Prevalence, Sensitivity and Specificity of Microscopy and Rapid Diagnostic Test in Malaria Diagnosis among Pregnant Women attending Antenatal Clinic in Some Parts of Nasarawa State and Federal Capital Territory, Nigeria

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ABSTRACT: Malaria during pregnancy is a major health problem in endemic countries with clinical consequences including death of both mother and child. In Nigeria, statistics shows that as many as 300,000 lives especially those of children and pregnant women are lost annually to malaria. The aim of this study was to evaluate the prevalence of malaria parasites among pregnant women attending antenatal clinic at the Mararaba Medical Centre, Mararaba-Nasarawa State and National Hospital, Abuja. The study was conducted between January and May, 2016. A total of four hundred and thirty seven (437) respondents were tested for malaria using microscopy and Care Start Malaria HRP2 (Pf) rapid test kit; 142(32.5%) patients tested positive for Microscopy and 98 (22.4%) for Rapid Diagnostic Test; giving an overall prevalence rate of 54.9% in the study population. Pregnant women between the ages of 21-29 recorded the highest prevalence rate of 59.2% (microscopy) and 56.1% (RDT). According to their marital status, those married recorded the highest prevalence rate of 76.8% (microscopy) and 86.7% (RDT). Based on their trimester, women in their second trimester recorded the highest prevalence rate of 56.35% (microscopy) and 59.2% (RDT). Pregnant women who are self-employed recorded the highest prevalence rate of 49.3% (microscopy) and 52.0% (RDT) and according to their level of education those in their tertiary education recorded the highest prevalence of 33.8% (microscopy) and 33.7% (RDT). The result showed that Microscopy test as compared to Rapid Diagnostic Test had a sensitivity and specificity of 90.6% and 84.7% respectively. The Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were 83.5% and 91.3% respectively with an accuracy of 87.4%. Following the high prevalence of malaria infection in pregnancy, more efforts are needed in the control of malaria in pregnancy. The people need public enlightenment in the importance of malaria diagnosis. Malaria should therefore be recognized as a global priority in health care more so in pregnancy.

Keywords: Malaria, Microscopy, Nigeria, Rapid Diagnostic Test, Pregnancy, Sensitivity.

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1. INTRODUCTION

Malaria is a life threatening parasitic disease. More than 40% of the world population lives in malaria-prone areas and it is considered a complex and overwhelming public health problem. The disease is caused by four species of *Plasmodium* parasites (*P. vivax*, *P. malariae*, *P. falciparum* and *P. ovale*), and it is transmitted through the bite of infected female Anopheles mosquitoes [1]. The burden of malaria infection during pregnancy is caused mainly by *Plasmodium falciparum*, the most common malaria species in Africa [1]. Each year, at least 3 million pregnancies occur among women in malaria-prone areas of Africa, most of who reside in areas of relatively stable malaria transmission [2].

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 [3]. Pregnant women and unborn children are particularly vulnerable to malaria, which is a major cause of prenatal mortality, low birth weight, and maternal anaemia [4].

Malaria control still remains a challenge in Africa where the disease is endemic in about 45 countries, including Nigeria; and about 588 million people are at risk [5]. The protection of pregnant women living in malaria-endemic countries has been of particular interest to many National Malaria Control Programs because of their reduced immunity. Most cases of malaria in pregnancy in areas of stable malaria transmission are asymptomatic; this is attributed to anti-disease immunity acquired during previous exposures, which protects against clinical malaria [5]. Unfortunately, this subclinical infection still poses great danger to both the mother and the foetus. The principal impact of malaria infection is due to the presence of parasites in the placenta causing maternal anemia (potentially responsible for maternal death when severe) and low birth weight. Other burdens associated with malaria during pregnancy include, but are not restricted to, spontaneous abortion, miscarriage, premature delivery and stillbirth. Socio-economic status of the family is affected in terms of using scarce resources on preventable conditions [6].

Malaria is a life threatening parasitic disease and the rate at which pregnant women contact malaria is a major risk to their health status. 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 [1].

Each year, many women get pregnant in malaria endemic areas such as Nigeria. A sizable percentage of these women also suffer from malaria at one stage of pregnancy. Report has shown that malaria has severe negative effects on health and birth outcomes such as intrauterine growth, retardation, incidence of low birth weight, abortions and in severe cases death of the women and foetus [5]. It is therefore necessary to determine the prevalence of maternal malaria in pregnancy among antenatal attendees. About 51% of malaria cases and deaths in Nigeria occur in rural villages away from effective diagnostic or treatment facilities [1]. 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 [7], hence, the need for routine diagnostic screening of

malaria parasites in endemic rural areas, urban slums, as well as urban areas.

Despite evidence of the cost effectiveness of improving treatment access and compliance, most victims of malaria still die because of lack of health care close to their homes or because their condition is not diagnosed by health workers [8]. Early diagnosis and prompt effective treatment of malaria illness has been a cornerstone of malaria control [7]. Diagnosis based on symptoms alone has inherent difficulties [7], although volunteer health workers in rural areas have practiced it with some success.

The reduction of morbidity and the interruption of parasite transmission by means of community-based antimalarial treatment require an accurate, rapid and practical method of diagnosis. The delivery of treatment in rural areas in Nigeria is complicated by the centralized nature of microscopy services, hence the need for this study. Over the past few years, developments in rapid field diagnostic techniques based on the demonstration of parasite antigens have opened new possibilities for improved rural malaria diagnosis that is independent of centralized diagnostic services [7].

There have been a considerable number of reports about knowledge, attitudes, and practices relating to malaria and its control from different parts of Africa. These reports concluded that misconceptions concerning malaria still exist and that practices for the control of malaria have been unsatisfactory [9]. However, epidemiological patterns of malaria are widely different from one place to another [10]. Specific data of a place collected can help in the making of a design of improved programme for strategic malaria control for a particular location. Part of the rationale for investigating malaria prevalence in pregnancy is to compare present with past situations especially with current efforts at controlling malaria during pregnancy [10]. The study was therefore, aimed at determining the prevalence of malaria parasite among pregnant women attending antenatal clinic in some parts of Nasarawa State and Federal Capital Territory, Nigeria, using conventional microscopy and Rapid Diagnostic Test (RDT) strips, as well as determining the sensitivity and specificity of these two techniques.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out at the Mararaba Medical Centre, Mararaba – Nasarawa State which lies between latitude $9^0$2' North and longitude $7^0$35' East, with an elevation of 448m (1,470 ft), and National Hospital, Abuja which lies between latitude 9.0407^0 North and longitude 7.4611^0 East. The Mararaba Medical Centre covers the rural as well as urban slum areas of Nasarawa State while National Hospital covers the urban areas of the Federal Capital Territory, Abuja.

2.2 Study Population

The study population for this research was pregnant women attending antenatal clinic at the Mararaba Medical centre, Mararaba-Nasarawa state and National Hospital, Abuja. Simple Systematic Sampling Technique was used for the selection of the study population. A total number of 437 participants were recruited for this study. Questionnaires were administered and blood sample of each respondent was collected to analyze for the presence of malaria parasite and antibodies.

2.3 Ethical Approval and Informed Consent

Informed consent was obtained from the study group, and the participants were enrolled consecutively. Ethical consent was

sought and gotten from the Research and Ethics Committees of the respective hospitals.

2.4 Sample Collection

Sample collection was carried out between the months of January and May, 2016. Anonymized socio-demographical data, as well as peripheral blood samples from pregnant women attending antenatal clinic in the study locations within the period of the study were obtained for this study. A total of 437 peripheral blood samples were collected using venous procedure (venipuncture) into sterile vaccutainer Sequestrine (EDTA) anticoagulant-coated tubes. Sample collection was done with the aid of qualified laboratory personnel, adopting standard and careful laboratory procedures. Samples collected were analysed shortly after collection to avoid alteration in the morphology of white blood cells (WBC) and malaria parasites. The consent of the patients was obtained prior to sample collection. Questionnaires concerning socio- demographic characteristics such as age, level of education, trimester, employment status and level of education were distributed to the sampled pregnant women.

2.5 Thick Blood Film Preparation and Microscopy

A drop of blood was smeared on a clean glass slide using the sharp edge of another glass slide to make a thick smear which covers about 15 x 15 mm. The slides were labeled. The blood film was allowed to air dry with the slide in a horizontal position.
Holding the slide with the dried thick film facing downwards, it was then dipped into the Field's stain A for 5 seconds and the corner of the slide was touched against the container to drain off excess stain; then it was wash gently for about 5 seconds by placing in clean water.
The washed slide was then dipped into Field's Stain B for 3 seconds and excess stain was drained off and then wash gently in clean water, the back of the slide was wiped clean and place upright in a draining rack for film to air dry. The films were then examined microscopically, first with ×40 objective to obtain the level of distribution of material and then with oil immersion ×100 objective to observe the microscopic appearance of the malaria parasites.

2.6 Rapid Diagnostic Test

After collection, the blood sample was poured into an EDTA tube, mixed properly and then centrifuged. The Rapid Diagnostic malaria test cassette was used for rapid test. A sterilized pipette was used to take out the serum from the EDTA tube, and dropped into the sample well of the cassette, and the result read after 5-10 minutes. The presence of a single band was recorded as a negative result, while the presence of a double band indicated that the patient was positive for malaria.

2.7 Determination of the Sensitivity and Specificity of Microscopy and RDT

Sensitivity (also called the true positive rate) is a measure of the proportion of positives that are correctly identified while Specificity (also called the true negative rate) measures the proportion of negatives that are correctly identified. The respective sensitivity and specificity of Microscopy and RDT from this study were determined using the formulae below:

Sensitivity or true positive rate (TPR) = $\frac{TP}{(TP+FN)} \times 100$

Specificity or true negative rate (TNR) = $\frac{TN}{(TN+FP)} \times 100$

Positive predictive value (PPV) = $\frac{TP}{(TP+FP)} \times 100$

Negative predictive value (NPV) = $\frac{TN}{(TN+FN)} \times 100$

Accuracy = $\frac{(TP+TN)}{(TP+FP+FN+TN)} \times 100$

TP- True Positive; TN- True Negative; FP- False Positive; FN- False Negative

3. RESULT

Plasmodium falciparum was the only malaria parasite observed in the blood samples of the pregnant women examined in the study. A total of 437 pregnant women attending antenatal clinic in Mararaba medical centre and National Hospital were tested for Malaria. Two different diagnostic techniques were carried out; Microscopy and RDT, of which 32.5 % (142) patients tested positive for Microscopy and 22.4 % (98) patients tested positive for RDT, giving an overall prevalence rate of 54.9 % in the study population.

Table 1 shows the distribution of malaria parasite among the pregnant women according to their association between demographic factors and number tested positive for Microscopy and RDT. The distribution of malaria parasite according to the age groups of the patients recorded the highest prevalence rate of 59.2% (84) and 56.1% (55) in the middle age group (21-29 years) respectively for both tests. According to their marital status, those married recorded the highest prevalence rate of 76.8% (109) and 86.7% (85) respectively. Based on their trimester, women in their second trimester recorded the highest prevalence rate of 56.35% (80) and 59.2% (58). Pregnant women who are self employed recorded the highest prevalence rate of 49.3% (70) and 52.0% (51) and according to their level of education, those that had tertiary level of education recorded the highest prevalence rate of 33.8% (48) and 33.7% (33) respectively.

Table 2 shows the distribution of malaria parasite among the pregnant women according to their association between risks factors and number tested positive for microscopy and RDT.

3.1 Sensitivity of Microscopy as compared to Rapid Diagnostic Test

The result showed that the Microscopy test had a sensitivity of 90.6% and Rapid Diagnostic Test had a specificity of 84.7% respectively. The Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were 83.5% and 91.3% respectively with an accuracy of 87.4%

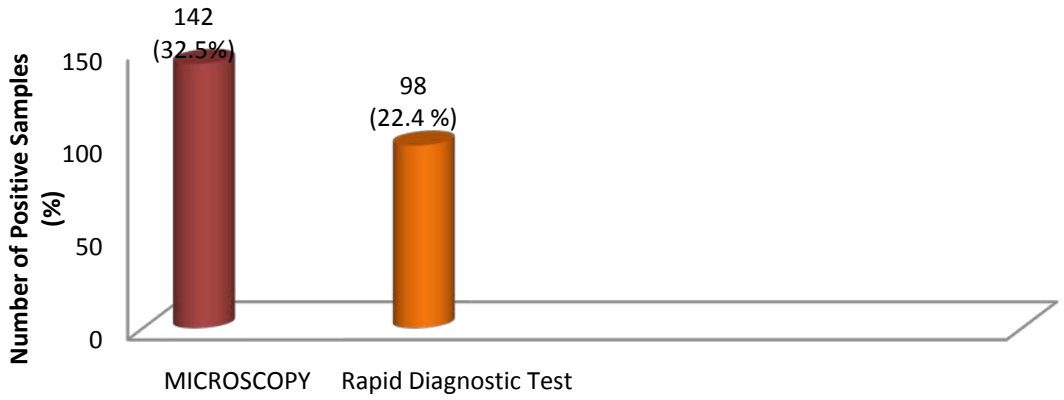

Fig. 1: Overall Prevalence of Malaria Parasite among the Study Population Using Microscopy and Rapid Diagnostic Test

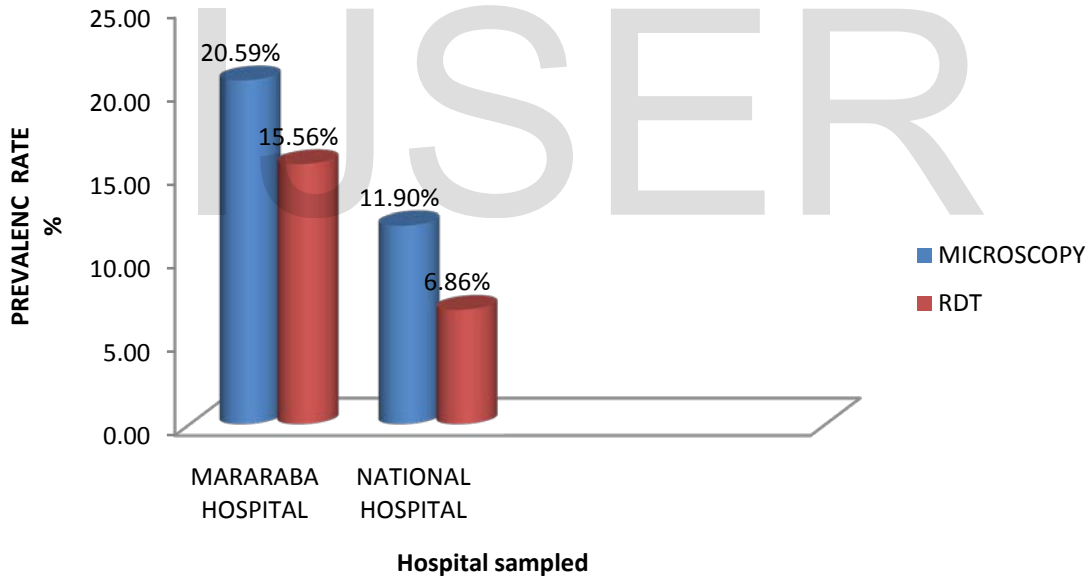

Fig. 2: Prevalence Rate of Malaria Infection of the Pregnant Women who Tested Positive through Microscopy and Rapid Diagnostic Test from Mararaba Medical Centre and National Hospital

Table 1: Association between Demographic Factors and Number Tested Positive for Microscopy and Rapid Diagnostic Test (RDT)

	Microscopy				RDT		
	Number examined	**Number positive (%)**	**X^2**	**P- value**	**Number Positive (%)**	**X^2**	**P- value**
AGE							
10 – 19	70	20 (14.1)	4.041	0.400	12 (12.2)	4.019	0.403
20 – 29	247	84 (59.2)			55 (56.1)		
30 – 39	104	36 (25.4)			29 (29. 6)		
40 – 49	14	2 (1.4)			2 (2.0)		
>50	2	0 (0)			0 (0)		
Total	**437**	**142 (100)**			**98 (100)**		
MARITAL STATUS							
Single	78	20 (14. 1)	3.087	0.378	11 (11.2)	4.756	0.095
Married	237	109 (76. 8)			85 (86.7)		
Divorced	116	11 (7.75)			1 (1.0)		
Widowed	6	2 (1. 4)			1 (1.0)		
Total	**437**	**142 (100)**			**98 (100)**		
TRIMESTER							
1st	96	29 (20. 4)	0.341	0.842	14 (14.3)	4.716	0.095
2nd	244	80 (56.3)			58 (59 .2)		
3rd	97	33 (23.2)			26 (26.5)		
Total	**437**	**142 (100)**			**98 (100)**		
EMPLOYMENT STATUS							
Civil servant	113	36 (25.4)	6.868	0.143	19 (19. 4)	7.027	0.134
Private sector	28	8 (5.6)			7 (7.1)		
Pensioners	9	2 (1.4)			1 (1. 0)		
Self employed	181	70 (49.3)			51 (52.0)		
Unemployed	106	26 (18.3)			20 (20.4)		
Total	**437**	**142 (100)**			**98 (100)**		
LEVEL OF EDUCATION							
None	39	12 (8.5)	3.727	0.444	10 (10.2)	3.398	0.494
Primary School	106	34 (32. 1)			24 (24.5)		
Secondary School	124	46 (32. 4)			30 (30.6)		
Tertiary	165	48 (33.8)			33 (33.7)		
Student	3	2 (1.4)			1 (1.0)		
Total	**437**	**142 (100)**			**98 (100)**		

Table 2: Association between Risk Factors and Number Tested Positive for Microscopy and Rapid Diagnostic Test (RDT)

		Microscopy			RDT		
	No Examined	**No (%) Positive**	**X^2**	**P- value**	**No (%) Positive**	**X^2**	**P- value**
ATDSIYA							
Yes	237	75 (52.8%)	0.170	0.680	55 (52.8%)	0.182	0.670
No	200	67 (47.2%)			43 (43.9%)		
Total	**437**	**142 (100%)**			**98 (100%)**		
HORTC							
Never	217	75 (52. 8%)	2.370	0.499	48 (49.0%)	1.704	0.636
Once a year	69	25 (36.2%)			19 (19.4%)		
Twice a year	61	17 (12.0%)			14 (14.3%)		
>than twice a year	90	25 (17.6%)			17 (17.3%)		
Total	**437**	**142 (100%)**			**98 (100%)**		
DYHRD/SITN							
Yes	264	84 (59.2%)	0.139	0.709	63 (64.3%)	0.793	0.373
No	173	58 (40.8%)			35 (35.7%)		
Total	**437**	**142 (100%)**			**98 (100%)**		
HOATD							
Never	200	68 (47.9%)	0.985	0.805	42 (42.9%)	3.139	0.371
Once a year	59	16 (11.3%)			12 (12.2%)		
Twice a year	46	15 (10.6%)			15 (15.3%)		
Quarterly/Every Week	132	43 (30.3%)			29 (29.6%)		
Total	**437**	**142 (100%)**			**98 (100%)**		
ITNRT							
Yes	162	55 (38.7%)	0.249	0.618	31 (31.6%)	1.602	0.206
No	275	87 (61.3%)			67 (68.4%)		
Total	**437**	**142 (100%)**			**98 (100%)**		
DYSWTMN							
Yes	240	76 (53.5%)	0.166	0.683	56 (57.1%)	0.252	0.616
No	197	66 (46.5%)			42 (42.9%)		
Total	**437**	**142 (100%)**			**98 (100%)**		

Key: ATDSIYA- Are There Drainage Systems in your Area, **HOATC** – How Often Are They Cleaned, **DYHR/SITN** – Do You Have Refuse/Silos in the Neighborhood, **HOATD** – How Often are They Disposed, **ITNRT** – Is the Neighborhood Road Tarred, **DYSWTMN** – Do You Sleep with Treated Mosquito Net

4. DISCUSSION

The diagnosis of malaria is confirmed by blood tests; and these tests can be divided into microscopic and non-microscopic tests (Rapid Diagnostic Test). Microscopy and RDT are the standard tools available for accurate routine diagnosis of Malaria. The use of RDT detects specific parasite antigen or enzyme in the infected individual. In Nigeria however, the diagnosis of malaria in pregnancy relies on microscopic examination (the current gold standard) of thick and thin blood films for parasites.

The sensitivity of Microscopy as compared to RDT from this study was 90.6% while the specificity was 84.7%. This result indicates or shows that Microscopy (the current gold standard) is more efficient than the use of RDT. This is in concordance with the WHO [8] report that RDTs are less sensitive than malaria blood film [8]. The positive and negative predictive values were 83.5% and 91.3% respectively with an accuracy of 87.4%.

The prevalence rate of *Plasmodium falciparum* infection among pregnant women in Mararaba Medical Centre and National Hospital for Microscopy and RDT were 32.5% (142) and 22.4% (98) respectively; giving an overall prevalence rate of 54.9 % in the study population. This is higher than the 13.7% recorded by Adam *et al*., [11] in Sudan and lower than the 57 % recorded by Bouyou-Akotet *et al*., [12] in Gabon. The high prevalence rate obtained from this study is pointer that malaria parasitaemia is high in pregnant women in both the urban areas of the Federal Capital Territory (Abuja) and the slums of Nasarawa state, Nigeria. The high susceptibility and infection of these pregnant women could be due to exposure to the vectors, as well as the decline in their immunity which comes with pregnancy.

In this study, the highest prevalence of malaria among the pregnant women in relation to their ages showed that middle aged women had the highest prevalence rate of 59.2% and 56.1% as regard to Microscopy and RDT respectively, while the younger women showed a prevalence rate of 14.1% and 12.2% (Microscopy and RDT); the older women had a prevalence of 25.4% and 29.6%. This finding is similar to that reported by other researches carried out in Nigeria by Nduka, *et al.*, [13], Rogerson *et al.*, [14] and Bouyou-Akotet *et al.*, 12]. Study carried out by Oduola *et al.*, [15] revealed a malaria prevalence rate of 52% in pregnant women living in a certain part of Lagos, those within the age bracket of 20 to 30 years recorded the highest number of positive result while those of the age group of above 40 years recorded the lowest or no result at all. This result supports the existing knowledge that high prevalence at lower ages and low prevalence at higher ages is due to the existence of natural immunity to infectious disease including malaria which the pregnant women acquire as the age increases [16]. In another study carried out by Onwere *et al.*, [17], younger women appeared to be susceptible to malaria as the prevalence was highest among age group 21 - 25 (68.8%). This contradicted the findings of Adefioye *et al.*, [18] that found 36 – 39 year old group to be more susceptible, but agreed with the findings of Dicko *et al.*, [19] who opined that adolescents and young adult pregnant women were more susceptible to malaria than older pregnant women, because of continuous development of malaria immunity in older women. The different malaria prevalence rate observed among these age groups could be attributed to the level of acquired immunity that increases with age, which may be associated with protection from malaria infection.

Women in their second trimester were found to have the highest prevalence rate of 56.3% and 59.2% (Microscopy and RDT), while those in their third trimester had a

prevalence rate of 23.2% and 26.5%; however those in their first trimester had the lowest prevalence rate of 20.4% and 14.3% respectively. The highest rate of prevalence (second trimester) could be due to the fact that at this stage, the infection is successfully established in the placenta, which is the main and most dangerous site of infection for a pregnant woman, and so can be detected even in the peripheral blood; this also in line with the studies where the highest level of parasiteamia was recorded at the second and early third trimester [13]. Second trimester prevalence in this study is in line with previous studies as [2] found in western Kenya that prevalence was highest at 13 – 16 weeks gestation (first trimester), and found similar number of recoveries in both groups during the second and third trimesters. The loss of immunity in early pregnancy was equivalent to an 11-fold decrease in the rate of recovery from infection [2]. The recovery seen in the late pregnancy suggests that the women mount a satisfactory immune response to malaria infection, re-acquiring their pre-pregnancy immune status at about the time of delivery [20]. The observation could also be as a result of constant intermittent preventive treatments in pregnancy (IPTp) given to pregnant women during antenatal care visit which usually commence during second trimester.

Employment status influenced the prevalence of malaria infection in this study area based as results obtained shows that self employed women recorded the highest rate of infection with 49.3% and 52.0% (Microscopy and RDT) respectively. The reason might be due to exposure to mosquito bites while staying outside longer at night due to their jobs, as compared to those who work in offices and private firms who do not stay out (in the open) late at night. There was however, no significant relationship between employment status and malaria infection ($P>0.05$).

Current study showed that respondents who reside in Mararaba recorded higher prevalence rate of the disease more than those that reside in Abuja. Most of the respondents resident in Mararaba agreed that there are poor drainage systems in their area, and they are not often cleaned, and they also agreed to the fact that they have refuse dumps/silos in their neighborhood which are rarely disposed. The neighborhood roads are also not tarred and most of the women do not sleep with treated mosquito net; hence, predisposing them to the disease. Similarly, environmental and host factors have previously been implicated in the incidence of the infection.

Chi-square analysis from this study however, revealed that the relationship between those who tested positive and negative to malaria with respect to age, marital status, trimester, employment status, level of education and associated risk factors was insignificant.

5. CONCLUSION

Despite incentives and measures to fight and check malaria, the prevalence of malaria is high in pregnant women in Abuja and Nasarawa State, Nigeria. The high prevalence of falciparum malaria among women in this study area could be due to poor environmental hygiene, which makes the environment more conducive for the parasite vector to multiply and high rate of illiteracy especially among the women in this study. This parasites invade and injure the host (in this case the pregnant women), resulting in socio economic burden, exorbitant cost of medical care, premature delivery, low productivity and worst, death of mother and child.

6. RECOMMENDATIONS

The following recommendations can be emphasized to serve as preventive and

control measures for malaria infection in the study area and the general public at large.

1. The federal government should provide malaria control programs and improve the distribution of treated mosquito nets and insectides.
2. Pregnant women should always use long lasting insecticides, treated nets and wear protective clothes.
3. Government should provide accessible malaria diagnostic facilities and affordable effective drugs to treat active infection.
4. Pregnant women should be educated on the preventive measures of malaria.
5. Government should provide preventive measures on malaria breeding by removing surface water, spraying insecticides or oil on stagnant waters, drain ditches.
6. Hospitals should make sure that Malaria test should be part of the routine test carried out for all pregnant women attending antenatal clinic.

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