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Research Article

Malaria Parasite Density and Associated Risk Factors in Pregnant Women Attending Antenatal Care in a Tertiary Healthcare Facility in Awka

Okeke, O. A.[1], Nnanwuba, S. P.[1], *Ishar, C. O.[1], Azaka, E. I.[1], Ikeh, M. I.[2], Imakwu, C. A.[3], Obiakor, U. A.[3], Udeh, N. P.[1], Okeke, C. J.[1], and Oraneli, U. B.[4]

[1]Department of Zoology, Nnamdi Azikiwe University, Awka, Nigeria
[2]Public Health and Environmental Research Group
[3]Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka, Nigeria
[4]Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Nigeria

***Corresponding Author's email*: co.ishar@spgs.unizik.edu.ng; Phone: +2347062662642**

ABSTRACT

Malaria remains a leading cause of morbidity and mortality in sub-Saharan Africa, particularly among pregnant women, who are at increased risk due to immunological and physiological changes. This cross-sectional hospital-based study was carried out to investigate malaria parasite density and associated risk factors among pregnant women attending antenatal clinic at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Anambra State, Nigeria. A total of 101 venous blood samples were collected and analysed for malaria parasite using microscopy, while urine samples were analyzed for biochemical markers using combi 9 test strips. Demographic information such as age, parity and gestation were obtained through structured questionnaires. Statistical analysis was done using chi-square tests and logistic regression to identify, associations between malaria parasite density and risk factors. Overall, malaria prevalence was 52.5%, with 11.9% of cases exhibiting high parasite density. Parasite density was significantly associated with gestational age, parity, and biochemical markers such as protein and blood presence in urine. Women in the third trimester and nulliparous women exhibited higher parasite densities. Higher malaria parasite density was also linked to lower haemoglobin levels, indicating an increased risk of malaria-induced anaemia. This study highlights the high burden of malaria among pregnant women and the urgent need for improved antenatal care services, routine malaria screening, and enhanced preventive strategies, including the use of insecticide-treated nets and intermittent preventive treatment in pregnancy.

Keywords: Haematological profile; Malaria; Nigeria; Parasite density; Pregnant women; Risk factors

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INTRODUCTION

Malaria is a major public health challenge, particularly in sub-Saharan Africa, where *Plasmodium falciparum* accounts for the highest morbidity and mortality rates (WHO, 2022). Despite substantial progress in medical sciences, malaria infection remains a serious health issue in world populations. In many endemic regions, malaria infection poses a significant threat to over 125 million pregnancies annually, with more than 25% of this burden coming from sub-Saharan Africa (Accrombessi *et al*., 2018). Acccording to Reddy et al. (2023), estimates showed that 46.1 million pregnancies were at risk of malaria infection in the WHO Africa region and 819,000 low birthweight babies in the sub-Saharan, 52.9 million in the South East Asia region, 6.7 million in the Americas and 11.1 million in the Eastern Mediterranean region. Pregnant women are more susceptible to malaria due to changes in their immune

system and increased attractiveness to mosquitoes (Rogerson *et al*., 2007; Lindsay *et al*., 2000). The consequences of malaria in pregnancy are profound, affecting both the mother and the fetus. These include maternal anemia, placental malaria, low birth weight, preterm delivery, intrauterine growth restriction and increased risk of neonatal and infant mortality (McClure *et al*., 2020) and cognitive developmental delays in infants (Garrison *et al*., 2022; Saito *et al*., 2020). The pathophysiology of malaria in pregnancy is complex and varies depending on the level of malaria transmission in a given area (Brabin and Green, 2018). In areas of high transmission, where individuals are often exposed to the malaria parasite from a young age, women typically acquire partial immunity before their first pregnancy (Rogerson *et al*., 2019). This immunity reduces the severity of malaria, though it does not completely prevent infection. In contrast, in areas of low transmission, women have less acquired immunity, making them more susceptible to severe malaria during pregnancy. Malaria in pregnancy (MiP) is characterized by the sequestration of infected erythrocytes in the placenta, leading to placental malaria. This condition impairs the exchange of nutrients and gases between the mother and fetus, contributing to poor pregnancy outcomes. The degree of placental malaria and the associated parasite density are critical determinants of the severity of maternal and fetal complications (Fried and Duffy, 2020). Several strategies have been recommended to prevent and manage malaria in pregnancy as stated by WHO (2023). These include the use of Insecticide Treated Nets (ITNs), Intermittent Preventive Treatment in Pregnancy (IPTp) with sulfadoxine-pyrimethamine and prompt diagnosis and effective treatment of malaria cases. However, despite these recommendations, the implementation of these strategies remains sub-optimal in many regions, including Nigeria, leading to continued high rates of malaria in pregnancy. Understanding malaria parasite density and its associated risk factors in pregnant women is essential for improving maternal and neonatal health outcomes. Therefore, this study evaluates malaria parasite density and identifies risk factors among pregnant women attending antenatal care at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka, Nigeria.

MATERIALS AND METHODS

Study Area

The study was conducted at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH), a public tertiary healthcare facility in Awka, Anambra State, Nigeria. The town has an estimated population of 301,657 as of the 2006 Nigerian census and over 2.5 million as of 2018 estimate. It is located at longitude 6.2220N and latitude 7.0821E. Awka is malaria-endemic and has a population highly susceptible to the disease (Okpala *et al*., 2022). Chukwuemeka Odumegwu Ojukwu University Teaching Hospital is a public hospital located in Amaku, Awka South Local Government Area of Anambra State. It was established on the 8th of August, 2019 and operates on a 24-hour basis. Chukwuemeka Odumegwu Ojukwu University Teaching Hospital Amaku is licensed by the Nigerian Ministry of Health, with facility code 04/06/1/1/1/0039 and registered as a tertiary health care centre.

Ethical Approval

Ethical clearance to carry out this study was obtained from the Health Research Ethics Committee of Chukwuemeka Odumegwu Ojukwu University Teaching Hospital Amaku, Awka, Anambra State, Nigeria. The ethical approval letter was assigned with reference number COOUTH/CMAC/ETH.C/HREC/Vol.1/361.

Study Design

The design was a hospital-based cross-sectional study which included 101 pregnant women attending antenatal clinic at COOUTH. Participants were selected using systematic random sampling.

Determination of Sample Size

The sample size of 101 patients for this study was determined using the Cochrane formula:

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

Where:

n = required sample size

z = standard normal deviation at 95% confidence level (1.96)

p= prevalence (7.7 %) based on previous studies by Gontie *et al*. (2020)

d= margin of error 5% (0.05)

$$\mathrm{n} = \frac{1.96^2 x\ 0.077(1-0.077)}{0.05^2}$$

$$= \frac{3.8416\ \ x\ 0.077(0.923)}{0.0025}$$

$$= \frac{0.2730}{0.0025}$$

= 109

The calculated sample size was 109, however, only 101 pregnant women completed the study, due to last minute withdrawals.

Inclusion and Exclusion Criteria

Pregnant women of all gestational ages attending antenatal care in the study hospital during the study period and who gave their consent were included in the study. On the other hand, pregnant women with chronic

illnesses that could confound the study's findings, those on IPTp treatment as well as those who did not consent were excluded from the study.

Sample Collection and Laboratory Analysis

A clean sterile syringe was used to collect venous blood samples from the patients and was dispensed into Ethylene Diamine-tetra-acetic Acid (EDTA) tubes. The EDTA blood collection tubes were arranged into a specimen box containing ice packs. Patients were also given clean, sterile, leakproof specimen containers and instructed to collect their urine samples. The urine samples were also arranged in another specimen box and all samples were immediately transported to the laboratory for parasitological examination.

The venous blood samples collected were analyzed for malaria parasites using thick and thin blood smears stained with 10% Giemsa solution according to the method of Okon *et al*. (2022). Parasite density was estimated by counting the number of parasites per 200 white blood cells and multiplying by 8,000 to convert the results to parasites per microliter of blood. In other words, Parasite Density (PD) was determined using the formula; PD =Number of parasites x 8000 / 200 as described by Folarin *et al*. (2021). Hemoglobin levels were measured using a portable hemoglobin meter. Urine samples were tested for biochemical markers such as protein, blood, and ketones using Combi 9 test strips according to manufacturer's instruction.

Data Analysis

Data were analysed using SPSS version 25. Descriptive statistics summarized demographic and clinical characteristics. Chi-square and logistic regression assessed associations between malaria parasite density and risk factors. A p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Prevalence of Malaria Parasite

Out of the 101 pregnant women in the study, 53 (52.5%) were infected with one plus (+) which was attributed as low levels of malaria parasites, while 12 (11.9%) were infected with higher parasite levels which was observed as two plus (++). Malaria parasites were not seen in 36 (35.6%) of the women. The result was statistically significant ($p < 0.001$) which is significantly lower than the alpha level of 0.05 as shown as in table. The 52.5% prevalence recorded in this study is similar to the 55.0% reported in Ibadan, southwest Nigeria by Awosolu *et al*. (2021). However, the result is higher compared to the 27% reported by Ikeh *et al*. (2024) among pregnant women in a university teaching hospital in Nnewi, southeastern Nigeria. The variations observed in the various studies could be attributed to immune status, ignorance of risk behaviours and level of exposure to mosquito bites.

Malaria Parasite Density

The infection levels of malaria were categorized into two groups: low infection (denoted as +) and high infection (denoted as ++). Among the respondents, 53 exhibited low malaria parasite density with a mean density of 7.15 ± 3.21, while 12 displayed high parasite density, with a mean density of 36.67 ± 16.24. The statistical analysis yielded a p-value of 0.00008, indicating a highly significant difference between the two groups. The parasite density ranges documented in this study are much lower than that reported by Awosolu *et al*. (2021) in Ibadan. Others are Oyibo *et al*. (2023) who also documented a higher parasite density among pregnant women in the six geo-political zones in Nigeria. Padilla-Rodriguez *et al*. (2020) also reported a higher geometric mean parasite density of 5,919 parasites/µl in Colombia. Epidemiologically, parasite density in the blood of infected individuals helps in understanding infection endemicity in any community (Hay *et al*., 2008). The study findings underscores the stark contrast in parasite densities, suggesting that those with high infection levels are at an increased risk for complications associated with malaria, thereby highlighting the need for targeted interventions and monitoring for this subgroup of pregnant women.

Table 1: Prevalence of Malaria parasite

Malaria infection level	Frequency	Percentage	X^2 value	P value
+	53	52.5	25.86	<0.001
++	12	11.9		
Not detected	36	35.6		
Malaria Parasite Density among Respondents		Parasite Density		
Low (+)	53	7.15± 3.21		0.00008
High (++)	12	36.67 ±16.24		

Relationship between Gestational Age and Malaria Parasite Density

The relationship between gestational age and malaria parasite density was analyzed to determine whether parasite density increases or decreases as pregnancy progresses. Table 3 shows the mean malaria parasite density for the different gestational age groups.

The parasite density appears to increase as pregnancy progresses, with the first trimester having an average of 10.50 parasites/µL, the second trimester rising to 15.75 parasites/µL, and the third trimester significantly higher at 30.25 parasites/µL. This trend suggests that as gestational age increases, the risk of higher malaria parasite density also rises, possibly due to the physiological changes in the immune response during pregnancy. This result contradicts the work of Accrombessi *et al*. (2018) that reported higher malaria density in early pregnancy in Benin. Higher malaria parasite infection had also been documented in early pregnancy by Ikeh *et al*. (2024) in Anambra State, contrary to the result of this study. Various studies have reported conflicting results of malaria infection in the different gestational ages. The increase in parasite density in the late stages of pregnancy as documented here could be due to factors such as immunological changes, parity-dependent immunity, delayed antenatal care and placental sequestration.

Relationship between Malaria Parasite Density and Parity

Table 4 shows that among the study participants, nulliparous women (40) had a higher average malaria parasite density of 25.00 parasites/µL compared to multiparous women (61), who had an average of 15.50 parasites/µL. The result of the present study is in-line with the work of Nnaji *et al*. (2006) that also reported lower malaria parasite density in multipara (1,950/ µL). However, result of the current study contradicts the work of Okoro *et al*. (2023) which documented higher malaria parasite density 431(6-6,200/ µL) in multiparous women. Another study conducted by Folarin *et al*. (2021) reported high prevalence (68.7%) of malaria parasitaemia in multiparous women. The findings of this study suggests that nulliparous women may be more susceptible to malaria infections, possibly due to a less experienced immune response compared to multiparous women who have been exposed to malaria in previous pregnancies.

Malaria Infection and Hemoglobin Levels

Table 5 illustrates the association between malaria infection and hemoglobin levels among pregnant women. The group with one plus (+) malaria infection level had an average hemoglobin level of 10.5 g/dL, which is indicative of mild anemia. In contrast, those with two plus (++) infection level exhibited a significantly lower average hemoglobin level of 8.3 g/dL, suggesting moderate anemia that can result from the higher parasite burden. Conversely, the group classified as "Not detected" had a relatively higher average hemoglobin level of 11.0 g/dL, reflecting better overall health. Hemoglobin is a vital protein found in red blood cells responsible for transporting oxygen from the lungs to tissues throughout the body and carrying carbon dioxide back to the lungs to be exhaled. Reports state that normal hemoglobin levels in pregnancy ranges from 11.0 to 12.0g/dL. Our result indicates that pregnant women with one plus and two plus infection level are probably suffering from anaemia which is detected when hemoglobin levels fall below 8.0-10.9g/dL (WHO, 2011). The observed differences in hemoglobin levels indicate a potential link between malaria severity and anemia, highlighting the importance of regular screening and management of anemia in pregnant women infected with malaria.

Malaria Parasite Density and Hemoglobin Levels

The group with low malaria parasite density (7.15 ± 3.21 parasites/µL) had an average hemoglobin level of 10.5 g/dL, indicating mild anemia. In contrast, those with high parasite density (36.67 ± 16.24 parasites/µL) exhibited a significantly lower average hemoglobin level of 8.3 g/dL, indicative of moderate anemia. This result mirrors that of Starck *et al*. (2021) that attributed decreased haemoglobin levels to acute malaria infection. The lower hemoglobin (HGB) in this study is a clear signal for anaemia which is due to mechanical destruction of parasitized red blood cells. This result corroborate the work of Ejike et al. (2022) who also reported lower hemoglobin levels among pregnant women in Abia State. According to Ejike and colleagues, physiological and pathological changes occur during pregnancy due to malaria infection and that these haematological anomalies are common complications in malaria infection, often playing a key role in malaria pathogenesis. The p-value of 0.0000832 in the present study demonstrates a statistically significant association between higher malaria parasite density and reduced hemoglobin levels, suggesting that increased parasite burden exacerbates anemia in pregnant women and can have potential large clinical implications. This finding emphasizes the need for effective malaria management to prevent severe anemia and its associated complications in this vulnerable population.

Association between Associated Risk Factors and Malaria Parasite Density

The results of various tests, including Blood, Protein, Nitrate, Ascorbic Acid, Bilirubin, Urobilinogen, and Keton, are presented in table 7. The analysis of laboratory test results revealed significant associations between various parameters and malaria parasite density among pregnant women. Specifically, the presence of blood and ketones in urine was linked to

higher parasite densities, with average densities of 25.00 parasites/µL and 25.00 parasites/µL, respectively, compared to those with nil results, who averaged 15.00 parasites**/**µL. Protein levels also showed a strong correlation, with higher densities observed in participants with trace (28.00 parasites/µL) and ++ levels (35.00 parasites/µL) compared to nil (12.00 parasites/µL). Similarly, nitrate, bilirubin, urobilinogen, and ascorbic acid levels exhibited significant associations, with their presence correlating with increased parasite density. Malaria infection, caused by *Plasmodium* species, has a significant impact on several biochemical markers due to the systemic effects of the parasite on the host's liver, kidneys, red blood cells and metabolism. For instance, Malaria can lead to proteinuria due to kidney involvement (malarial nephropathy), particularly in severe infections. The immune response, including glomerular inflammation and damage, results in protein leakage into the urine (Enejoh et al., 2019). On the other hand, Boutlis *et al*. (2003) stated that nitrate levels during malaria infection increases as a result of the host's immune response. Nitric oxide (NO), a defense molecule, is synthesized during infection and converted into nitrate and nitrite. Elevated levels are associated with oxidative stress and inflammation. Similarly, In malaria infection, especially caused by *Plasmodium falciparum*, there is increased hemolysis of red blood cells, leading to elevated serum and urinary bilirubin. This may present as jaundice in severe cases (Kochar, et al., 2003). According to Park and Murray (2006), urobilinogen, a breakdown product of bilirubin, may also increase in levels in urine of patients due to the same hemolytic process. Excessive bilirubin metabolism leads to increased production and urinary excretion of urobilinogen. Furthermore, in severe malaria cases, ketone bodies may be elevated due to metabolic stress, starvation, or hypoglycemia, which can occur during acute illness. The body switches to fat metabolism, increasing ketone production (English *et al*., 1998). As part of its impact on the body system of an infected individual, malaria infection further induces reactive oxygen species (ROS), against which ascorbic acid commonly known as vitamin C, is a key defender. As such, Nweneka and Odaibo (2009) posited that Ascorbic acid levels may decrease due to oxidative stress and increased utilization as an antioxidant during malaria infection. This finding correlate with the work of Okon *et al*. (2022) that reported a progressive decrease in serum ascorbic acid concentration with increase in malaria parasite density. Similar results had earlier been equally reported in southeastern Nigeria by Onyesom *et al*. (2010). According to Hassan *et al*. (2001), supplemental doses of ascorbic acid in malaria-infected patients may be vital in boosting immune system and as well as protection from the destructive action of oxidant compounds released during red cell rupture that accompanies malaria parasite infection. In-line with these findings, a study in Ghana by Ephraim *et al*. (2015) also reported higher significant values of urine blood, ketones, bilirubin, protein and urobilinogen in *P. falciparum* infected individuals. Reports of liver dysfunction and renal impairment in severe malaria infection show that malaria infection have significant effect on urine composition (Ugwuja and Ugwu, 2011). These findings underscore the importance of monitoring these biochemical parameters as potential risk indicators for malaria infection, highlighting their role in identifying pregnant women at higher risk for malaria or other health conditions and emphasizing the need for targeted preventive strategies to safeguard maternal and fetal health.

Table 3: Malaria Parasite Density by Gestational Age Group

Gestational age(weeks)	Parasite Density (parasites/µL)	Frequency	P value
12-14	10.50	6	0.0015
15-27	15.75	39	
28-38	30.25	56	

Table 4: Malaria Parasite Density by Parity

Parity	Frequency	Parasite Density (parasites/µL)	P value
Nulliparous	40	25.00	0.0001
Multiparous	61	15.50	

Table 5: Association between malaria infection, hemoglobin levels and parasite density in pregnant women

Malaria infection	Frequency	Hemoglobin Level (g/dL)	Parasite Density	P-value
+	53	10.5	7.15 3.21	0.0000832
++	12	8.3	36.67 16.24	
Not detected	36	11.0	-	

Table 7: Distribution of Biochemical Parameters in Urine Samples among Female Respondents

Parameter	Frequency	Percent (%)	Parasite Density	P value
Blood				
Trace	10	9.9	25.00	0.005
Nil	91	90.1	15.00	
Protein				
(+)	11	10.9	28.00	0.0001
(++)	3	3.0	35.00	
Nil	87	86.1	12.00	
Nitrate				
(+)	2	2.0	30.00	0.003
(++)	70	69.3	12.00	
Nil	29	28.7	20.00	
Ascorbic Acid				
(+)	28	27.7	18.00	0.002
(++)	11	10.9	22.00	
(+++)	3	3.0	30.00	
Nil	59	58.4	15.00	
Bilirubin				
(+)	8	7.9	25.00	0.001
(++)	1	1.0	35.00	
Nil	92	91.1	12.00	
Urobilinogen				
(+)	9	8.9	30.00	0.004
Nil	92	91.1	12.00	
Ketones				
(+)	7	6.9	25.00	0.0001
Nil	94	93.1	1200	

CONCLUSION

This study demonstrates a high burden of malaria among pregnant women in Awka, Nigeria, with significant associations between malaria parasite density, gestational age, parity, and anemia. Biochemical parameters such as proteinuria, bilirubin, urobilinogen, ketones, blood, nitrate and ascorbic acid were all found to be risk indicators of malaria parasite infection among the pregnant women.

Routine malaria screening should be incorporated into antenatal visits. Insecticide-treated nets (ITNs) and IPTp should be promoted to reduce malaria risk. Early anemia detection through hemoglobin level monitoring should be implemented. Public health education on malaria prevention should be strengthened. Further research is needed to explore long-term maternal and neonatal health outcomes associated with malaria parasite density.

Competing Interest

The authors declare that they have no competing interests regarding the publication of this manuscript.

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