https://dx.doi.org/10.4314/dujopas.v10i3a.14
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 10 No. 3a September 2024*

Malaria Infection in relation to Parasitological Indices, Blood Group and Anaemia in Pregnant Women attending Two Health Facilities in Adamawa State, Nigeria

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Abstract

Decline of maternal immunity and susceptibility to malaria had posed a detrimental effect on the maternal health and foetal outcomes. A cross-sectional study was carried out in two different secondary health facilities at General Hospital Ganye and Cottage Hospital Toungo with the aim of determining the malaria burden in relation to parasite species, parasite density, blood group and anaemia in pregnant women who booked for antenatal care as first timer irrespective of their gestational stage. A total of 471 randomly selected pregnant women who consented to the study were enrolled for the study. Blood samples were collected from each of the consented pregnant women. Thick and thin blood smear were prepared using Giemsa and examined microscopically to detect malaria parasite and infecting species, respectively. The overall prevalence of malaria was 22.9% (n=108). Plasmodium falciparum and P. malariae were the two malaria species encountered with a prevalence of 78.7% and 19.4%, respectively. Mixed infection had a 1.9% prevalence. Parasite density was recorded as low (67.6%), moderate (11.1%) and severe (21.3%) with a statistical significance (P=0.000). Prevalence of malaria in relation to blood group showed that pregnant women with blood group O (51.9%) had the highest burden of malaria infection and blood group AB (6.5%) had the least, although there was no statistical significance. Non-anaemic pregnant women recorded the highest infection prevalence (37.0%). This

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study revealed that malaria infection is significantly associated with the infecting parasite species and parasite density. It was discovered that P. falciparum was the most predominant species in the region during the period of this research.

Keywords: Anaemia, Ganye, *Plasmodium*, Pregnancy, Toungo

INTRODUCTION

The burden of malaria infection in pregnant women is a significant public health concern, particularly in malaria-endemic areas. Malaria impacts in pregnancy is enormous, include profound lasting effects and susceptible to higher risk of malaria in the early stage of infant life as might impact innate immune responsivity in infants (Jagannathan, 2018). Humans and female *Anopheles* mosquitoes are infected with the protozoa parasite of the genus *Plasmodium*, which is the causative agent for malaria, hence a vector-borne infectious disease (Ramdzan *et al*., 2020; Azizi *et al*., 2020). According to Gontine *et al*. (2020), *P. falciparum*, *P. vivax*, *P. malariae, P. knowleshi*, and *P. ovale* are the species of *Plasmodium* that cause malaria in humans. These parasites need a tropical climate to breed and infect humans (Ramdzan *et al*., 2020).

About 70% of the global malaria burden is concentrated in 11 countries: Burkina Faso, Cameroon, The Democratic Republic of Congo, Ghana, India, Mali, Mozambique, Niger, Nigeria, Uganda and Tanzania (Venkatesan, 2024). According to the World Malaria Report, Africa has the highest risk of contracting malaria (Hadiza *et al*., 2018) and has the greatest burden of malaria (Abe and Olusi, 2014; Hadiza *et al*., 2018). Four African countries accounted for just over half of all malaria deaths worldwide: Nigeria (31.3%), Democratic Republic of Congo (12.6%), Tanzania (4.1%) and Niger (3.9%). In Sub-Saharan Africa, 11 million pregnant women contracted *Plasmodium* infection in 2019, leading to 872,000 low birth weight (LBW) deliveries, according to WHO data (WHO, 2019a). Roman *et al*. (2019), reported that there are about 125 million pregnancies worldwide each year in areas where *P. falciparum* and/or *P. vivax* are transmitted, and about 25 million pregnant women in sub-Saharan Africa are at risk of contracting *P. falciparum* malaria during their pregnancy (Tecla, 2018).

Pregnant women are more susceptible to malaria infection due to reduced immunity, which increases the risk of illness, anaemia, severe disease, and death for both the mother and the unborn child. Malaria during pregnancy can lead to adverse outcomes such as spontaneous abortion, stillbirth, premature delivery, and low birth weight, which are major causes of child mortality (WHO, 2023). After delivery, malaria during pregnancy can have a long-term effect on the developing child, such as delayed development, behavioral issues, inadequate stature, neurological impairments, and a higher chance of infections in childhood (Fana *et al*., 2015; Accrombessi *et al*., 2018).

The risk of malaria infection in pregnant women is influenced by various factors, including the infecting parasite species, parasite density, blood group and a host of other factors. Pregnant women are more susceptible to malaria caused by *P. falciparum*, as these parasites have a unique ability to sequester in the maternal blood spaces of the placenta, thereby avoiding clearance by the immune system (Rogerson, 2017). In a region of low malaria transmission, pregnant women mostly infected had uncomplicated malaria with parasitaemia ≤ 20,000 parasites/μL, however, severe malaria were only presented among a few pregnant women who were presented with second malaria episode (Lopez-Pere *et al*., 2016). A study conducted in Ghana by Dosoo *et al*. (2020), pregnant women attending their first ANC visit

the geometric mean parasite density was 442 parasites/ μL, with 31% (having very low, 1-199 parasites/ μL) and 3% (having high density, ≥ 10,000 parasites/ μL).

The ABO blood group has been suggested as a genetic factor contributing to malaria susceptibility. A systematic review and meta-analysis showed that primiparae with different ABO blood groups had a comparable risk of malaria, but multiparae with blood group B had a significantly higher risk than non-B group, while multiparae with blood group O had a significantly lower risk than non-O group (Ai *et al*., 2022).

This study is aimed at assessing the burden of malaria infection and to determine the association between malaria infection and parasite density, parasite species, blood group type and anaemia among pregnant women. It is essential to establish knowledge about malaria from the perspective of its burden in the study areas and its association with the study variables. Finding will help provide a useful insight that would enhance epidemiological understanding of factors associated with malaria infection, developing treatment strategies that would help track drug resistance which is crucial for adapting treatment protocols, and development of new diagnostic tools at the initiation of ANC visit.

MATERIALS AND METHODS

Study Area

The study was carried out in Ganye and Toungo Local Government Areas (LGAs) headquarters of Adamawa State, Nigeria. Ganye and Toungo LGAs are neighbouring LGA. Toungo LGA is located between latitude 7^{o} 23′ – 8^{o} 23′N, longitude 11^{o} 20′ – 12^{o} 02′E, it shares borders with Ganye LGA to the north, Taraba State to the west and southwest, and Cameroon Republic to the east and south. While Ganye LGA is located between latitude 8^{o} 12′ – 8^{o} 40′N, longitude 11^{o} 37′ – 12^{o} 15′E, it shares borders with Jada LGA to the north, Mayo-Belwa LGA to the northwest, Toungo LGA to the south, Taraba State to the west and Cameroon Republic to the east (Adebayo *et al*., 2020). The predominant tribes consist of Chamba, Fulani and Verre. The main occupations are farming, trading and civil services. The climate of the area is categorized as tropical wet and dry seasons. The region experiences relatively low humidity between January to March, while this increases from April and reaches it highest peak of 83% in August due to the humid maritime air mass. From October the relative humidity starts to decline as a result of cessation of rainfall (Adebayo *et al*., 2020).

Study Design and Study population

A cross-sectional hospital-based study was carried out in two health facilities consisting of General Hospital, Ganye and Cottage Hospital, Toungo both in Southern Adamawa State, Nigeria. The target population consist of pregnant women who came for ANC care as first-timers. Participants were briefed on details concerning the research and objectives and their consent was obtained before being recruited into the study. Those pregnant women who agreed to participate were subjected to laboratory diagnostic test for malaria speciation and density, anaemia and blood group.

Ethical Clearance

The Ethical Clearance was secured from Adamawa State Ministry of Health (ASMoH). Permission from the hospital management's of General Hospital, Ganye and Cottage Hospital, Toungo were also sought and obtained.

Inclusion and Exclusion Criteria
Pregnant women who enrolled into the facility for ANC care for the first time from the inception of their pregnancy, irrespective of their stage of trimester were enrolled into the study while pregnant women who had enrolled into the ANC facilities prior to the inception of this study or elsewhere and transferred to these present ANC facilities were excluded.

Sample Size
The 2021 projected population of Ganye and Toungo LGAs of women of child bearing age is 73,007 with the state specific growth rate of 2.9% and 22% population proportion (Adamawa State Ministry of Health, 2021). The minimum sample size required for the study was determined to be 384, using a Sample Size Calculator with a 95% Confidence Level and 5% Confidence Interval/Margin of Error. A total of four hundred and seventy one (471) pregnant women participated in this study.

Blood Sample Collection, Parasitological and Haemotological Analyses
A trained Laboratory Scientist and two Laboratory Technicians assisted in the collection of blood samples. Approximately 2-3ml of blood was taken from each participant, using venipuncture technique. Blood samples were utilized for both malaria diagnostic testing, haematocrit (PCV) and blood group analyses. Upon collection, the blood was promptly mixed in an Ethylene Diamine Tetraacetic Acid (EDTA). Furthermore, each blood sample was meticulously labeled with the participant's relevant data, including their identification number, age, and the date of collection, to prevent any mix-up (Cheesbrough, 2009). Giemsa-stained thin blood smear microscopy was used in identifying parasite species, while thick blood smear microscopy was used for parasite density and parasites counted using the gold standard (WHO, 2016):

$$\text{Parasite denity/µl of blood} = \frac{\text{Parasite count x 8000}}{\text{No. of WBC counted}}$$

To assess for anaemia, the PCV also known as haematocrit, was measured as outlined by Cheesbrough (2006). The haemoglobin (HB) levels to diagnose anaemia at sea level (g/dl), based on criteria for pregnant women was used, and is as follows: 11.0 g/dl or higher (non-anaemia); 9-10.9g/dl (mild anaemia); 7-8.9g/dl (moderate anaemia); lower than 7g/dl (severe anaemia). (WHO, 2011). The gold standard for determining blood group as described by Cheesbrough (2006) was used for detecting visible agglutination or hemolysis when blood sample is mixed with antibodies against A and B antigens.

Data Analysis
The data generated was analyzed using Statistical Package for Social Sciences (SPSS) Version 26.0. The analyzed results were presented in tables using frequency and percentages, Pearson's Chi-Square ($\chi 2$) was used to determine the association between dependent and independent variables. A $P<0.05$ was considered statistically significant.

RESULTS
The distribution of malaria infection according to parasite species, parasite density, blood group and anaemia is presented in Table 1. The overall prevalence of malaria infection was 22.9% (n = 108). Two species of *Plasmodium* were detected, with *P. falciparum* (78.7%, n = 85) recording the highest prevalence, followed by *P. malariae* (19.4%, n = 21). Mixed infections of *P. falciparum* and *malariae* (1.9%, n = 2) recorded the least malaria prevalence (P=0.000).

Malaria infection based on parasite density in pregnant women showed that those with low parasite density (67.6, n = 73) and moderate parasite density (11.1%, n = 12) had the highest and the lowest malaria prevalence, respectively. Statististical analysis indicated a highly significant association (0.000). Blood group O (51.9%, n = 56) had the highest malaria prevalence, while blood group AB (6.5%, n = 7) had the least. There was no statistical significant difference (P = 0.535). Pregnant women with normal anaemia (37.0, n = 40) recorded the highest malaria prevalence, while those with severe anaemia (17.6%, n = 19) recorded the least malaria prevalence. There was no statistical significant difference (P = 0.493).

Table 1: Malaria Burden and its Distribution According to Parasite Speciation, Parasite Density, Blood group and Anaemia

Variable	Characteristics	No. Examined	No. Infected (%)	χ^2	*P*-value
Malaria Status	Positive	108	108 (22.9)		
	Negative	363	363 (77.1)		
Plasmodium species	*P. falciparum*	85	85 (78.7)	471.000[a]	0.000
	P. malariae	21	21 (19.4)		
	P. falciparum and malariae	2	2 (1.9)		
Parasite Density	Low	73	73 (67.6)	471.000[a]	0.000
	Moderate	12	12 (11.1)		
	Severe	23	23 (21.3)		
Blood Group	A	104	23 (21.3)	2.186[a]	0.535
	B	100	22 (20.4)		
	AB	19	7 (6.5)		
	O	248	56 (51.9)		
Anaemia	Normal	179	40 (37.0)	2.403[a]	0.493
	Mild anaemic	142	29 (26.9)		
	Moderate anaemia	87	20 (18.5)		
	Severe anaemia	63	19 (17.6)		

DISCUSSION

The study revealed an overall prevalence of malaria infection among pregnant women to be 22.9%. This prevalence closely resembles findings from a study conducted in Abakiliki where the author's reported a malaria prevalence of 22.4% (Felix *et al*., 2019). In Northern Ghana, Ahenkorah *et al*. (2020) reported 21.6% of pregnant women to be malaria positive during their first antenatal visit. The overall prevalence observed in this study surpasses the reported figures from other regions. For instance, a study done in Kogi State, Nigeria, reported a prevalence of 13.0% was documented by Yaro *et al*. (2017), while other study in Burkina Faso, the prevalence reported was 15.7% (Yaro *et al*., 2021). In contrast, higher malaria infection reported in various studies across different regions of Nigeria. For instance, a study in Argungu, Northwestern Nigeria found a prevalence of 41.6% (Fana *et al*., 2015), while another conducted in Aboh Mbaise LGA of Imo State reported 70.8% (Amala and Nwibani, 2015). In Kenya, 35.0% of pregnant women had asymptomatic malaria at their first ANC visit (Desai *et al*., 2015). The high malaria prevalence reported in this study, might suggest an association with low health literacy, lack of access to vector control methods or limited access to antimalarial drugs, or some local ecological variables like temperature, humidity, housing condition and vegetation (Felix *et al*., 2019).

The two species of malaria identified in this study *were P. falciparum* and *P. malariae*. The most prevalent species was *P.falciparum*, whereas mixed infection by caused *P. falciparum* and *P. malariae* had the lowest prevalence. The results of this study correspond with those of Oladosu and Adeniyi (2023), who found that *P. falciparum* (92.4%) was the most common malaria

species in Iwo, Osun State, Southwest Nigeria. The variation from this study, however, was that *P. falciparum* and *P. ovale* were the mixed species, and approximately three (3) different species of *Plasmodium*: *P. falciparum*, *P. malariae*, and *P. ovale*, were detected (Oladosu and Adeniyi, 2023). In Bossuso city, Somalia, Jama *et al*. (2022) reported *P. falciparum* and *P. vivax* alongside mixed infection with the proportion of 75.3%, 22.4% and 2.4%, respectively, in their study. Several studies carried out in Nigeria have reported *P. falciparum* as the increasingly dominant malaria species responsible for malaria cases during pregnancy. This was observed in Sokoto, Nigeria (Osaro *et al*., 2019), Southwest Nigeria, particularly in Ekiti State (Simon-Oke *et al*., 2019), and in Aboh Mbaise, Imo State, Nigeria (Valentine *et al*., 2020).

The findings in this study revealed an association between parasite density and malaria prevalence among pregnant women. However, those pregnant women with low parasite density showed a higher prevalence of malaria, whereas those with moderate parasite density exhibited the lowest prevalence. This difference in malaria prevalence in relation to parasite density was found to be highly significant (P = 0.000). In our study, pregnant women with mild, moderate and severe parasites densities denote an increasing presence of parasite, thus, if left undetected they might be at a high risk of developing complications associated with malaria and might serve as potential reservoir for parasite transmission. Therefore, the importance of Sulfadoxine-Pyrimethamine and other malaria preventive measures during ANC cannot be over emphasized. This further implies that understanding the association between malaria infection and parasite density is crucial for identifying women at high risk especially the severe parasite density that might lead to complications of malaria such as anaemia, low birth weight and maternal mortality.

The results of this study also indicate that those women with blood group O had the highest prevalence of malaria infection than the other blood group types. This is consistent with the results of another study which reported highest malaria infection among pregnant women with blood group O at 25.50% (Muhammed *et al*., 2022). Similarly, other studies have also reported high malaria prevalence within blood group O, followed by blood groups A, B, and AB, with prevalence of 68.8%, 55.7%, 45.5%, and 14.6%, respectively (Awosolu *et al*., 2021). Other studies have reported different findings, with some observing high malaria parasitaemia among study participants with blood group A (57.1%), followed by blood group O (48.8%), AB (20.0%), and B with 18.2% (Gomerep *et al*., 2017). The high malaria prevalence within the blood group O might be attributed to the higher occurrence of blood group O among pregnant mothers, representing 52.6% (n = 248) of the entire participants, which is in agreement with the findings of Muhammad *et al*. (2022). The inhomogenous distribution of blood group ABO in the population, the O allele has a significantly higher occurrence in malarious regions, especially in Sub-Saharan Africa, as this attributed factor might interact with malaria prevalence (Ai *et al*., 2022). Although, it has been reported that individuals with blood group O were less likely to come down with severe manifestations of malaria infection (Afoakwah *et al*., 2016).

Results from this study highlighted a significant association between haemoglobin levels and susceptibility to malaria despite the higher prevalence of malarial infection among non-anaemic pregnant women. A study by Nega *et al*. (2015) found a significant association between anaemia and asymptomatic malaria during pregnancy. However, a lower prevalence of malaria was associated with mild, moderate and severe anaemia among pregnant women, as presented in this study. This might suggest the importance of iron supplementation in enhancing haemoglobin levels and managing anaemia in pregnancy during routine ANC

services. Similar findings to this study found an overall prevalence of anaemia among pregnant women to be as high as 65.5%, with only a few pregnant women found to have severe anaemia at their first ANC visit (Mlugu *et al*., 2020). Additionally, another study showed an alarming prevalence of anaemia to be 81.4% among pregnant mothers (Olukosi and Afolabi, 2018). Furthermore, it is important to note that malaria and anaemia at any stage of pregnancy have a devasting effect on the foetus, and the mother, and by extension to the family and the community at large (Olukosi and Afolabi, 2018).

CONCLUSION

The prevalence of malaria in this study showed the level of endemicity of malaria in the study setting and its association with parasite species and density to be highly significant. Of the two species identified, *P. falciparum* is the most dominant species. The study also highlighted insignificant association between susceptibility to malaria infection and blood group types as well as haemoglobin level, though higher malaria prevalence among blood group O and non-anaemic pregnant women was observed.

ACKNOWLEDGEMENT

We acknowledge the ASMoH and the respective hospital management's for granting research ethical clearance and permission respectively. We express our sincere gratitude to all the participants volunteered, the staff of ANC units and laboratory units of the respective hospital's who have assisted in sample collection, storage and blood sample analysis and Mr. Joseph Medugu (Medical Laboratory Scientist, Modibbo Adama University Teaching Hospital, Yola, Adamawa State, Nigeria) for parasitological examination of the sample's collected.

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