



Research Article

Prevalence of Malaria Parasitaemia in Peripheral and Placental Blood Samples of Pregnant Women in Relation to their Age Group and Gravidity, Katsina State

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ABSTRACT

Malaria during pregnancy is a serious public health problem in sub-Saharan Africa, accounting for an estimated 10,000 maternal deaths and about 200,000 infant mortalities annually. Pregnant women living in areas with stable malaria endemicity often remain asymptomatic, thus making placental malaria difficult to recognize and diagnose. The present study aimed to analyse the prevalence of malaria parasitaemia in both peripheral and placental blood of pregnant women living in the vicinity of Katsina State, in relation to their age groups and gravidity. A cross-sectional study was conducted involving 300 consented pregnant women. The analysis of malaria parasitaemia was carried out using the thick blood smear (peripheral and placental blood samples). The results revealed that the majority of the participant 222 (74.0%), showed positive results for malaria parasite in their placental blood smear, while 180 (60.0 %) of the peripheral blood smears indicated a malaria positive result. Malaria parasitaemia was observed to be more virulent among primigravidae 95.8% (peripheral blood) and 100% (placental blood), followed by secundigravidae 37 (72.5%) and 41 (80.4%), and among participants who are especially within the age group of 14 – 25 and 26 – 35 years. From the results of the present study, the high level of malaria parasitaemia that was observed within the placental blood samples is a result of the sequestered parasites embedded within the intervillous space of the placenta, thus indicating a high level of placental malaria, especially among the young participants and primigravida.

Keywords: Age groups; Gravidity; Peripheral blood; Placental blood; Placental malaria; Prevalence

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INTRODUCTION

Placental malaria is a pregnancy-specific manifestation of malaria characterized by the sequestration of Plasmodium falciparum-infected erythrocytes within the intervillous spaces of the placenta (Clark, 2019). This phenomenon is mediated by parasite adhesion to chondroitin sulfate A on placental syncytiotrophoblasts around the third month of gestation (Chua *et al.* 2021). Placental malaria has been recognized as a complication of malaria in pregnancy in areas of stable transmission and is particularly frequent and more severe in

primigravidae (Takem and D'Allessandro, 2013). Women in zones of high malaria transmission are often asymptomatic (Fried *et al.*, 2012), leading to chronic untreated placental malaria (PM) with insidious consequences to the mother that could include severe anaemia (Matangila *et al.*, 2014), pulmonary oedema, hypoglycaemia, puerperal sepsis (Guin *et al.*, 2012) and death. While consequences of placental malaria in the foetus include abortion, stillbirth, intrauterine growth retardation (IUGR), premature delivery, and low birth weight (LBW) (Menendez *et al.*, 2000; Sharma and Shukla, 2017).

These sequels result in an estimated 10,000 maternal deaths and 200,000 infant mortalities annually in Africa (Omer *et al.*, 2017). According to a report by the World Health Organization (WHO) in sub-Saharan Africa each year, more than 50 million pregnant women are reportedly exposed to the risk of malaria (WHO, 2017c). Nigeria bears the highest malaria burden globally and accounts for a substantial proportion of malaria-related morbidity and mortality in pregnancy, making placental malaria a critical public health concern (WHO, 2023).

The prevalence of placental malaria in Nigeria varies widely depending on geographical location, transmission intensity, gravidity, and diagnostic method employed. In clinical practice, in Nigeria diagnosis of malaria generally relies upon conventional methods which include microscopic examination, which is viewed as the gold standard (Gueneuc *et al.*, 2017), immune-chromatographic lateral flow assay otherwise known as rapid diagnostic test (RDT), (Maltha *et al.*, 2013). However, the use of clinical diagnosis as an indicator for fever has shown to be a sensitive indicator only in children < 5 years but not in adults and pregnant women (Mutanda *et al.*, 2014). The thick microscopic smear only allows for estimating parasite density in maternal peripheral blood but does not reveal the level of infected erythrocytes (IEs) sequestration (Gueneuc *et al.*, 2017). RDTs on the other hand may seem to be a better option as reported by the WHO (2022), but only to nonpregnant individuals (Fried *et al.*, 2012).

Studies using placental blood microscopy have reported prevalence rates ranging from 20% to 40% in parts of Southern Nigeria, where Babalola *et al.* 2015 reported 19.0% in Ogun State, 21.3% in Imo State (Okoro *et al.* 2023), to 40.2% in Cross River (Emechebe *et al.* 2022), whereas majority of investigations from northern Nigeria the likes of Lawal, S. *et al.* 2023 from Katsina metropolis reported a lower prevalence of 40.67%, another study from the same area by Sani, N. *et al.* 2023, reported a prevalence of 44.7%, Okoli, C. G. *et al.* 2017, from Daura reported a prevalence of 44.23%, Abdullahi *et al.* 2020 from Dutsinma reported a prevalence of 72%, only provide insight on the prevalence of malaria in pregnancy using peripheral circulation.

Despite the over whelmed researches and initiatives meant to combat malaria in pregnancy in Katsina state and Nigeria at large, more data needs to be collected on the prevalence of placental sequestration of malaria parasites with in the placenta, so as to ascertain the magnitude of

malignancies that could be caused by the disease to both the mother and especially the baby just born with minimal immunity. The present study was carried out to determine the prevalence of malaria parasites in both the peripheral blood circulation and the placental blood in relation to age variations and parity level.

MATERIALS AND METHODS

Study Design

The study was a cross-sectional study, where 300 consenting pregnant women were involved as study subjects. From each of the study subjects 2ml - 3 ml of peripheral blood and placental blood samples each were aseptically collected. The samples collected from each of the study subjects were designated and labelled as follows, after which thick and thin smear was carried to a certain the level of parasitaemia.

Study Area

The study was conducted in Katsina metropolis of Katsina State. Katsina State is located in the North-Western part of Nigeria, bordering Niger Republic to the North, Zamfara state to the South, Kano state to the East and Jigawa state to the West (El-Ladan *et al.*, 2014). The climate of Katsina state is a tropical continental climate classified by Koppen as AW climate (winter dry Season) (Abaje *et.al* 2014). The dominant vegetation is Sudan savannah, which combines the characteristics and species of both Guinea and Sahel savannah (Tukur and Amadi, 2014). The mean annual temperature ranges from 29°C – 31°C, the highest air temperature normally occurs in April/May and the lowest occurs in December through February. Annual rainfall in Katsina state normally falls between July and October with a range of 500mm – 920mm (Ogungbenro and Morakinyo, 2014). Malaria transmission is endemic in Katsina state with all year-round transmission at levels below the National average, with a seasonal peak (60% of annual malaria cases) coinciding with the raining season (Stranchan *et al.*, 2016).

Study Population

The study populations (subjects) involved were drawn from pregnant women (Primigravidae, Secundgravidae and Multigravidae) with a gestation period of between 39 to 40 weeks. Majority of people in Katsina predominantly speak Hausa and Fulfulde language with Islam as the dominant religion practiced. The populace largely engaged in subsistence farming (James *et al.*, 2018), trading and cattle rearing (Ladan, 2014). For the study all the 300 study subjects that were recruited involved only those attending antenatal care and delivering at

either the maternity units of General Hospital Katsina, Federal Medical Centre Katsina and Turai Yaradua maternal Hospital Katsina.

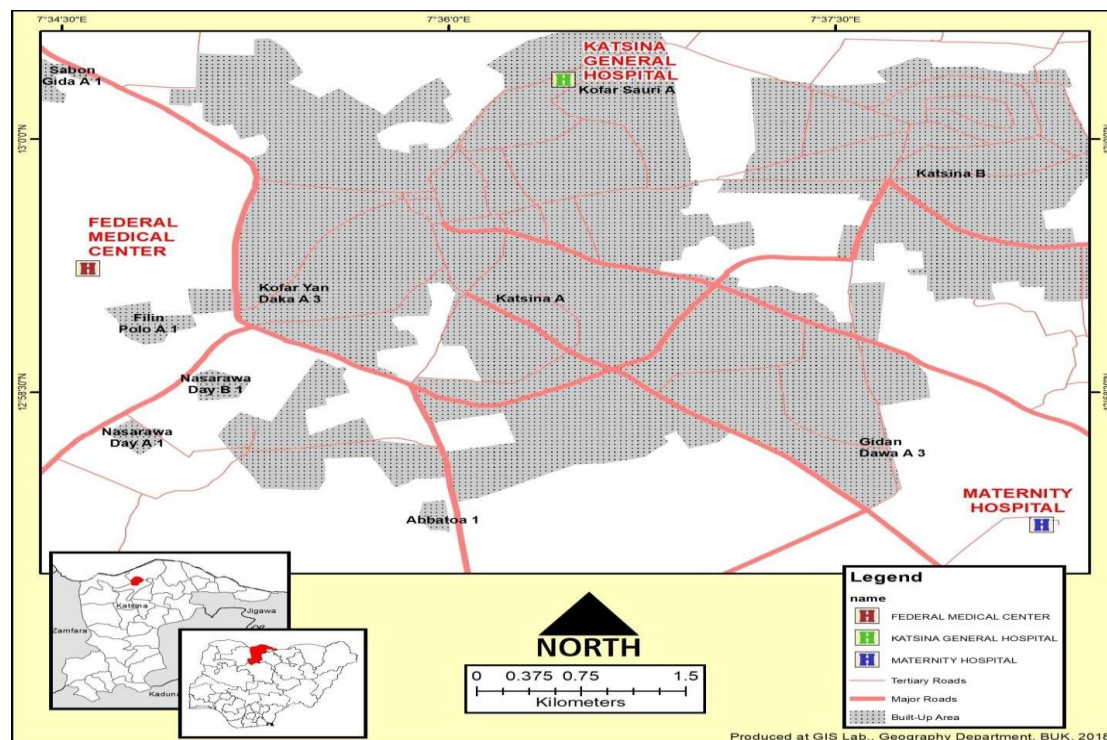


Figure 1: Map of Katsina Metropolis showing the three hospitals

Produced at GIS Lab. Geography Department BUK

Selection of Study Subjects

The study subjects were selected using a purposive sampling method, where pregnant women in their 3rd trimester between 39 to 40 gestational weeks and are willing to participate by delivering at either of the respective hospital units were involved. The selection of the study subjects relies on pregnant women fulfilling the inclusion and exclusion criteria.

Inclusion Criteria

The pregnant women were included irrespective of their age, educational background, and socio-economic status, cultural or religious ideology. Pregnant women in their last trimester and are about to deliver were recruited for the study.

Exclusion Criteria

For the study, only pregnant women with live birth and singletons delivered vaginally were involved in the study, also pregnant women with a history of pre-eclampsia and diabetics were not included among the study subjects. In addition, women that are hypertensive or have had a caesarean section before were also not included.

Determination of Sample Size

The sample size of the study was calculated based on Fisher 's population formula as reported by Charan and Biswas, (2013), using an expected prevalence of 22.4% as reported by Aliyu *et al.*, (2017). The formula used for the calculation of the sample size is; $n = \frac{Z^2 P (1- P)}{d^2}$

Where;

n = Sample size

Z = Confidence level (95%) = Z-value is 1.96

P = expected prevalence or proportion (22.4% = 0.224)

(Aliyu *et al.*, 2017), d = precision (5% = 0 .05)

Informed Consent

Both written and oral informed consent was obtained from the participants prior to collection of samples, in the event where the pregnant women are indisposed any close relative of the participant were allowed to fill in the consent form and the semi structured questionnaire.

Collection of Clinical Samples

Between the months of June - August of 2024, 300 peripheral and 300 placental blood samples were collected simultaneously from each of the study

participant and stored appropriately. Before delivery, either at the inception of the program or during labor, 2 - 3mls of the peripheral blood sample was aseptically collected (using hand gloves and alcohol cotton swabs) by puncture of the antecubital vein of the forearm of each study subject with a sterile syringe by a certified nurse (Adikwu *et al.*, 2017). After the safe delivery of the baby and the expulsion of the placenta, at the maternal side of the placenta using a sterile blade an incision is made where the placental blood is collected using a sterile syringe. Immediately after collection 1ml of each of the blood samples was stored in a screw cap bottle already treated with ethylene diamine tetra-acetic acid (EDTA) and gently mixed to prevent clotting.

Blood staining and Microscopy

The thick blood smear was used to determine the level of malaria parasitaemia in each of the peripheral and placental blood samples stored in EDTA bottles respectively using Giemsa stain in accordance with the guidelines provided by the World Health Organization WHO, (2016). Parasite density was determined by counting using haemocytometer. The number of asexual parasites relative to at least 200 leucocytes in each thick blood film and assuming a mean leucocyte count of 800. Parasitaemia was graded as low (Parasite < 1000), moderate (Parasite > 1000 – 9,999) and severe (Parasite > 10,000) (WHO, 2010).

Data Analysis

Statistical analysis for this research was carried out at 99% level of significance using Microsoft excel and statistical package for Social Sciences (SPSS) software (IBM SPSS Statistics v.23.0). The values obtained after malaria microscopy of both peripheral and placental blood samples collected, descriptive statistics of frequency was used to present the level of malaria parasitaemia, then Paired Sample t-test was used to statistically find out the relationship between level of malaria parasitaemia in all the peripheral blood samples and parasitaemia in all of the placental blood samples obtained.

RESULTS AND DISCUSSIONS

Malaria in endemic regions such as Nigeria is one of important public health issues especially among pregnant women due to the disease asymptomatic nature. From the results of microscopy in this study the study participants were as observed from the results of Table 1 to harbour statistically significantly ($p = 0.000$) higher placental parasitaemia (mean value of 6279.19) than peripheral blood samples (mean value of 3700.95), which coincides with result from

similar studies (Emechebe *et al.* 2022) conducted in cross rivers where the parasitaemia in placental circulation was observed to be higher (70.2 %) as compared to the peripheral parasitaemia (40.2%). The high prevalence of placental parasitaemia observed from the present study as compared with the peripheral parasitaemia could be attributed to the ability of the malaria infected erythrocytes to express unique variant surface antigens (VSA) (Chandrasiri *et al.*, 2016) which are known to mediate placental adhesion.

When clinically observing pregnant women, from the results obtained in Figure 1 and 2, from both the peripheral and placental blood smear, shows that malaria parasitaemia is more severe among primigravidae 89 (95.8%) and 72 (100%), followed by secundgravidae 37 (72.5%) and 41 (80.4%), and that of study participants that are especially with in the age group of 14 – 25 and 26 – 35 years. The high prevalence of malaria parasitaemia obtained among the primigravidae and young pregnant mothers is in tandem with the works of Babalola *et al.* 2015 from Ogun state (Being within the age range of 18-22 years [OR = 4.4, 95% CL = 1.1-17.4, P = 0.046], being primigravid [OR = 2.1, 95% CL = 0.9-5.1, P = 0.028] as significant risk factors for placental malaria). and studies from pregnant women are often reported with high placental parasitaemia but with minimal peripheral parasitaemia (Ezebialu *et al.* 2012; Bassey *et al.* 2015). The results obtained from this study are also in line with the work of Iwuchukwu and Vincent, 2021 from Imo state whose work further explains that complexity of malaria infection showed an increase in young pregnant women (prevalence of 70.2%) and primigravidae (prevalence of 71.3%).

To further stress the variation that exist between the level of malaria parasitaemia in placental and peripheral blood samples, the results of Table 2 and 3 indicate statistically significant ($p = 0.000$) difference in level of placental parasitaemia and peripheral parasitaemia, where placental parasitaemia was observed to be higher among all gravidity level and across all age groups of the study participants. Thus, it is recognized from different studies that peripheral parasitaemia may remain below the levels of microscopic detection although parasites harboured the placenta (Adam *et al.* 2017). This could be attributed to lower levels of acquired immunity among the young women as well as lack of pregnancy-associated and naturally acquired immunity against the placenta-binding parasites in primigravids. Studies have shown that immunity against the placenta binding parasites develops with

subsequent pregnancies as a result of previous exposure to the placenta binding strain.

Table 1. Prevalence of Malaria parasite in both peripheral and placental blood Samples

Blood Samples	Prevalence NO. (%)	Mean	Std. Dev.	Df	R	T	P. Value
Placental	Positive 222 (74.0)	6279.19	3499.00	299	0.107	10.54	0.000
	Negative 78 (26.0)						
Peripheral	Positive 180 (60.0)	3700.95	2786.03				
	Negative 120 (40.0)						

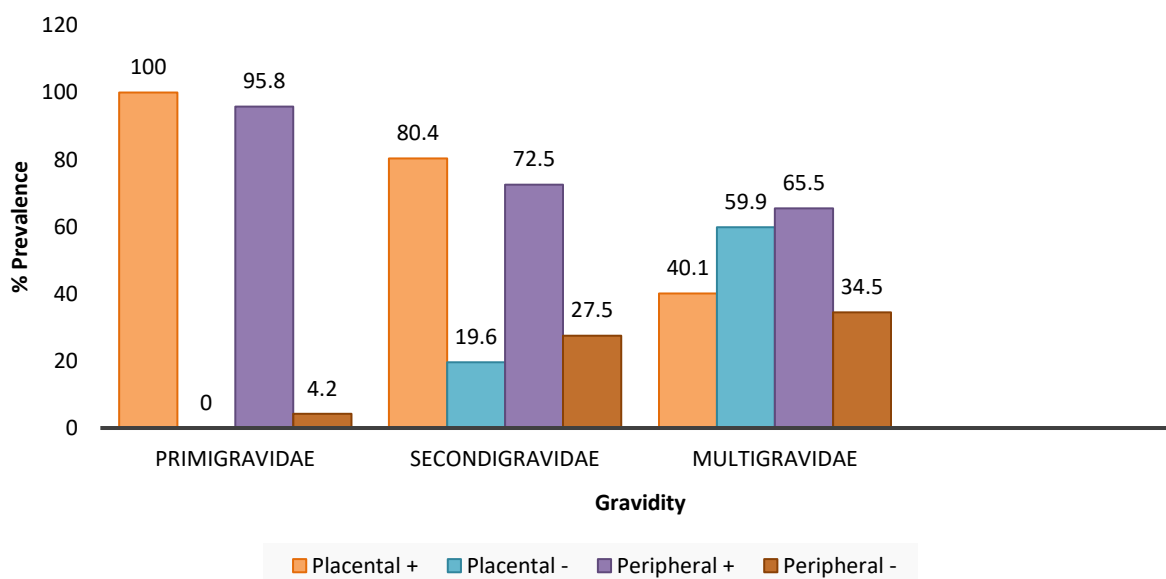


Figure 2. Prevalence of Malaria parasitaemia in Relation to Gravid level among Study participants

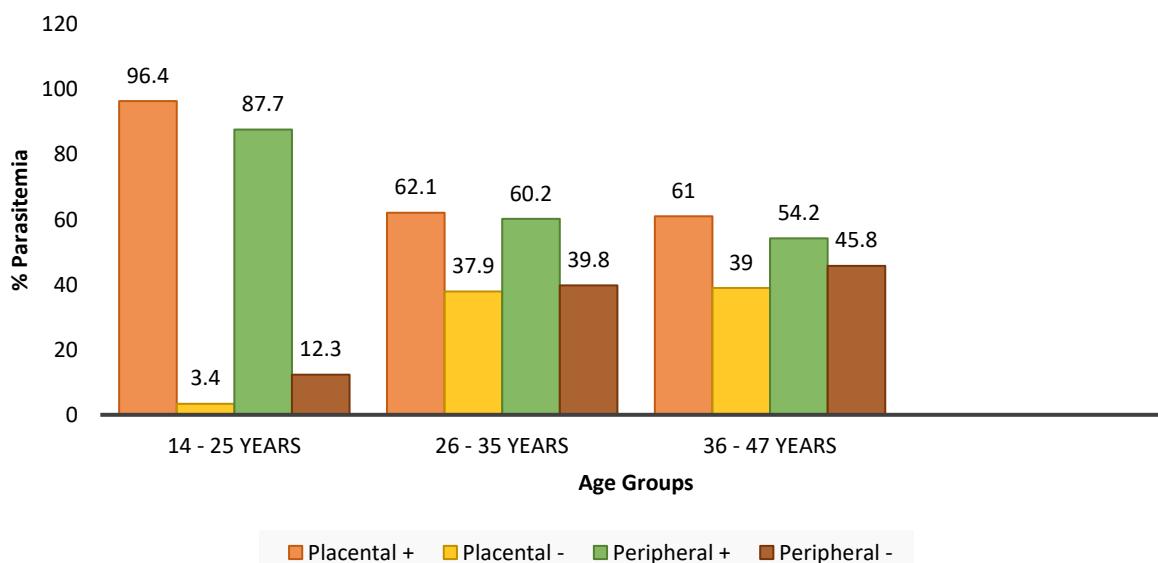


Figure 3. Prevalence of Malaria parasitaemia in pregnancy in Relation to Age group of the study participants

Table 2: Differences of Malaria Parasitaemia present in Peripheral and Placental Blood Samples based on the Age Group of Study participants (N = 300)

Age Group	Blood Sample	Mean	Std. Dev.	Df	R	T	P
14 – 25 Years (n = 138)	Placental	6653.9	3562.3	137	0.074	6.71	0.000
	Peripheral	4314.7	2299.6				
26 – 35 Years (n = 103)	Placental	5856.9	3438.6	102	0.099	6.53	0.000
	Peripheral	2947.6	3293.8				
36 – 47 Years (n = 59)	Placental	6139.8	3416.8	58	0.093	4.83	0.000
	Peripheral	3580.2	2554.2				

Table 3: Differences of Malaria Parasitaemia in Peripheral and Placental Blood Samples of Patients based on the Parity Level of study participants

Parity Level	Blood Sample	Mean	Std. Dev.	Df	R	T	P
Primigravidae (n = 72)	Placental	6937.78	3100.87	71	-0.04	4.45	0.000
	Peripheral	4958.03	1999.38				
Secundgravidae (n = 51)	Placental	6568.95	2940.63	50	0.09	4.48	0.000
	Peripheral	4346.34	2261.25				
Multigravidae (n = 177)	Placental	5927.80	3760.01	176	0.09	8.51	0.000
	Peripheral	3003.63	2977.43				

CONCLUSION

Among the 300 study subjects that were recruited majority 222 (74%) of the participants harbor malarial parasites in their placental circulation. Significant difference (p<0.01) was observed between the level of malarial parasitaemia in the placental and peripheral circulation. The level of malarial parasitaemia was found to be severe among primigravids, where 100% of them had placental parasitaemia whereas 95.8% of them had peripheral parasitaemia.

The high level of placental malaria observed in the presents study reiterate the need to introduce and sustain mass literacy classes among not only pregnant women. This will help in mass enlighten of the different dangers posed by placental malaria and the different malignancies caused by the disease severity, especially among rural dwellers. Other measures to reduce the burden of placental malaria in the study area should include provision of treated mosquito nets to the environs and improvement of environmental sanitation and proper enlightenment regarding advantages of proper personal and environmental hygiene practices.

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