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Abstract

Background: According to World Health Organization Malaria are a major public health problem and an important cause of maternal and infant morbidity in sub-Saharan Africa, including Nigeria. Malaria during pregnancy remains a major public health threat in sub-Saharan Africa where about 125 million pregnancies are at risk of malaria each year, and up to 200,000 babies die as a result. In malaria endemic areas, Plasmodium infections during pregnancy tend to remain asymptomatic yet causing significant problems like maternal anemia, low birth weight, premature births, miscarriage and still birth. Besides, asymptomatic carriers serve as silent reservoir of gametocytes for transmission by mosquito vectors. Early and accurate diagnosis of malaria with effective treatment and use of long-lasting insecticidal nets (LLINs) is the best strategy for prevention and control of complications during pregnancy and infant morbidity and mortality. However, laboratory diagnosis has relied on the identification of malaria parasites and parasite antigens in peripheral blood using Giemsa-stained microscopy or rapid diagnostic tests (RDTs). The aim of this study was to determine the prevalence of asymptomatic malaria parasite among pregnant women attending antenatal clinic at Mariya Sunusi maternity hospital Kano, Nigeria.

Methodology: A facility based cross-sectional study was conducted in Mariya Sunusi maternity hospital Kano, Nigeria from June, 2020 to July, 2020. Socio-demographic data were collected by using a semi-structured questionnaire. Plasmodium parasites were diagnosed by using Giemsa-stained blood smear microscopy and a rapid diagnostic test. Descriptive analysis was performed to obtain the frequency distribution of the variables.

Results: The result shows that of the total 248 pregnant women participated in this study, **10.8%** and **7.6%** were confirmed to be infected with Plasmodium species by microscopy and rapid diagnostic tests (RDTs), respectively.

Conclusion: The present study showed asymptomatic malaria is prevalent in pregnant women. The findings of the present study suggests the need of further malaria control strategies to screen the pregnant women for asymptomatic Plasmodium infection followed by prompt malaria treatment in the Antenatal care (ANC) service package. Such initiatives not only safeguard the pregnant women from malaria associated morbidities and mortalities but also the fetus and the newborns. As part of Antenatal care (ANC) service package, educating on the appropriate usage and benefits of the bed nets, and encouraging early Antenatal care (ANC) attendance among pregnant women could enhance benefits for the women's health. RDTs can be used sufficiently for diagnosis of the asymptomatic malaria in areas where there is no access to light microscopy.

Keywords: Anaemia, Kano, Mariya Sunusi, Plasmodium species, pregnant women, Prevalence

1. INTRODUCTION

Malaria is a disease caused by the protozoan parasites of the genus Plasmodium. The five species that commonly infect humans are: Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae and Plasmodium knowlesi [1]. P. falciparum is found in the tropics and sub- tropics and it is the most important species as it is responsible for 50% of all morbidity and mortality from severe malaria. P. vivax is seen in tropics and subtropical areas and is less dangerous but more widespread. It is transmitted to humans by the bite of infected female Anopheles mosquito of more than 30 species [1]. In sub-Saharan Africa, Anopheles gambiae, Anopheles arabiensis and Anopheles funestus are the primary vectors of malaria parasites and show highly anthropophagic tendencies [1]. Malaria during pregnancy remains a major public health threat in sub-Saharan Africa where about 125 million pregnancies are at risk of malaria each year, and up to 200,000 babies die as a result [2]. Plasmodium falciparum is the principal cause of severe malaria while *Plasmodium vivax* is increasingly recognized as capable of causing severe disease [3-6]. According to the latest World Health Organization report of 2015, there were an estimated 214 million cases of malaria worldwide with 438,000 deaths. Ninety per cent of the deaths occurred in sub-Saharan Africa where pregnant women and children are significantly affected [3]. Every year about 30 million African women in malaria endemic areas become pregnant and are at risk of infection with malaria, and an estimated 75,000-200,000 infant deaths are reported due to malaria infection in pregnancy [7, 8]. Malaria, particularly due to P. falciparum, in pregnant women increases the risk of maternal death, miscarriage, stillbirth and neonatal death [9, 10]. The impact of malaria during pregnancy may vary within a country depending on the intensity of malaria transmission. In areas of seasonal malaria transmission, pregnant women are three times more likely to suffer from severe malaria as compared to non-pregnant counterparts. In areas of stable malaria, adult pregnant women would have considerable acquired immunity and infection during pregnancy typically does not cause symptomatic malaria. The effect of malaria in pregnancy is primarily low birth weight and maternal anaemia [11, 12].

The World Health Organization (WHO) recommends a three-pronged approach to reduce the burden of malaria in pregnancy: (1) provision and promotion of insecticide treated bet nets (ITN) or long-lasting insecticidetreated bed nets (LLINS); (2) administration of intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTp-SP) after the first trimester of pregnancy in areas with stable malaria transmission; and (3) prompt diagnosis and appropriate treatment of malaria [7, 13]. However, because of rising P. falciparum resistance to SP in sub-Saharan regions, the use of rapid diagnostic tests to screen women for malaria at the first or each antenatal visit and then treat is likely more sustainable than IPTp without diagnosis [14]. Currently, WHO strives to increase access to IPTp-SP for pregnant women in all areas with moderate to high malaria transmission in Africa, as part of antenatal care (ANC) service package [11].Early and accurate diagnosis with effective treatment is the best strategy to decrease malaria-related pregnancy complications and infant mortality. The current malaria diagnostic methods include Giemsa-based microscopy, rapid diagnostic tests (RDTs), polymerase chain reaction (PCR) and placental histology depending on the setting [15]. The poor performance of routine malaria diagnostic techniques including RDTs and microscopy contribute to the burden of malaria in pregnant women [16, 17]. This is in large part due to the sequestration of the parasite in the placenta making the parasitaemia lower than usual in the peripheral blood. Therefore, nPCR which targets the small subunit ribosomal RNA (ssrRNA) is the better alternative diagnostic technique due to its high sensitivity (as low as 0.1 parasite/µl of whole blood). However, nPCR it is not widely used for the diagnosis of malaria in resource-limited settings as it requires a very well equipped laboratory, and the cost of diagnosis is more expensive [18, 19].

Globally there are an estimated 219 million malaria cases and 435,000 deaths due to malaria each year with the highest mortality reported in Africa [20]. Malaria infection during pregnancy is a major public health problem in tropical and subtropical regions [21]. It affects an estimated 24 million pregnant women in sub-Saharan Africa annually [22]. In malaria-endemic areas, malaria is the cause for almost 25% of maternal deaths each year, with the greatest risk of morbidity infection and occurring in primiparous women, adolescents, and those co-

infected with human immunodeficiency virus (HIV) [23]. Malaria during pregnancy may cause a variety of adverse consequences maternal including anaemia. placental accumulation of parasites, and low birth weight from prematurity and intrauterine growth retardation, congenital infection and infant mortality [24]. Asymptomatic infection with Plasmodium species is common in malaria endemic areas [25, 26]. The prevalence of asymptomatic parasitaemia reaches over 90% in children, in highly endemic areas of Africa [27]. Asymptomatic individuals, whether with a detectable parasitaemia by microscopy or below the microscopic detection level, can be a reservoir for transmission by Anopheles mosquitoes and may progress to symptomatic Moreover, disease [27, 28]. untreated asymptomatic malaria evolves in a chronic infection characterized by marked dyserythropoietic changes in the red cell precursors and increased erythrophagocytosis [29]. The efforts to reduce the burden of malaria require moving beyond the treatment of clinical infections to targeting transmission in the community by accurate identification of asymptomatic infections [30]. Each year at least 3 million pregnancies occur among women in malarious areas of Africa, most of who reside in areas of relatively stable malaria transmission [31]. The symptoms and complications of malaria during pregnancy differ with the intensity of malaria transmission and thus with the level of immunity the pregnant woman has acquired [32].

Malaria is the most deadly tropical infectious disease disproportionately affecting the poor, children under the age of 5 years, and pregnant women. There were an estimated 216 million episodes of malaria worldwide in 2010, of which approximately 81% or 174 million cases were in the African region [33].

In malaria endemic areas, a significant proportion of individuals have asymptomatic infection with Plasmodium species [34, 35] among whom pregnant women are at higher risk [36]. The sequestration of Plasmodium species in placenta is believed to be associated with low birth weight, preterm delivery, miscarriage, and still birth [37, 38]. Besides, asymptomatic carriers serve as silent reservoir of gametocytes for transmission by mosquito vectors [39, 40].

In Nigeria, malaria in pregnant women is a major public health problem where it accounts

for more cases and deaths than any other country in the world. Malaria is a risk for 97% of Nigeria's population. The remaining 3% of the population live in the malaria free highlands. There are an estimated 100 million malaria cases with over 300,000 deaths per year in Nigeria. This compares with 215,000 deaths per vear in Nigeria from HIV/AIDS. Malaria contributes to an estimated 11% of maternal mortality [41]. Beyond the impact of malaria on children and pregnant women, it affects the general population. 100% of the total population of Nigeria is at risk of malaria and at least 50% of the total population suffers from at least one episode of malaria each year [42]. About 51% of malaria cases and deaths in Nigeria occurs in rural villages away from effective diagnostic or treatment facilities [42]. Malaria cases and deaths have been increasing in the country, mainly due to injudicious use of anti-malarial drugs, delayed health seeking, and reliance on the clinical judgment without laboratory confirmation in most of the peripheral health facilities [43].

To the best of our knowledge there has been no published study to attest the epidemiological data regarding asymptomatic malaria parasite among pregnant women in the study area. Therefore, the present study determined the prevalence of asymptomatic malaria parasite among pregnant women attending antenatal clinic at Mariya Sunusi maternity hospital Kano, Nigeria.

2. METHODOLOGY

2.1. Study Area And Study Design

The study was conducted at Mariya Sunusi maternity hospital Kano, Nigeria. Mariya Sunusi maternity hospital Kano is a government secondary health facility serving a population of about 1.5 million and having a patronage of about 300/day. It is located along Katsina road in Kano metropolis. The hospital caters for all cases. maternity A facility-based crosssectional study was conducted among apparently healthy pregnant women in medical laboratory department at Mariya Sunusi maternity hospital Kano, Nigeria from June, 2020 to July, 2020. Socio-demographic data were collected by using a semi-structured questionnaire. Plasmodium infection was diagnosed by using Giemsa-stained blood smear microscopy and a rapid diagnostic test (SD BIOLINE Malaria Ag Pf/Pv POCT, standard diagnostics, inc., Korea).

2.2. Sample Size Determination

In this study, manual calculation of the sample size using Morgan and Krejcie (1970) formula was used for sample size determination as stated below:

 $S = X^{2}NP (1-P) \div d^{2} (N-1) + X^{2}P (1-P)$

Where:

S = Required sample size

 X^2 = The table value of the chi-square at desired confidence (3.841)

N =Study Population size (700)

P = Population proportion assumed to be 0.50 since this would provide maximum sample size

 d^2 = Degree of accuracy of the result expressed as proportion 0.050

3.841×700×0.5×0.5

 $0.0025 \times 699 + 3.841 \times 0.5 \times 0.5$

<u>672.175</u>= 248

2.70775

Hence 248 respondents

2.3. Inclusion And Exclusion Criteria

Pregnant women aged 18 years and above with absence of disease symptom/sign within the past 48 hours, axillary temperature $\leq 37.5^{\circ}$ c, permanent residents in the study area, and those willing to participate in the study and signed the informed consent were included. Individuals having taken anti-malarial drugs in the past six weeks prior to data collection, those undergoing any kind of long term medical treatments, and unwilling individuals were excluded from the study.

2.4. Data Collection

A pre-tested semi-structured questionnaire was administered by trained interviewer to obtain data on socio demographic characteristics of the pregnant women. Capillary blood samples were collected by a finger pricking using disposable lancet.

2.5. Laboratory Investigations

Thin and thick blood smears were prepared, stained with 3% Giemsa and examined microscopically from each asymptomatic pregnant woman. Thin smears were considered positive for malaria if one or more malarial parasites were seen; and, negative if no asexual form of *Plasmodium* was observed in 200 highpower fields. On the other hand, thick blood films were taken as positive if one or more malaria parasites have been observed; and, negative if no parasites were seen after examining 1000 white blood cells.

The rapid diagnostic test used in this study was SD BIOLINE Malaria Ag P.f/P.v POCT standard diagnostics, Inc., Korea. This test is one step, rapid, qualitative, and differential test for the detection of HRP-II (Histidine-rich protein II) specific to P. falciparum, and pLDH (Plasmodium lactate dehydrogenase) specific to P. vivax in a human blood sample.

2.6. Quality Control

Two experienced medical laboratory scientist individually examined the microscopic slides. Hundred microscopic fields of the thick smear were examined before concluding as negative. Discrepancy between the first and second readings was settled by a third senior microscopist, whose readings were considered final. The manufacturer's instruction was strictly followed for the RDTs. Blood smear microscopy readers were blinded to the result of RDTs.

2.7. Data Analysis

Data were analyzed using Statistical Package for Social Science (SPSS) software version 16.0 at that time with the help of the Statistician. The descriptive statistical method was used to analyze frequencies and percentages.

3. RESULTS

3.1. Socio-demographic characteristics

A total of 248 respondents were interviewed, giving 100% response rate. Among all, 110(44.4%) of respondents were 26-30 years of age. Majorities 198 (79.8%) of the pregnant women were married.

96 (38.7%)of the subjects were Secundigravidae, 101(40.7%) of the pregnant women were in the second trimester.168 (67.7%) of the pregnant women uses Insecticide Treated Mosquito Net (ITN). The socioeconomic characteristics of the study showed that, among all respondents, 171(68.9%) of respondents attended formal education, among this 101(40.7%) of respondents were primary school completed, 70(28.2%) of respondents were secondary school completed, while 77(31%) of respondents reported that they were took informal education (were illiterate and only

read and write). Similarly, results of occupational status of respondents indicated, 122(49.2%) of respondents were house wives, 60 (24.2%) were Government employee, 16(6.4%) company employee and 50(20.2%) were Merchants (**Table 1**).

Table 1. Socio demographic characteristics ofrespondents (n=248)

Characteristics	Frequencies	Percentages %
Ages		
20-25	72	29.0
26-30	110	44.4
31-35	50	20.2
36+	16	6.4
Marital status		
Married	198	79.8
Divorced	33	13.3
Widowed	17	6.9
Parity		
Primigravid	90	36.3
Secundigravid	96	38.7
Multigravid	62	25
Gestational		
age		
1 st trimester	87	35.1
2 nd trimester	101	40.7
3 rd trimester	60	24.2
Education		
Secondary and above	70	28.2
Primary	101	40.7
Can read and	60	24.2
write		
Illiterate	17	6.9
Occupation		
House Wife	122	49.2
Government	60	24.2
Employee		
Company	16	6.4
Employee		
Merchants	50	20.2
ITN use		
Use always	168	67.7
Use rarely	19	7.7
Do not use	61	24.6

According to age, pregnant women aged 26-30 years had highest infection rate of 11(4.4%) by microscopy and 9(3.6%) by RDT, followed by 8 (3.2%) by microscopy and 5 (2.0%) by RDT for age group 20-25 years. 5 (2.0%) by microscopy and 3 (1.2%) by RDT for age group 31-35 years, 3 (1.2%) by microscopy and 2 (0.8%) by RDT for age group 36+ (**Table 2**).

Table 2. Prevalence of malaria parasite amongpregnant women according to age group (n=248)

Ages	No. Exami ned (%)	Micros copy No. Positive (%)	Total (%)	RDT No. Positi ve (%)	Tota l (%)
20-	60	8	8	5	5
25	(24.2	(3.2%)	(3.2	(2.0	(2.0
	%)		%)	%)	%)
26-	110	11	19	9	14
30	(44.4	(4.4%)	(7.6	(3.6	(5.6
	%)		%)	%)	%)
31-	50	5	24	3	17
35	(20.2	(2.0%)	(9.6	(1.2	(6.8
	%)		%)	%)	%)
36+	28	3	27	2	19
	(11.3	(1.2%)	(10.8	(0.8	(7.6
	%)		%)	%)	%)

According to parity, primigravids had the highest infection rate with 14 (5.6%) by microscopy and 9 (3.6%) by RDT being infected. This was followed by women of 2nd pregnancy with prevalence of 8 (3.2%) by microscopy and 7 (2.8%) by RDT. Women of 3^{rd} pregnancy and above had the least prevalence of 5 (2.0%) by microscopy and 3 (1.2%) by RDT (Table 3).

Table 3. Prevalence of malaria parasite amongpregnant women according to Parity (n=248)

Parity	No. Exam ined (%)	Micros copy No. Positiv e (%)	Tota l (%)	RD T No. Posi tive (%)	Tot al (%)
Primigra vid	90 (36.3 %)	14 (5.6%)	14 (5.6 %)	9 (3.6 %)	9 (3.6 %)
Secundi gravid	96 (38.7 %)	8 (3.2%)	22 (8.8 %)	7 (2.8 %)	16 (6.4 %)
Multigra vid	62 (25%)	5 (2.0%)	27 (10.8 %)	3 (1.2 %)	19 (7.6 %)

It was also observed that according to gestational age of pregnancy, women of second trimester had the highest prevalence of 12 (4.8%) by microscopy and women of 1^{st} trimester 10 (4.0%) by RDT. This was followed by first trimester 9 (3.6%) by microscopy and second trimester 6 (2.4%) by RDT. The least was 6(2.4%) by microscopy and 3 (1.2%) by RDT for third trimester (Table 4).

Table 4. Prevalence of malaria parasite amongpregnant women according to Gestational age(n=248)

Gestati onal age	No. Exami ned (%)	Micros copy No. Positiv e (%)	Tota l (%)	RDT No. Posit ive (%)	Tot al (%)
1 st	88	9	9	10	10
trimest	(35.4	(3.6%)	(3.6	(4.0	(4.0
er	%)		%)	%)	%)
2 nd	110	12	21	6	16
trimest	(44.4	(4.8%)	(8.4	(2.4	(6.4
er	%)		%)	%)	%)
3 rd	50	6	27	3	19
trimest	(20.2	(2.4%)	(10.8	(1.2	(7.6
er	%)		%)	%)	%)

4. DISCUSSION

The present study aimed at determined the prevalence of asymptomatic malaria parasite among pregnant women attending antenatal clinic at Mariya Sunusi maternity hospital Kano, Nigeria.

In the present study, the prevalence of asymptomatic *Plasmodium* infection among pregnant women was **10.8%** and **7.6%** by using Giemsa-stained blood smear microscopy and RDT respectively. This prevalence is in agreement with the report of malaria prevalence among pregnant women ranged from 10% to 65% in malaria endemic areas [44]. However this prevalence was in line with the finding reported from the rural surroundings of Arbaminch town, South Ethiopia, which was **9.1%** and **9.7%** by microscopy and RDT, respectively among pregnant women [45].

The finding of the present study corroborates the findings of similar studies reported from other parts of Ethiopia [46, 47]. However it is lower than the finding of 27% by RDTs and 23% by microscopy reported from Democratic Republic of the Congo [48], and is higher than the prevalence of 3.1% by microscopy and 4.8% by RDTs reported from Nigeria [49]. This might be due to differences in geographical locations or transmission pattern. In different geographical locations, there are unique malaria transmission patterns that result in different immune acquisition capacity of the residents. Individuals living in higher malaria transmission areas have greater chance of developing asymptomatic malaria because they get frequent infections that can highly boost immunity against malaria, while those in low transmission areas have low infection frequency thus there may occur low prevalence of asymptomatic malaria [50].

The prevalence of asymptomatic *Plasmodium* infection was higher using microscopy than RDT which disagrees with a report from the rural surroundings of Arbaminch Town, South Ethiopia [45]. However, the finding was consistent with the study findings reported from Tanzania and Myanmar [51, 52].

The use of Insecticide Treated Mosquito Net (ITN) in the present study is 67.7%, though usage pattern differs among the study subjects. This is lower than the report of 89.6% by Getachew and his colleagues [46]. There was a strong association between increasing gravidity and decreasing rates of parasitemia.

This agrees with findings of similar studies from sub-Saharan African countries where the asymptomatic Plasmodium prevalence of significantly higher infection was in primigravidae than the multigravidae [53, 54]. It be linked to infection-specific might immunological factors. Some Plasmodium infected erythrocytes sequester in the maternal placenta by producing surface antigens, mainly variant surface antigen, that adhere to chondroitin sulphate-A (CSA) receptors expressed by syncytiotrophoblasts in the placenta. Primigravidae and secundigravidae are more susceptible to infection, as they lack these anti-adhesion antibodies against CSA binding parasites, which develop only after successive pregnancies [55].

In the present study there was significant difference in the rate of asymptomatic malaria parasitemia with respect to age of the pregnant women. However, the findings of some similar studies [56, 57] suggested that peripheral parasitemia was higher in pregnant women of younger age groups than old ages. This contrast might be due to differences in the sample size, technique, sampling physiologic and biochemical factors of pregnant women and the study setting such as geographical differences, altitude, temperature, and age categorization scheme.

Malaria has moderate to high transmission pattern in Nigeria. In countries where malaria has moderate to high transmission, WHO recommends intermittent preventive treatment with sulfadoxine pyrimethamine (IPTp-SP) for pregnant women as part of the Antenatal care (ANC) services, which does apply for Nigeria[58]. Hence, screening of pregnant women for asymptomatic Plasmodium infection should be the way forward to fight against malaria in pregnancy and its consequences on fetus.

5. CONCLUSION

The present study showed asymptomatic malaria is prevalent in pregnant women. The findings of the present study suggests the need of further malaria control strategies to screen the pregnant women for asymptomatic Plasmodium infection followed by prompt malaria treatment in the Antenatal care (ANC) service package. Such initiatives not only safeguard the pregnant women from malaria associated morbidities and mortalities but also the fetus and the newborns. As part of Antenatal care (ANC) service package, educating on the appropriate usage and benefits of the bed nets, and encouraging early Antenatal care (ANC) attendance among pregnant women could enhance benefits for the women's health. RDTs can be used sufficiently for diagnosis of the asymptomatic malaria in areas where there is no access to light microscopy.

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ETHICAL CONSIDERATIONS

This study was conducted only after obtaining approval from research ethics committee of the hospital.

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