letting the smear air dry, it was doused with 10% Giemsa stain and left to dry for around ten minutes. To get rid of any leftover stain, the slide was water washed. Using an oil-immersion objective lens at 10x magnification, the entire smear was studied. A thick smear can be used to identify mixed illnesses and to screen for parasites. A clean slide was centred with a drop of blood, which was then dispersed over a 15 mm area with a spreader. After letting the smear air dry, it was doused with 10% Giemsa stain and left to dry for around ten minutes. To get rid of any leftover stain, the slide was water washed. Using an oil-immersion objective lens at 10x magnification, the entire smear was studied. (Cheesbrough, 2005). Thin smear is useful for species identification of parasites already detected on thick smears. A small drop of blood was placed at the right end of a grease-free microscope slide. The blood was spread using the edge of another clean slide which was held at an angle of 30 degrees and placed to the left, until the blood was exhausted making a tail end. It was then allowed to dry. The smear was then fixed in absolute methanol for 2-3mins. It was screened at low magnification (10x or 20x objective lens). The smear was then examined using the 100x oil-immersion objective lens (Cheesbrough, 2005).

Molecular Analysis

DNA extraction

Deoxyribonucleic Acid (DNA) was extracted using DNA Extraction kit (Accu prep Genomic DNA extraction kit, Bioneer Inc, USA, 2018) following the manufacturers protocolsby inserting filter paper that was spotted with positive samples inside GB buffer in a 1.5 ml tube to dissolve, using a heating block, overnight. Then 20 µL of Proteinase K was added and mixed by vortexing. After incubation at 60°C for 10 minutes, it was then spun down briefly to remove drops from the tube. Ethanol (400 µL) was added and mixed by vortexing. It was then centrifuged for 8,000 rpm for 1 min. The tube containing the filtrate was then discarded. The DNA bounded to the filters in the spin columns. The column was placed in a clean 2 ml collection tube. Thereafter, 500µL Buffer AW1 was added without hitting the rim. The cap was closed and centrifuged at 8,000 rpm for 1min.The column was placed in a clean 2 mL collection tube which was then poured into a disposal bottle. Carefully, without hitting the rim, 500 µL Buffer AW2 was added and centrifuged at 8,000 rpm for 1min. It was centrifuged once more at 12,000 rpm for 1 minute to completely remove ethanol. The binding column was further placed in a clean 1.5 mL tube for elusion, 200 µl Elution buffer was added unto the binding column, and allowed to stand for 1 minute until EL was completely absorbed into the glass fibre of binding column tube. Before storing, it was centrifuged at 8,000 rpm for 1 minute to elute.

Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction was carried out in a total 20 µL reaction volume using Kit Start Premix Kit type (Bioneer, 2018). Detection and speciation of *Plasmodium* was carried out with a two-step nested PCR. In the first step, 16µLof water and 2µL of primers (1µL PF1 and 1µL PF2) (Table 1) were added to 2µL of DNA template. In the second step, 17µLof water and 2µL of primers (1µL PF1 and 1µL PF2) were added to 1µL of first step PCR product. The reactions include pre- de-naturation: 5min at 95ºC, denaturation: 40sec at 94ºC, annealing: 40sec at 54ºC, extension: 40sec at 72ºC, final extension: 5min at 72ºC.

Second step amplified DNA products were separated by 2% agarose gel electrophoresis, stained for 15 min with ethidium bromide and visualized by UV light.

Table 1: Primer Sequences of the Amplified Genes

Primers	Sequences of Primers	Expected Amplicon Size (bp)
PF1	AAT GAA GAG CTG TGT ATC	200-400
PF2	GGA ATC TTA TTG CTA ACA C	200-400

(Jill *et. al.*, 1999)

Data Analysis

Data obtained was analysed using Statistical Package for Social Sciences (SPSS version 24). Prevalence between hospitals were determined using Chi-square test at a statistical significance of $p<0.05$.

RESULTS

A total of three hundred and nine (309) pregnant women enrolled across the three hospitals for the study, 103 participants per hospital. Of these, 180 (58.3%) were between the age of 18 and 29 years, 117 (37.9%) were of age 30 to 39 years, while 12 (3.9%) were 40 years or older. The demographic status of the pregnant women sampled indicated that the highest proportion of the women enrolled in the study were self-employed (138/309, 44.7%); 103 (33.3%) were unemployed while 68 (22.0%) were employed. Forty-nine (15.9%) of the women were in their first trimester, 164 (53.1%) in the second trimester, while 96 (31.1%) were in their third trimester of pregnancy. Results are presented in Table 2.

Table 2: Demographic Status of the Pregnant Women Sampled Percentages are given in parenthesis

Hospital sampled	Number Examined	Demographics								
		Trimester			Occupation			Age		
		First	Second	Third	Employed	Self-employed	Unemployed	18 - 29	30 - 39	40 and above
Gen. Hospital, Kawo	103	16 (15.5)	64 (62.1)	23 (22.3)	17 (16.5)	42 (40.8)	44 (42.7)	78 (75.7)	24 (23.3)	1 (1.0)
BarauDikko Hosp.	103	14 (13.6)	69 (67.0)	20 (19.4)	20 (19.4)	55 (53.4)	28 (27.3)	49 (47.6)	51 (49.5)	3 (2.9)
NDA Hospital	103	19 (18.4)	31 (30.1)	53 (51.5)	31 (30.1)	41 (39.8)	31 (30.1)	53 (51.5)	42 (40.8)	8 (7.8)
Total	309	49 (15.9)	164 (53.1)	96 (31.1)	138 (44.7)	103 (33.3)	68 (22.0)	180 (58.3)	117 (37.9)	12 (3.9)

The results of prevalence of *Plasmodium falciparum* malaria among the pregnant women sampled in all the hospitals is presented in Table 3. An overall prevalence of 65.0% of *Plasmodium falciparum* malaria was recorded among the pregnant women as 201 of the 309 pregnant women were positive for the infection via microscopy. The results indicated that there was significant difference ($p < 0.05$) in the occurrence of *Plasmodium falciparum* malaria in pregnant women attending the selected hospitals: the highest cases of infection was recorded among pregnant women visiting General Hospital Kawo with a prevalence of 76.9% as 79 of the 103 women enrolled at the hospital were positive for the parasite; pregnant women visiting NDA hospital recorded a prevalence of 65.0% as 67 of the 103 women enrolled at that hospital were infected with *Plasmodium falciparum*; at BarauDikko Hospital, the prevalence of *Plasmodium falciparum* malaria among the pregnant women was 53.4% as 55 of the 103 pregnant women that were enrolled for the study were positive for *Plasmodium falciparum* malaria.

Table 3: Prevalence of *Plasmodium falciparum* Malaria among Pregnant Women Attending Selected Hospitals in Kaduna North

Hospital Sampled	Number Examined	Number Infected	Prevalence (%)
General Hospital,Kawo	103	79	76.9
Barau Dikko Hospital	103	55	53.4
NDA Hospital	103	67	65.0
Total	309	201	65.0

x^2 = 12.299; *p* = 0.002

Prevalence of *Plasmodium falciparum* based on stages of pregnancy (Trimester) shows that the prevalence of *Plasmodium falciparum* malaria among the pregnant women was highest among women in their second trimester (68.9%), followed by those in their third trimester(65.6%), with the least recorded among those in their first trimester (51.0%). The results however indicated that the difference in prevalence of *Plasmodium falciparum* malaria among women at different trimesters of pregnancies was not statistically significant ($p>$ 0.05). The result is presented in Table 4.

Table 4: Prevalence of *Plasmodium falciparum* Malaria among Pregnant Women of Different Stages of Pregnancy (Trimesters)

Trimester	Number Examined	Number Infected	Prevalence (%)
First	49	25	51.0
Second	164	113	68.9
Third	96	63	65.6
Total	309	201	65.0

x^2 = 5.327; *p* = 0.070

Prevalence of *Plasmodium falciparum* based on age shows that among the 180 pregnant women between ages 18 and 29 years, the prevalence of *P. falciparum* malaria was 66.7%, being the highest, while in the 117 women between ages 30 and 39 years, the prevalence was 65.0 percent. A prevalence of 41.7% was recorded among the pregnant women that were 40 years and older, as 5 of the 12 pregnant in this age category presented *P. falciparum* malaria. The difference in prevalence of *P. falciparum* malaria among the pregnant women based on their ages was, however, not statistically significant ($p>$ 0.05) (Table 5).

Table 5: Prevalence of *Plasmodium falciparum* Malaria among Pregnant Women of Different Age Groups

Age groups (years.)	Number Examined	Number Infected	Prevalence (%)
18-29	180	120	66.7
30-39	117	76	65.0
40-49	12	5	41.7
Total	309	201	65.0

x^2 =3.093; *p* = 0.213

Prevalence of *Plasmodium falciparum* based on occupation shows that the prevalence of *Plasmodium falciparum* malaria among the pregnant women did not differ significantly ($p>$ 0.05) on the bases of the occupation of the women. Among the 68 pregnant women that were employed, the prevalence was 66.2 percent as 45 of such presented with the infection. Of the 138 pregnant women that were identified as self-employed, ninety (65.2%) presented with the infection; among the 130 unemployed pregnant women, 64.1 percent were infected with of *Plasmodium falciparum* (Table 6).

Table 6: Prevalence of *Plasmodium falciparum* Malaria among Pregnant Women of Different Occupation

Occupation	Number Examined	Number Infected	Prevalence (%)
Employed	68	45	65.2
Self-employed	138	90	66.2
Unemployed	103	66	64.1
Total	309	201	65.0

$x^2 = 0.082$; $p = 0.960$

The results of the molecular detection of *Plasmodium falciparum*shows that out of 12 samples having high parasite density of >3000/µL during microscopy, only 5 (41.7%) were positive for *P. falciparum* DNA.*Plasmodium falciparum* was detected at 400bp band sizes (Plate I).

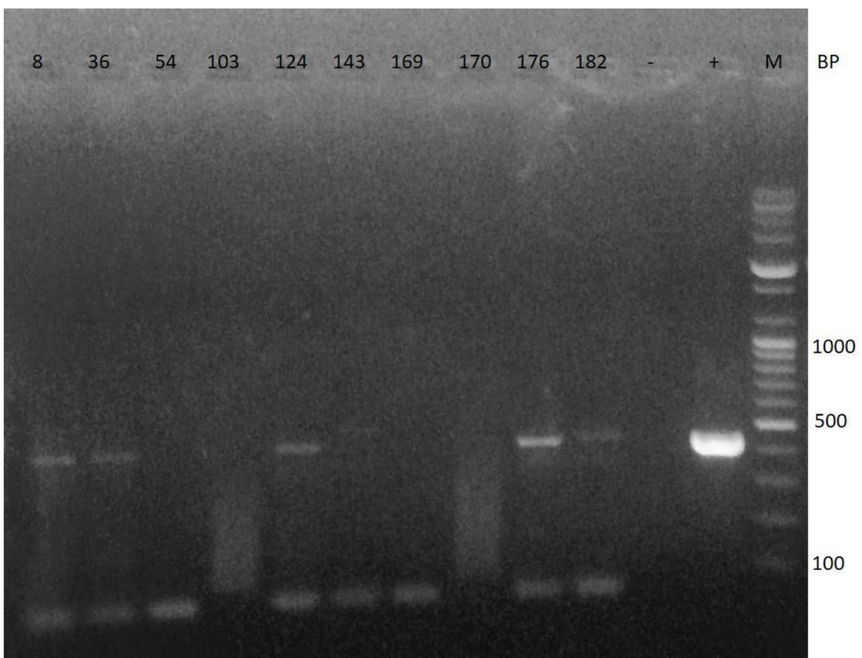


Plate I: Electrophorogram of Amplified *Plasmodium falciparum* DNA. Lane M; Molecular Ladder (10200bp), Lane 1; Positive Control, Lane 2; Negative Control, Sample 182; Positive Sample, Sample 176; Positive sample, Sample 170; Negative Sample, Sample 169; Negative Sample, Sample 143; Negative Sample, Sample 124; Positive Sample, Sample 103; Negative Sample, Sample 54; Negative Sample, Sample 36; Positive Sample, Sample 8; Positive Sample.

DISCUSSION

The high prevalence of *P. falciparum* (65.0%) recorded among pregnant women in this study could be due to poor adoption of malaria prevention and control strategies, or the endemicity of malaria. This high prevalence closely agreed with those of Iwuchukwu *et al.* (2021) who reported a prevalence of 65.5% in their study of the prevalence of malaria and its faetal outcomes among women attending antenatal care at the Federal Medical Centre Owerri. Similarly, Frank *et al.* (2016) reported that 66.7% of all pregnant women attending health centers in Ideato South Local Government Area of Imo State were positive for *Plasmodium*. The significantly high prevalence (76.9%) of *Plasmodium falciparum P. falciparum* malaria recorded at General Hospital Kawo might be due to infrequent use of insecticide treated nets and adoption of poor environmental sanitation practices. The present result contradicted the findings of Ebenezer *et al.* (2016) who noted that differences in prevalence by location were not significant in their study.

In this study, the relatively higher prevalence of *P. falciparum* malaria recorded among women in the second trimester might be connected to the period of presentation at the facility as some women delay registration at the antennal clinic until almost or in the second trimester due to financial, personal, traditional or religious reason (Diorgu*et al.*, 2021).

This was in conformity with the work of Aliyu *et al*. (2017) who had noted higher prevalence of *P. falciparum* malaria among pregnant women in their second trimesters than in other trimesters of pregnancy.

The higher prevalence in *P. falciparum* malaria amongst young aged pregnant women might be due to the absence of specific immunity to placental malaria which is usually acquired after exposure to malaria parasites during pregnancy. Among younger women, this immunity is lacking as it might be the first pregnancy for most of the women in that age category; this makes them more susceptible to malaria infection than older women who are most likely multigravidae (Abe and Olusi, 2014 and Jean-Claude *et al*., 2018). The findings of this work was in consonance with the reports of Jean-Claude *et al*. (2018) who recorded higher prevalence of *P. falciparum* malaria among younger aged pregnant women. However, it is contrary to the previous finding of Muhammad and Muhammad (2022) who reported higher prevalence of *P. falciparum* malaria among older pregnant women.

The relatively higher prevalence (65.2%) of *P. falciparum* recorded among self-employed participants in this study might be attributed to the fact that self-employed individuals usually stay outdoors at late hours of the night and exposure conducting business and consequently gets bitten repeatedly by mosquitoes. This is in accordance with the work of Aliyu *et al*. 2017 who recorded higher prevalence of *P. falciparum* in pregnant women that engaged in trading.

The 41.7% positive *P. falciparum* samples that were confirmed using molecular analysis might be because molecular analysis is more sensitive than microscopy (Kiyonga *et al*., 2020), and might be from degradation of extracted DNA due to poor storage conditions and the activity of DNase that digests DNA molecules. This observation contradicts those of Mbuyi *et al*. (2014) who reported that *P. falciparum* DNA was amplified in all the samples shown to be infected by microscopy examination. According to Uba *et al*. (2021), reliability of microscopy suffers from technicalities of the microscopy including the staining quality of the blood film, inability to differentiate between the parasitized cells and artifacts. The *Plasmodium falciparum* DNA detected at 400bp is similar to that of Anyirékun *et al*., (2018) in which some alleles of *P. falciparum* were also detected at 400bp.

CONCLUSION

It can be concluded from this study that *Plasmodium falciparum* is prevalent among pregnant women attending selected hospitals within Kaduna North LGA with 65% of the women infected. The positivity of *P. falciparum* malaria varied with sampling location, age of women, trimester of pregnancy, and occupation; location-based prevalence showed significant ($p < 0.05$) association: the highest prevalence of the *P. falciparum* malaria was observed among pregnant women attending General Hospital, Kawo (76.9%), women in their second trimester (68.9%), younger age (66.7%), and self-employed (66.2%). Molecular confirmation revealed five samples positive for *P. falciparum* at 400 base pair each.

Author's Contribution

Fatimah Sanusi BabanTakko conceptualized and designed the study, participated in fieldwork and data collection, performed the data analysis and interpreted the data. All authors participated in the preparation of the manuscript, reviewed it and contributed to the development of the final manuscript and approved its submission.

Declaration of Competing Interest

Authors have declared no competing interests exist.

Acknowledgements

Glory is to Almighty Allah who in His Graciousness spares our life and gave us the health and capacity to undergo this study.

We are grateful to the authority of the Nigerian Army University Biu for sponsoring the research and the managements of all the hospitals involved in the study.

REFERENCES

Abe A. F. and Olusi T. A. (2014). Seroprevalence of Malaria Parasite Infection among Pregnant Women Attending Two Tertiary Health Facilities in Akure Ondo State Nigeria. *Journal of Bacteriology and Parasitology*; 5. **[Crossref]**

Abdullahi M. S., Abdulazeez M. A., Mudassir L. and Aminu A. (2020). Prevalence of Malaria amongst Pregnant Women Attending Comprehensive Health Centre Dutsin-Ma Local Government Area, Katsina State, Nigeria. *International Journal of Research and Reports in Hematology*; 3(2): 53-59.

Aliyu M. M., Idris A. N., Umar Y. A., Vanstawa P. A., Jessy T. M., Anthony U. E. and Dele O. A. (2017). Prevalence, risk factors, and antimalarial resistance patterns of falciparum plasmodiasis among pregnant women in Kaduna metropolis, Nigeria. *TZU CHI Medical Journal*; 29(2):98-103. **[Crossref]**

Anyirékun F. S., Thomas B., Issaka Z., Serge Y. R., Frédéric N., Cathérine N., Liz K. T. and Jean-Bosco O. (2018). *Plasmodium falciparum msp*1 and *msp*2 genetic diversity and allele frequencies in parasites isolated from symptomatic malaria patients in Bobo- Dioulasso, Burkina Faso. *Parasites and Vectors*; 11:323. **[Crossref]**

Bauserman, M., Conroy, A. L., North, K., Patterson, J., Bose, C., & Meshnick, S. (2019). An overview of malaria in pregnancy. *Seminars in Perinatology* 43(5), 282-290. **[Crossref]**

Caraballo H. and King K. (2014). "Emergency department management of mosquito-borne illness: malaria, dengue, and West Nile virus". *Emergency Medicine Practice*. 16 (5): 1–23.

Cheesbrough M. (2005). *District Laboratory Practices in Tropical Countries*.Part 1. Pp. 239-258. **[Crossref]**

Dahalan F. A., Churcher T. S., Windbichler N. and Lawniczak M. K. (2019). "The male mosquito contribution towards malaria transmission: Mating influences the Anopheles female midguttranscriptome and increases female susceptibility to human malaria parasites". *PLoS Pathogens*. 15 (11). **[Crossref]**

Diorgu F. C., Iwu R. C. and Iwuanyanwu P. K. C. (2021).Prevalence of Malaria Infection across Trimesters of Pregnancy and Number of Births in Pregnant Women in South-South Nigeria. *Nursing Primary Care*; 5(1): 1-4. **[Crossref]**

Ebenezer A., Stephen D. P. and Enaregha E. B. (2016).Patterns of *Plasmodium falciparum* Malaria among Pregnant Women Attending Antenatal Clinics in the Communities along the Epie Creek, Bayelsa State.*Nigeria. Annals of Biological Research*; 7 (6):1-5.

Fikrie, A., Mihiret K. and Bekele H. (2020). Malaria prevention practices and associated factors among households of Hawassa City Administration, Southern Ethiopia. *PLos ONE. 10.1371*. **[Crossref]**

Frank M. D., Robinson-Bassey G. C. and Akaeze G. O. (2016).Prevalence of malaria parasitaemia among pregnant women attending three selected health centers in Ideato South Local Government Area, Imo State.*Obstetrics and Gynecology International Journal*; 4(3):103–106. **[Crossref]**

Frankline K. (2021). How to determine sample size for a research study.Retrieved 13th Feb, 2022.www.geopoll.com

Iwuchukwu I. C. and Vincent C. N. (2021). Studies on prevalence of malaria and its adverse fetal outcomes in Federal Medical Centre (FMC), Owerri, IMO State, Nigeria. *Arch Community Medical Public Health* 7(2): 151-163. . **[Crossref]**

Jean-Claude M. K., Bienfait M. M., Simon I. K. and Jean-Baptiste K. S. Z. (2018). Epidemiological Aspects of Malaria in Pregnant Women: Prevalence and Risk Factors in MweneDitu, DR Congo. *Open Access Library Journal*, 5: e4676. **[Crossref]**

Jill M. T., Szu H. L.,Theresa M. C. T., Robert C. Y. T.and Ursula A. K. K. (1999). Detection and Species Determination of Malaria Parasites by PCR: Comparison with Microscopy and with ParaSight-F and ICT Malaria Pf Tests in a Clinical Environment. *Journal of Clinical Microbiology*. 37(5): 1269-1273. **[Crossref]**

Kiyonga A. K., Lengu T. B., Nsibu C. N., Umesumbu S. E., Ngoyi D. M. and Chen T. (2020). Molecular detection and species identification of *Plasmodium sp*. infection in adults in the Democratic Republic of Congo: A population-based study. *PLoS ONE* 15(11). **[Crossref]**

Mbanefo, A. and Kumar N.(2020). Evaluation of Malaria Diagnostic Methods as a Key for Successful Control and Elimination Programs. *Tropical Medicine and Infectious Disease* 5(2):102. **[Crossref]**

Mbuyi M. L. T., Bouyou-Akotet M. K. and Mawili-Mboumba D. P. (2014). Molecular detection of *Plasmodium*

falciparum infection in matched peripheral and placental blood samples from delivering women in Libreville, Gabon. *Malaria Research and Treatment*; Article ID 486042, 6 pages. . [Crossref]

Muhammad K. S. and Muhammad I. (2022). Malaria Prevalence Among Pregnant Women Attending Kwadon Primary Health Care, YamaltuDeba Local Government Area, Gombe State, Nigeria. *International Journal of Advanced Biological and Biomedical Research*; 10(2): 139-148.

O'Kane S. (2015). "Scientists found a way to turn deadly female mosquitoes into harmless males". *The Verge*.

Pascual, Z. N. and Langaker M. D. Physiology, Pregnancy. 2023 May 16. In: StatPearls[Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. PMID: 32644730.

Samina, N. M., Madiha S., Gul Su., Kashif Shaikh., Arshi N. and Tahir S. S. (2017). Laboratory Diagnosis of Malaria: Comparison of Manual and Automated Diagnostic Tests. *Canadian Journal of Infectious Diseases and Medical Microbiology* 7 pages. 9286392. [Crossref]

Shigeharu S. (2021). Plasmodium- a brief introduction to the parasites causing human malaria and their basic biology. *Journal of Physiological Arthropology*; 40:1. [Crossref]

Tse, E. G., Marat K. and Matthew H. T. (2019). The past, present and future of anti-malarial medicines. *Malaria Journal*93. . [Crossref]

Uba C. C., Ikegbunam M. N., Anagor I. S., Eleanya L. C., Ezeumeh E. N., Ezekwueche S. N., Okechukwu E. C. and Esimone C. O. (2021). Comparative performance of difference malaria diagnostic tools among pegnant cohorts in Onitsha South-East Nigeria.*Advances in Infectious Diseases*; 11(4): 384-394. [Crossref]

WHO (2020) World Malaria Report 2020. Switzerland: World Health Organization. ISBN 978-92-4-001579-1.

WHO (2022). World Malaria Report 2022. Tracking Progress and gaps in the global response to malaria.

Yusuf S. (2015). Kaduna: Physical and Human Environment.