Investigating the *Plasmodium falciparum* infection prevalence and intensity and two methods of malaria diagnosis in North Western Nigeria.

Abstract

Aims: The study was undertaken to estimate the intensity of *Plasmodium falciparum* malaria in randomly selected areas of North Western Nigeria and to evaluate the efficiency of microscopy and rapid diagnostic test (RDT) in detecting and determining intensity of -*P. falciparum* infection.

Study Design: The study was a hospital based; conducted in some randomly selected North Western States of Nigeria during the period of April-August 2013. The states are Kano, Kaduna, Katsina, Kebbi and Jigawa States,.

Methodology: A total of one thousand four hundred and seventy (1470) samples were collected. About 2-5ml of blood was drawn (venepuncture) into EDTA sample bottles. Rapid one step malaria HRP2 Rapid test was carried out according to the manufacturer's instructions to determine presence of *Plasmodium falciparum,* and also Giemsa stained thick and thin blood smears were examined using a light microscope.

Results: A total of eight hundred and thirty four 834(56.5%) cases were positive microscopically while two hundred and eighty seven 287(19.52%) were positive with the RDT which showed high significance ($P<0.01$) in the efficiency of the diagnostic methods. Low intensity (+) was higher in 542 (36.87%) and statistically different within the states ($P<0.01$). The RDT showed high specificity of 68.21% while a higher sensitivity of 47.68% was observed from the microscopy results with high statistical difference ($P<0.01$) between the two methods.

Conclusion: The study area was found to be *P. falciparum* endemic with a certain level of intensity; microscopy remains the most reliable method of malaria diagnosis. However discordance between microscopy and RDT requires further investigations.

Key Words: *Plasmodium falciparum,* Microscopy, Rapid Diagnostic Test (RDT), Northwest, Nigeria

Introduction

Malaria remains one of the major health problems in sub-Saharan Africa (1, 2). Though there are encouraging reports that malaria morbidity and mortality are declining, malaria is still an overwhelming public health problem, with an estimated 207 million cases and 627,000 deaths every year worldwide (3). *Plasmodium falciparum* are the species responsible for 85% of all malaria cases, as results of its ability to induce severe malaria and in some cases multiple organ dysfunctions.

Proper diagnosis is important in the epidemiological screening of malaria infection for research purposes and treatment to provide informed indices for control strategies. It improves the management of all patients with febrile illness and may also help reduce the emergence and spread of resistance by reserving the antimalarials for those with the actual disease (4). In 2005 the Federal Ministry of Health recommended that all fevers be treated presumptively where confirmation cannot be made (5) but the use of presumptive treatment for malaria has the potential for facilitating resistance by greatly increasing the number of people who are treated unnecessarily thus exerting a selective pressure on the circulating parasite population (6). As microscopy is the gold standard method for malaria diagnosis (7), in Nigeria its efficiency has been hampered by lack of trained technicians, short supply of reagents and the delay in obtaining

results in most health facilities (8). These shortcomings of microscopic diagnosis favoured the reliance on rapid diagnostic test (RDT) for malaria diagnosis, where some facilities confirm the RDT results with microscopy. However like the microscopy, RDT also has its limitations and short comings in the identification of malaria parasites. Therefore this study was carried out to investigate the prevalence and intensity of *P. falciparum* infection and the efficiency of RDT and microscopy in malaria diagnosis.

MATERIALS AND METHODS

Area of study

The study was conducted in some randomly selected north western states of Nigeria during the period of April-August 2013. The states are Kano, Kaduna, Katsina, Kebbi and Jigawa States,. Malaria is meso to hyper-endemic in the whole states and it is seasonally transmitted, with the main peak of transmission from early June to late August and second/minor peak from early October to mid November (9). *Plasmodium falciparum* is the most common malaria causing species ~~(4).~~ (10).

Ethical Consideration

Scientific and Ethical permit/clearance were obtained from Ministries of Health/Hospital Management Board (MOH/HMB) of Kano, Kaduna, Katsina, Kebbi, and Jigawa State before commencement of the research.

Participation Consent

Written/informed consent was obtained from patients prior to recruitment into the study. Consent for the children was provided by the parents/guardians.

67 **Blood Collection**

68 Venous blood samples were collected for *Plasmodium falciparum* screening, from the subjects in
69 the hospitals by clinicians and medical laboratory scientists after obtaining their bio data. About
70 2-5 ml of blood was then drawn (venepuncture) with a sterile disposable syringe and transferred
71 into sample bottles containing EDTA as anti-coagulant and examined within six hours of
72 collection.

73 **Screening blood samples for *P. falciparum***

74 Rapid one step malaria HRP2 Rapid test was used according to the manufacturer's instruction, to
75 determine samples positive for *Plasmodium falciparum.* Using the supplied plastic pipettes, one
76 drop (5µl) of the blood sample was collected and added to the well labeled S. Two drops (60µl)
77 of the assay buffer then added to well labelled A. The result was then read after 20-30mins; the
78 presence of two (2) colour bands, test and control indicates positive result while one (1) band,
79 control only indicates negative result.

80 **Microscopic Examination**

81 Both thin and thick smears of each blood sample were prepared for the microscopy. The blood
82 films were allowed to dry thoroughly, especially the thick film and Giemsa stained as described
83 by WHO,(11). Light microscope was used to examine the blood films. A drop of oil immersion
84 was placed on each of the films and viewed using X100 power objective of the microscope (12).
85 The numbers of asexual parasites per 200 white blood cells (WBCs) were counted and parasite
86 intensities were computed assuming a mean WBC count of 8,000/ µl. A slide was defined as
87 negative if no asexual forms were found after counting 1,000 WBCs (13). Thin films were used

88 for the species identification of *Plasmodium* parasites. Two microscopists differently carried out
89 the microscopy to ascertain the results.

90 **Sensitivity and Specificity of Rapid Diagnostic Test (RDT) and Microscopy**

91 The following formulae were used to calculate the sensitivity and specificity of the two (2)
92 diagnosis methods.

93 Sensitivity = TP / (TP + FN)

94 Where TP = True positive, FN = False negative

95 Specificity = TN / (FP + TN)

96 Where TN = True negative, FP = False positive

97 **Data Analysis**

98 Chi-square was used to determine any significance association in prevalence between the states,
99 gender, age, and intensity at $P \leq 0.05$. Paired T-test was used to determine significant association
100 between Rapid Diagnostic Tests (RDT) and microscopy, specificity and sensitivity of the two
101 methods.

102 **Results**

103 **Prevalence of *Plasmodium falciparum* in the study area**

104 *Plasmodium falciparum*, which causes the most serious form of malaria especially in sub
105 Saharan Africa, was studied in this work, among the population of North Western part of

106 Nigeria. *Plasmodium falciparum* was the only species found in causing malaria in the population

107 with a high prevalence of 56.5% using MIC and a low prevalence (18.6%) using RDT (Table 1).

108 Table1. Prevalence of *Plasmodium falciparum* in the study area.

State	Number Examined	RDT	MIC
		Number Positive (%)	Number Positive (%)
Kebbi	300	51 (17.0)	228 (76.0)
Katsina	301	94 (31.2)	188 (62.5)
Jigawa	269	35 (13.0)	136 (50.6)
Kaduna	300	52 (17.3)	147 (49.0)
Kano	300	41 (13.7)	132 (44.0)
Total	**1470**	**273 (18.6)**	**831 (56.5)**
	Chisquare	42.956	80.578
	Df	4	4
	P value	0.000**	0.000**
Paired T-test Between RDT and MIC			
	T- value	Df	P value
	-6.792	4	0.002**

109

110 RDT- Rapid Diagnostic Test, MIC- Microscopy, ** - Highly significant at P≤0.00)

111

112 Based on gender the *P.falciparum* was more prevalent among the males' 19.4% (129/665) by
113 RDT method and by microscope 58.0% (386/665). However there was no significant difference
114 between the two methods and the sexes P≥0.5 (Table 2).

115 Table 2: Prevalence of *Plasmodium falciparum* by gender

Gender	Number Examined	RDT	MIC
		Number Positive (%)	Number Positive (%)
Male	665	129 (19.4)	386 (58.0)
Female	805	144 (17.9)	445 (55.3)
Total	**1470**	**273 (18.6)**	**831 (56.6)**
	Chi square	0.549	1.133
	df	1	1
	P value	0.459ns	0.287ns
	Odd Ratio	1.105	1.119
	C.I.	0.849 - 1.438	0.910 - 1.377

116 ns – not significant at P≥0.05

117
118 Based on age, the highest *P. falciparum* prevalence was observed among age group 1-5 years
119 27.0% (105/389) and 59.6% (232/389) using RDT and MIC methods of diagnosis respectively.
120 The least prevalence 9.5% (23/241) was in the age group of above 40 years of age by RDT and
121 51.6% (127/241) among age group of 6-15 years by microscopy. Differences in prevalence by
122 the RDT was significant (P<0.1) but not significant by microscopy (P >0.5) (Table 3).

123 Table 3: Prevalence of *Plasmodium falciparum* by age

Age	Number Examined	RDT	MIC
		Number Positive (%)	Number Positive (%)
1 - 5 years	389	105 (27.0)	232 (59.6)
6 - 15 years	245	49 (20.0)	127 (51.8)
16 - 25 years	288	52 (18.1)	152 (52.8)
26 - 40 years	307	44 (14.3)	186 (60.6)
>40 years	241	23 (9.5)	134 (55.6)
	1470	**273 (18.6)**	**831 (56.6)**
	Chi square	35.259	7.517
	Df	4	4
	P value	0.000**	0.111ns

124 ns – not significant at P≥0.05, * *- significant at P≤0.01.

125 **Intensity of *Plasmodium falciparum***

126 The highest *P. falciparum* infection intensity (+++) 9.6% (29/301) was seen in Katsina State,
127 lowest in Kebbi State 0.66% (2/300), followed by moderate intensity (++) 27.9% (84/301) in
128 Katsina state and lowest in Kano State 10.0% (30/300), while the highest lowest intensity
129 (+)58.0% (174/300) was observed in Kebbi State and lowest 24.92% 75/301). These differences
130 were highly significant (P<0.1) (Table 4)

131 Table 4: Intensity of Plasmodium falciparrum

132

Location	No. Examined	Intensity (%)		
		+ve	++ve	+++ve
Kano	300	95 (31.67)	30 (10.00)	7 (2.33)
Kaduna	300	102 (34.00)	37 (12.33)	4 (1.33)
Katsina	301	75 (24.92)	84 (27.91)	29 (9.64)
Kebbi	300	174 (58.00)	47 (15.67)	2 (0.66)
Jigawa	269	96 (35.69)	27 (10.04)	13 (4.83)
Total	**1470**	**542 (36.87)**	**225 (15.31)**	**55 (3.74)**
Chisquare	91.777			
Df	8			
P value	0.000**			

133

134 **Sensitivity and specificity of RDT and MIC methods of diagnosis**

135 In terms of specificity and sensitivity of the RDT and MIC methods of diagnosis employed, RDT
136 was found to be more specific (68.21%) and least sensitive (23.04%) while MIC was more
137 sensitive and least specific (53.38%). Significantly high difference ($P \leq 0.01$) was observed
138 between the sensitivity and specificity of the two methods (Table 5).

Table 5 ~~4.7~~: Sensitivity and Specificity of RDT and Microscopy Diagnosis

Diagnostic Test	Sensitivity	Specificity
RDT	23.04%	68.21%
MIC	47.68%	53.38%

Chi-square	df	P value
10.169	1	0.001**

** - highly significant at P≤0.01.

Discussions

Plasmodium falciparum was the only species found in infecting people in the study area. This corroborates WHO reports that *Plasmodium falciparum* is the most common species in Nigeria (14, 15).

The *Plasmodiun falciparum* prevalence of 56.5% obtained in the study area is regarded as high, according to WHO endemicity classification, and suggests hyperendemicity (9). Generally malaria prevalence has been observed to vary across Nigeria. Lower prevalences of 36.1% and 36.6% were observed in Abia State (southeast) and Plateau (north central) states respectively (16). High prevalences of 72% and 70% among pregnant women have been reported in Oyo State, by Adefioye (17) and Gabon (18) respectively. A high prevalence of 76% was also reported by Aribodor (19) in Anambra State, south east Nigeria. These findings have generally showed that Nigeria has a high prevalence of malaria which is a leading cause of morbidity and mortality in the country (20, 21).

The findings that the prevalence of infection in males was not significantly higher than in females suggest that both males and females stand equal chance of being infected regardless of

the gender. This is in agreement with the observation of (22), in similar study in southeastern Nigeria which showed that males were more infected than the females. Moreover Gilles and Warren (23) noted that there is no scientific evidence to prove that higher prevalence is influenced by gender.

The age group of 1-5 years had a significantly high prevalence of *P. falciparum* than the rest of the age groups. This may be attributed to low immunity in the children within this age group as shown by WHO (15), that low immunity in children leads higher prevalence of malaria within that age group. These results are also in agreement with those of (15, 19, 24).

It was observed that Kebbi State had highest prevalence of *P. falciparum* and Katsina State had highest intensity of infection (+++) 9.6%. This implies that in Katsina State, the infection is not as widespread as in Kebbi State, and the parasites are more concentrated in Kebbi State than in Katsina State. Just as observed in prevalence of *P. falciparum* infection according to gender, although the intensity was higher among males than the females, the difference was not significant. This implies the physiological differences in males and female does not have any effect on the intensity of infection. This is in line with (25) who obtained a higher parasite load of 24.8 x 103parasites/microlitre of blood among the males than the females with 4.9 x 103 parasites/microlitre of blood.

178 Based on age, all the ranges of the *P. falciparum* intensity (+++, ++, and +) were higher among
179 age group 1-5 years and lower among the older age groups with significant difference. This
180 result could be due to low maternal immunity in the children within that age group. It also
181 showed that as children grow older, after continued exposure from multiple infections with
182 malaria parasites over time, they build up and acquire immunity becoming relatively protected
183 against disease (26). It is also in line with the work of (27) and (28), which showed high
184 prevalence and intensity among 1-5 age groups. However, (29) obtained a higher prevalence of
185 asymptomatic infection among children aged 6 -10 yr in the study population. Also the work of
186 Millicent and Uyaiabasi (30), showed higher prevalence of malaria infection among age groups
187 5-15 years.

188

189 The sensitivity of the RDT was found to be affected by the parasite intensity of the samples as
190 has been the case of Katsina and Kebbi States. Although highest RDT positive result was seen in
191 Katsina State samples (31.2%) and Kebbi State was highest in terms of prevalence level as per
192 microscopy, the highest parasite intensity value was quite low (0.7%) as compared to Katsina
193 State value (9.6%). This finding was in line with the work of Vander Jact *et al.,(*31), who showed
194 that while the microscopy of a thick blood film has a remarkable sensitivity capable of detecting
195 parasitaemia as low as 0.0001% (5 parasites/µl of blood or one parasite/100 thick film fields – 1
196 +), the best antigen detection assays described have a maximum sensitivity of 0.01 – 0.001%
197 (5000, 500 parasites/µl of blood – 3 +) parasitaemia and are 5- 10 times inferior to good quality
198 microscopy. A similar study carried out to compare microscopy and RDT sensitivity, only those
199 samples with 4+ and 3+ parasite intensity showed positive results with the RDTs (32).

Conclusions

Plasmodiun falciparum is endemic 56.5% in the study area with a significantly high intensity of the infection which is influenced by age, but not by gender. There is also a high significant difference between RDT and microscopy, RDT was found to be more specific (68.21%) and least sensitive (23.04%) while MIC was more sensitive (47.68%) and least specific (53.38%). However these differences require further investigation under different conditions and transmission settings.

References

1. Nevill CG .Malaria in sub-Saharan Africa. *Social Sciences Medicine*.1990.31:667–669.
2. Kebede S, Aseffa A, Medhin, G, Berhe N, & Velavan TP. Re-evaluation of microscopy confirmed *Plasmodium falciparum* and *Plasmodium vivax* malaria by nested PCR detection in southern Ethiopia. *Malaria Journal*, 2014. 13:48.
3. O'Meara WP, Mangeni JN, Steketee R & Greenwood B. Changes in the burden of malaria in sub-Saharan Africa. Lancet Infectious Diseases. 2010:10:545–555.
4. World Health Organization. Assessment and monitoring of antimalarial drug efficacy for the treatment of uncomplicated *falciparum* malaria 2014.
5. Federal Ministry of Health (FMOH). National Antimalarial Treatment Policy. FMOH, National malaria and Vector Control Division, 2005: Abuja, Nigeria
6. Oliver M, Develoux M, Abari A C, & Loutan L. Presumptive diagnosis of malaria results in a significant risk of mistreatment of children in urban Sahel. Transactions of the Royal Society of Tropical Medicine & Hygiene.1991: **85**:729–730.

7. Chansuda W, Maize JB, Sinuon M, Awalludin S and Walther HW. A review of malaria Diagnostic Tools: Microscopy and Rapid Diagnostic Test (RDT). Am J. Trop. Med. Hyg. 2007. 7 (6) 119-127

8. Amexo M, Tolhurst R, Barnish,G, Bates, I: Malaria misdiagnosis: effects on the poor and vulnerable. Lancet. 2004, 364. 6736(04)17446-1.

9. World Health Organization. Control of tropical diseases: (1992) WHO malaria report 4-14.

10. World Health Organization. Guidelines for the treatment of malaria. (2013) 2nd Ed. Geneva, World Health Organization

11. World Health Organization Expert Committee on Malaria, 20th Report, WHO Technical Report Series, (2000).Geneva 892.

12. Cheesbrough M. District laboratory practice manual in Tropical Countries. 2000. pt 2. Cambridge University press.

13. World Health Organization: Guidelines for the treatment of malaria second edition. [http://www.who.int/malaria/publications/atoz/9789241547925/en/index.html].2010 Geneva.

14. World Health Organization. Expert committee on malaria: 2012.Twentieth report Geneva Switzerland

15. World Health Organization.World Malaria Report. Geneva, Switzerland: Fact sheet; 2013.

16. Noland, G.S, Graves, P.M, Sallau, A., Eigege, A., Emukah, E. and Patterson, A.E. (2010). Malaria prevalence, anemia and baseline intervention coverage prior to mass net distribution in Abia and Plateau States, Nigeria", BMC Infectious Diseases, 14: 168.

17. Adefioye OA,Adeyaba OA, Hassan WO,Oyeniran OA. Prevalence of malaria parasite infection among pregnant women in Ogsogbo, Southwest, Nigeria. Ame Eus J.Sci. Res. 2007.2:43-45
18. Marielle K B, Denisa E I, Modeste M, Eric K, Pierre-Blaise M, Elie M, and Maryvonne K. Prevalence of *Plasmodium falciparum* infection in pregnant women in Gabon. 2003 Malaria Journal **2**:18. **DOI:** 10.1186/1475-2875-2-18.
19. Aribodor UD, Njoku OO, Eneanya CI & Onyali IO. Studies on prevalence of malaria and management Practices of the Azia community, Ihiala LGA, Anambra State, Southeast Nigeria. Nigerian Journal of Parasitology. 2003. **24**:33-38.
20. Sowunmi,A, Salako LA, Walker O, & Ogundahunsi OA. Clinical efficacy of Mefloquine in children suffering from CRPF in Nigeria. Transactions of Royal Society and Tropical Medicine and Hygiene. 2004. 84: 761-764.
21. Ademowo OG, Nneji CM & Adedapo AD. In vitro antimalarial activity of methylene blue against field isolates of *Plasmodium falciparum* from children in Southwest Nigeria. Indian Journal of Medical Research. 2006. 126:45-49.
22. Ibekwe AC, Okonko IO**,** Onunkwo AI, Ogun AA, & Udeze AO. Comparative Prevalence Level of *Plasmodium* in Freshmen (FirstYear Students) of Nnamdi Azikwe University in Awka, South-Eastern,Nigeria. Malaysian J. Microbiol. 2009. 5(1): 51-54.
23. Gilles HM ,& Warrell DA .Diagnostic Methods in Malaria. In Bruce-Chwatt's Essential Malariology. 1993. (Eds) London 78-95.
24. Ukpai OM, Ajoku EI. Prevalence of malaria in Okigwe and Owerri areas of Imo state. Nig. J. Parasitol. 2002.22(1 & 2): 43-48.

25. Umeanaeto PU., Dioji V C., Ifeanyichukwu MO., Onyido AE., Irikannu KC., Okwelogu IS. Malaria and Hepatitis B virus co-infection in Enugwu-ukwu community, Njikoka local Government Area, Anambra State, Nigeria. Ewemen Journal of Epidemiology & Clinical Medicine . 2007. 2(1) 21-28.

26. Plebanski, M. and Hill, A.V. (2000). The Immunology of Malaria Infection. Current Opinion Immunology 12(4): 437-441.

27. Oyeyi TI, Muhammad R H., and Muhammad A D. Slide Positivity rate of Malaria among Patients attending two Hospitals in Kano Metropolis. Bayero Journal of Pure and Applied Sciences, 2(2): 194 – 196.

28. Abdullahi, K., Abubakar, U., Adamu, T., Daneji, A. I., Aliyu, R. U. Jiya, N., Ibraheem, M. T. O. and Nata'ala, S. U. (2009). Malaria in Sokoto, North Western Nigeria. African Journal of Biotechnology Vol. 8 (24), pp. 7101-7105.

29. Rupashree, S., Igboanua, I. G., Sanjay S., Rajendra, B. S., Nike, T. I. and Ukatu V. E.(2014). High prevalence of asymptomatic malaria in apparently healthy school children in Aliero, Kebbi state, Nigeria Journal of Vector Borne Disease *(*51)128–132.

30. Millicent, L. U. and Uyaiabasi G N. (2015) Prevalence of Malaria in Patients Attending the General Hospital Makarfi, Makarfi Kaduna State, North-Western Nigeria. American Journal of Infectious Diseases and Microbiology, 3 (1), pp 1-5.

31. Vander Jagt T A Ikeh E I Ujah IOA Belmonte J Glew R H Vander Jagt DJ. Comparison of the OptiMAL rapid test and microscopy for detection of malaria in pregnant women in Nigeria. Tropical Medicine and International Health. 2005. 10: 39 -41.

32. Peletiri IC, & Ibecheozor NK. Malaria Status amongst some Patients at an Elite Hospital in Abuja, Nigeria. International Journal of Malaria Research Revision, 2013. 1: 12-21.