



Prevalence of *Plasmodium* Species Infection Among Pregnant Women in Igabi Local Government Area, Kaduna State

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ABSTRACT

Malaria is a disease caused by *Plasmodium* species that are haemo-protozoans, single-celled, transmitted by mosquito vector. The aim of this study was to determine the prevalence of *Plasmodium* species infection among pregnant women in Igabi Local Government Area, Kaduna State. A cross-sectional study designed was conducted among four Primary Health Care Centers (PHCC); Miyetti Allah, Taro-Taro, Dan-Mani and Mando in Igabi Local Government Area of Kaduna State. Blood samples were collected intravenously from 210 pregnant women. The samples were examined for *Plasmodium* species using Rapid Diagnostic Test (RDT), Microscopy and confirmed using Polymerase chain reaction test. Data was analyzed using IBM Statistical Package for Social Sciences (version 26) and Chi-square was used to determine the association at a P-value > 0.05. Result showed that, *Plasmodium falciparum* caused the infection among the pregnant women in the area. The overall prevalence was 66/210 (31.4%). The highest prevalence was recorded in Taro-Taro PHCC (40.0%). It was found that fever (51.5%) was the most frequent chief complaint and the least was body-ache (11.8%) out of the four encountered. It can be concluded that *Plasmodium falciparum* is responsible for infection in pregnant women characterized by fever, headache, chills and body-ache.

Keywords: Prevalence, *Plasmodium*, Infection, Pregnant, Women, Igabi, Kaduna State.

INTRODUCTION

Plasmodium species are haemoparasitic, single-celled microorganisms belonging to the genus *Plasmodium*. The genus causes malaria disease in humans and animals and is transmitted by the vector, mosquito (WHO, 2014). The disease, malaria is ubiquitous in accordance with the widespread of the causative parasite and mosquito vector in tropical regions of the world (NMIS, 2010). The species known to infect and spread among human population are: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale* and most recently implicated *Plasmodium knowlesi*. *Plasmodium falciparum* and *Plasmodium*

vivax account for the majority of malaria cases, globally. *Plasmodium falciparum* is responsible for more deaths while *Plasmodium vivax* is the most widespread of all *Plasmodium* species, causing severe, even fatal infections that results in significant global morbidity and mortality (Lacey and Walter, 2022). As reported by WHO (2020), malaria is mostly widespread in areas where there is destitution and the systems for identifying, documenting, and reporting the disease are inadequate

In Nigeria, malaria in pregnancy is a major public health challenge and a priority for the Roll back malaria partnership, because it is a health risk for the mother, her foetus and the

neonate (Igbenghu and Odaibo, 2013). Most cases of malaria in pregnancy in endemic areas are asymptomatic. Pregnant women and their unborn children are particularly vulnerable to malaria, which is a major cause of prenatal mortality, low birth weight and maternal anaemia. Pregnancy, also appears to interfere with the immune processes in malaria, a disease which itself alters immune reactivity (Saidu *et al.*, 2015). The severity of complications and prevalence of *Plasmodium* infection during pregnancy depend on parasite species, level of parasitemia, age of host and level of antibodies of placental-sequestered *Plasmodium* parasites (Akhtar *et al.*, 2012). In late pregnancy, it is a devastating and fulminant disease that is very difficult to manage with a high mortality for both mother and foetus. It requires teamwork between physicians and Obstetricians to manage (WHO, 2018). Clinical diagnosis is necessary and very vital prior to laboratory diagnosis. The main clinical features of *Plasmodium* infection is fever, which is known as malaria fever and is characterized by rise in body temperature, headache, nausea, vomiting, abdominal pain, diarrhea, chills, dizziness, anorexia, sweating, weakness, arthralgias, myalgias, back and joint pains, abortion and even death (WHO, 2014; Joana *et al.*, 2013).

This study aimed at determining the prevalence of *Plasmodium* species infection in association with chief complaints among pregnant women in Igabi Local Government Area, Kaduna State.

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

Where n = Sample size

P = expected prevalence = 84% from WHO (2017)

Z = standard normal distribution at 95% confidence interval = 1.96.

MATERIALS AND METHODS

Study Area

Igabi Local Government Area (LGA) is one of the four LGAs that constitute Kaduna Metropolitan city out of a total of 23 LGAs in Kaduna State, Nigeria. Its geographical coordinates are; latitude 10° 47' 0" North, longitude 7° 46' 0" East and is 608 meters above sea level as reported by Dikwa *et al.* (2021). The LGA occupies an area of 3,222 Kilometres square (Igabi, 2020).

Sampling Locations

Blood samples of pregnant women attending antenatal clinics at four (4) Primary Health Care Centres (PHCCs) chosen randomly, were collected. The PHCCs were: Miyetti Allah Centre in Makarfi road Rigasa, Mando Centre, Taro-Taro Centre in Rigasa and Hayin Dan-Mani Centre all situated in Igabi Local Government Area, Kaduna State.

Ethical Approval and Informed Consent

Ethical approval; MOH/ADM/744/VOL.1/111024 for this work was obtained from the Health Research Ethics Committee (HREC), Ministry of Health, Kaduna State. The approval was accepted by Igabi Local Government Health Authority (ILGHA). Informed consent was obtained from the participants prior to their inclusion in the study.

Sample Size Determination

The sample size was determined using Naing *et al.* (2022) method for calculating sample size for prevalence studies.

$D = \text{Precision (allowable error)} = 5\% (0.05)$

$n = \frac{(1.96)^2 \times 0.84 \times (1 - 0.84)}{(0.05)^2}$

$= 206.52 \approx 210$

$n = 210 \text{ samples.}$

Samples Collection

Two hundred and ten (210) blood samples were collected from pregnant women during antenatal clinics as follows: Miyetti Allah PHCC (55), Taro-Taro PHCC (50), Dan-Mani PHCC (53), and Mando PHCC (52) using standard method (Cheesebrough, 2010). The collection was done under the assistance of medical personnel's in the health centres from January to April, 2024 (4 months).

Five (5) mL syringe with 21G needle was used to aseptically withdraw 5mL of blood into an Ethylene Diamine Tetra-acetic Acid (EDTA) bottle for examination and identification of *Plasmodium species* present (Igiri *et al.*, 2018).

Clerking of Pregnant Women

During “clerking” of the patients (questioning on feelings) by the attending doctor, the main complains presented by the patients were recorded in the patients' hospital card/ folder and also quoted on the Laboratory Examination request form, such complains are known as “chief complains” and usually 2-3. Examples: “I am weak”; “I have headache”, “I am cold” etc. The complains were collected from the Laboratory Exam form, organised and analysed based on pregnant patients' infection status and by location of the PHCCs (Almaw *et al.*, 2024).

Samples Analysis

Rapid Diagnostic Tests (RDTs)

The RDTs was carried out in accordance with the manufacturer's instructions using Rapid

Malaria Antigen Detection Test; Detection of Pf (HRP-II) Antigen (AdvDx™, ADVY chemical PVT Ltd, India).

Microscopic Examination of Blood

The preparation, staining and examination of Thin and Thick Blood Films were done using a drop of sampled anticoagulated blood, stained with Giemsa reagent, and examined for *Plasmodium specie* microscopically at x40 and x100 (oil immersion) objectives for Thin and Thick Blood Films respectively as described by (Cheesbrough 2010).

Molecular Analysis

Blood samples of the pregnant women from the four Primary Health Care Centres (Miyetti Allah, Taro-Taro, Dan-Mani and Mando) that were positive for *Plasmodium falciparum* infection from RDT and microscopy tests were pooled and subjected to molecular test.

Deoxyribonucleic Acid Extraction (DNA)

Plasmodium falciparum genomic Deoxyribonucleic Acid (DNA) was extracted from pooled positive blood samples of the pregnant women using Serum Viral DNA/RNA Extraction kit (XIAORUI®, Biotechnology Co. Ltd, India) according to manufacturer's instructions.

Polymerase Chain Reaction (PCR)

The PCR was conducted as described by Abdel Hamid *et al.*, (2013). The protocol used for the PCR was based on genus-specific primer for *Plasmodium falciparum* (PF1 and PF2). Specie-specific primers for *P. falciparum* was used for the PCR assay.

Briefly, 25µL of the mixture in the tube consisted of 250nm of the primer (PF1 and PF2) and 1uL of genomic DNA. The amplification and detection were performed under the following conditions; Pre-denaturation: 5 minutes at 94°C, Denaturation: 30 seconds at 94°C, Annealing: 30 seconds at 58°C, Extension: 40 seconds at 72°C, Final extension: 10 minutes at 72°C for 35 cycles using GeneAMP® PCR system 9700 (Applied Biosystems). All PCR assays included positive control (genomic DNA from *Plasmodium falciparum*) and negative control (ultrapure water).

Agarose Gel Electrophoresis

Agarose gel electrophoresis was conducted and the products were visualized under short UV light. The number and size of resulting DNA bands were analysed using DNA ladder of 1,500bp (Abdel Hamid *et al.*, 2013). 2 gram of agarose powder was weighed and 100mL of TAE buffer (tris acetate) was added in a flat bottom flask. The mixture was placed on an oven and heated gently until the agarose dissolve completely. The mixture was removed from the oven and 6uL of ethidium bromide was added and mix gently. The gel containing ethidium bromide was poured into a gel cast with an inserted comb and allowed to solidify. The comb was removed and gel cast transferred to an electrophoretic tank. 15 uL of the PCR products and 5uL of ladder were poured and the tank was closed and ran for 40 minutes at 400mA. The products were visualized under short UV light.

Data Analysis

The data obtained from the study were entered into Microsoft Excel programme and

was analysed using IBM Statistical Package for Social Sciences (IBM SPSS version 26) and Chi-square test was used to determine the statistically significant association at a P-value > 0.05.

RESULTS

Prevalence of *Plasmodium species* infection among the pregnant women from each of the Primary Health Care Centers and the Overall Prevalence

Findings from Microscopy, Serology using Rapid Diagnostic Test (RDT) kits showed that only *Plasmodium falciparum* was prevalent out of the; *P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax* and *P. knowlesi* targeted in the area. Confirmatory Molecular test (PCR) using pooled positive samples also showed that only *P. falciparum* was detected.

The prevalence of *Plasmodium falciparum* infection among pregnant women from Miyetti Allah Primary Health Care Center (PHCC) was 21.8%. In the case of pregnant women from Dan Mani PHCC was 32.1%, Mando PHCC was 32.7% and from Taro-Taro PHCC showed the highest prevalence of 40.0% out of the pregnant women involved in the study in the four PHCC.

The overall prevalence of *Plasmodium species* infection among the pregnant women from the four Primary Health Care Centers was observed to be 31.4% with *Plasmodium falciparum* as the only specie encountered in the study area. There was no significant association between prevalence of *Plasmodium falciparum* infection among the pregnant women and the different Primary Health Care Centers in the Local Government Area of the State (Table 1).

Table 1: Distribution of pregnant women by their status of *Plasmodium falciparum* infection in the Primary Health Care Centers in Igabi Local Government Area.

Location of Primary Health Care Centers	Negative cases	Positive cases	Df	p-value
	No. Examined (%)	No. Examined (%)		
Miyetti Allah	43 (78.2)	12 (21.8)	3	0.250
Dan Mani	36 (67.9)	17 (32.1)		
Taro-Taro	30 (60.0)	20 (40.0)		
Mando	35 (67.3)	17 (32.7)		
Overall	144 (68.6)	66 (31.4)		

Keys: Values in parenthesis are percentages (%), Df: Degree of freedom

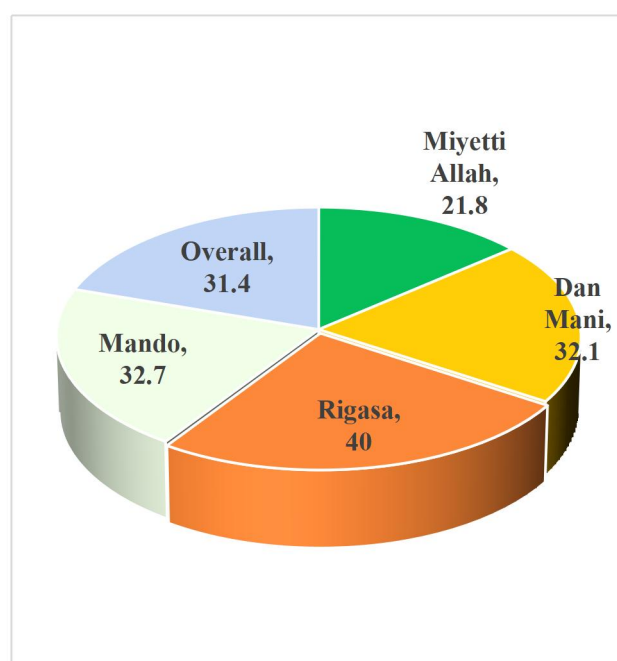


Figure 1: Percentage prevalence of *Plasmodium falciparum* infection among pregnant women attending the selected Primary Health Care Centers in Igabi Local Government Area.

Results of Molecular Confirmation of *Plasmodium falciparum*

A total of 16 pooled positive samples were subjected for confirmation of *Plasmodium falciparum*. Molecular analysis confirmed *Plasmodium falciparum* in all samples at 200 base pair (Plate I).

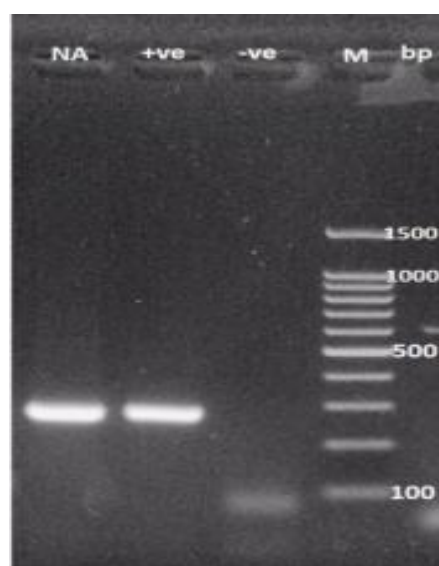


Plate I: Amplified product of *Plasmodium falciparum* gene.

Lane NA: pooled positive samples (200bp), Lane +ve: Positive control, Lane -ve: Negative control, Lane M: Molecular Ladder (1500 bp)

Determination of patients' Chief Complaints

Four major chief complaints were observed among the pregnant women in relation to *Plasmodium falciparum* infection from the four Primary Health Care Centers (PHCC). These are; Body ache, Chills, Fever and Headache.

Table 2 shows the distributions of the chief complains amongst pregnant women with

Plasmodium falciparum infection from the four Primary Health Care units in the Local Government Area. It shows that pregnant women from Miyetti Allah PHCC, Dan Mani PHCC and Taro-Taro PHCC were positive for *Plasmodium falciparum* infection but did not experience body-ache. The only infected pregnant women with such complain, were those from Mando PHCC 2(11.8%) of the chief complaints encountered in Mando representing 2(3.0%) of overall complaints. The most common chief complain among the pregnant women in all locations was fever; pregnant women from Miyetti Allah PHCC 9(75.0%) had fever as chief complain. To Dan Mani and Mando PHCC, fever as chief complain was observed in 8(47.1%) patients in each of the locations and 9(45.0%) of pregnant women from Taro-Taro had fever also. The overall number of pregnant women

with *Plasmodium falciparum* infection who complained of fever were 34 (51.5%) of the total infected with *Plasmodium falciparum*. The next most common complain, was headache with 6(35.3%) among pregnant women in Dan Mani PHCC, 6(30.0%) in Taro-Taro PHCC, 4(23.5%) in Mando PHCC and 1(8.3%) in Miyetti Allah PHCC had headache as chief complain. The overall number of pregnant women with headache were 17 (25.8%) of the total infected with *Plasmodium falciparum*. Pregnant women who complained of Chills from Miyetti Allah PHCC were 2(16.7%). Chills among those who attended Dan Mani, Taro-Taro and Mando PHCC, the figures were 3(17.6%), 5(25.0%) and 3(17.6%) respectively. The overall prevalence of chills in pregnant women was 13(19.7%).

Table 2: Prevalence of chief complains associated with *Plasmodium falciparum* infections in pregnant women by location of Primary Health Care Centers.

Location	Body-ache	Chills	Fever	Headache	Total(n)	Df	p-value
Miyetti Allah	0(0.0)	2(16.7)	9(75.0)	1(8.3)	12	9	0.355
Dan Mani	0(0.0)	3(17.6)	8(47.1)	6(35.3)	17		
Taro-Taro	0(0.0)	5(25.0)	9(45.0)	6(30.0)	20		
Mando	2(11.8)	3(17.6)	8(47.1)	4(23.5)	17		
Overall	2(3.0)	13(19.7)	34(51.5)	17(25.8)	66		

Keys: Values in parenthesis are percentages (%)

df: Degree of freedom

DISCUSSION

Findings from Microscopy, Rapid Diagnostic Test (RDT) kits and Polymerase Chain

reaction (PCR) on pooled positive samples revealed that only *P. falciparum* was prevalent which was the only specie that

causes malaria infection in Igabi Local Government Area. WHO (2012) reported that globally, *P. falciparum* infection is responsible for majority of cases of morbidity and mortality in human especially in African region (Lacey and Walter, 2022). Additionally, *P. falciparum* thrives well in high temperature and humid climate that supports mosquitos' growth and survival as seen in Kaduna State (KDGHS, 2015). These strongly suggest that *Plasmodium falciparum* is the only parasite specie causing malaria among pregnant women in Miyetti Allah, Taro-Taro, Dan-Mani and Mando PHCC of Igabi Local Government Areas of Kaduna State.

The overall prevalence of *Plasmodium falciparum* infection among the pregnant women is 31.4%. The report of this study is higher when compared with the record of Oluwasola *et al.* (2018) and Abigail *et al.* (2021) who reported 14.4% and 23.5% respectively in Ibadan State and Zaria, Kaduna State. It shows that pregnant women in the study area were exposed to heavy malaria parasite burden and may result in health complications such as still birth, spontaneous abortion, maternal anemia, placental parasitaemia and death of the mother (Abigail *et al.* 2021).

However, the present prevalence of *P. falciparum* infection recorded among the pregnant women in the study area is relatively lower when compared with similar studies by Ogbu *et al.* (2015) who recorded 38.8% in North Central Abuja. Foghi *et al.* (2021) reported 62.4% in Delta and Shuaibu *et al.* (2019) reported 60% in Zaria. This finding suggest that the prevalence of the infection have no specific pattern and can vary by location. The *P. falciparum* infection among the pregnant women differed from one PHCC to another with pregnant women from Taro-Taro PHCC having the highest prevalence of

40.0%, The least prevalent was from pregnant women in Miyetti Allah PHCC with 21.8%. The difference in the prevalence rate among the different PHCCs can be due to several factors which include socio-economic factors, educational attainment status of the individuals, poor environmental hygiene and attitude of households towards adequate protection measures which encourages the creation of breeding sites for mosquito propagation.

The overall prevalence of *Plasmodium falciparum* infection among the pregnant women in Igabi LGA is 31.4%, making it a high and endemic condition. *Plasmodium falciparum* infection is highest in Taro-Taro PHCC and least in Miyetti Allah PHCC area signifying that the prevalence value varies from place to place in Igabi LGA due, possibly to variations in climate, human activities and socio-economic status of the people.

This study revealed that, the most common complaints among the *Plasmodium falciparum* infected pregnant women were fever and headache. The finding is consistent with the report of Zekar and Sharman (2023) who reported fever and headache as the most common characteristics of *Plasmodium falciparum* infection. The symptoms may be mild for some people, especially for those who have had the infection before and may even lead to death if left untreated (CDC, 2024).

Incidence of fever among the *Plasmodium falciparum* infected pregnant women was particularly high among pregnant women from Miyetti Allah Primary Health Care center. The prevalence of fever was comparatively equal among pregnant women from Dan Mani, Mando and Taro-Taro. Other complaints included Chills which though was low but cut across the pregnant women in all

the Primary Health Care Centers. But pregnant women from Miyetti Allah, Dan Mani and Taro-Taro Primary Health Care Centers who were infected with *Plasmodium falciparum* infection did not have experience of body-ache. The study revealed that the only pregnant women with such a complain, were those from Mando Primary Health Care center. The study did not reveal significant variability in a test of association between the different Primary Health Care Centers and incidence of the different complains which means that the complains could not be associated with incidence of *Plasmodium falciparum* infection in any particular Primary Health Care center in the Local Government area of the state.

CONCLUSION

It was concluded that *Plasmodium falciparum* was responsible for malaria infections among pregnant women in Miyetti Allah, Taro-Taro, Dan-Mani and Mando PHCCs of Igabi Local Government Areas. The *Plasmodium falciparum* infection is significant and endemic with an overall prevalence of 31.4% among the pregnant women. The prevalence of *Plasmodium falciparum* infection across the study areas in Igabi LGA have no specific pattern by locations; it varies. The most frequent chief complaint due to *Plasmodium falciparum* infection amongst the pregnant women were fever and headache while chills and body ache were less frequent in that order of the four (4) complains generally. Therefore, all pregnant women in the Local Government Area be encouraged through communities and social gatherings to be attending Antenatal clinic towards better treatment of *Plasmodium falciparum* infection so as to prevent morbidity and mortality rate of mothers and fetus in the society.

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