

Investigating the *Plasmodium falciparum* infection 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 the diagnosis of *P. falciparum* infections.

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

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 (CareStart™) was carried out according to the manufacturer's instructions to determine presence of *Plasmodium falciparum* and also examined by the Giemsa stained thick and thin blood smears using a light microscope.

Results: A total of eight hundred and thirty four 834(56.73%) cases were positive microscopically while two hundred and eighty seven 287(19.52%) were positive with the RDT which showed high significance ($p<0.001$) in the efficiency of the diagnostic methods. Low intensity (+) was higher in 542 (36.87%) and statistically different within the states ($p<0.001$). 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 ($p<0.001$) between the two methods.

Conclusion: The study area was found to be *P. falciparum* endemic with some degree of intensity; microscopy remains the most reliable method for 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* is 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 in Nigeria has been hampered by lack of trained technicians, short supply of reagents and the delay in obtaining results in most

health facilities (7). These shortcomings of microscopic diagnosis favoured the reliance on rapid diagnostic test (RDT) for malaria diagnosis, where some facilities had to confirm the results using 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 intensity of *P.falciparum* infection and the efficiency of RDT and microscopy in malaria diagnosis being the most affordable when compared to recent technology like the PCR.

MATERIALS AND METHODS

Area of study

The study was conducted in some randomly selected North West States of Nigeria, these were Kano, Kaduna, Katsina, Kebbi and Jigawa States, during the period of April-August 2013. 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. These transmission periods corresponds to the rainy and dry season when the mosquito population is high (8). *Plasmodium falciparum* is the most common malaria causing species (4).

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

65 Written/informed consent was obtained from patients prior to recruitment into the study. Consent
66 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 malariaHRP2 Rapid Test (CareStartTM Malaria HRP2) was used according to the
75 manufacturer's instruction, to determine samples positive for *Plasmodium falciparum*.

76 **Microscopic Examination**

77 Both thin and thick smears of each blood sample were prepared for the microscopy. The blood
78 films were allowed to dry thoroughly, especially the thick film and Giemsa stained as described
79 by WHO,(10). Light microscope was used to examine the stained blood films. A drop of oil
80 immersion was placed on the each of the films and viewed using X100 power objective of the
81 microscope (11). The number of asexual parasites per 200 white blood cells (WBCs) was
82 counted and parasite densities were computed assuming a mean WBC count of 8,000/ μ l. A slide
83 was defined as negative if no asexual forms were found after counting 1,000 WBCs (12). Thin
84 films were used for the species identification of *Plasmodium* parasites. Two microscopists
85 differently carried out the microscopy to ascertain the results.

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

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

89 $\text{Sensitivity} = \text{TP} / (\text{TP} + \text{FN})$

90 Where TP = True positive, FN = False negative

91 $\text{Specificity} = \text{TN} / (\text{FP} + \text{TN})$

92 Where TN = True negative, FP = False positive

93 **Statistical Analysis**

94 Prevalence was calculated and expressed as percentage. Paired T-test was used to determine
95 significant association between Rapid Diagnostic Tests (RDT) and microscopy, specificity and
96 sensitivity.

97 **Results and Discussions**

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

99 *Plasmodium falciparum* was the only specie of *Plasmodium* found in the study population. It is
100 not surprising that *Plasmodium falciparum* was 100% found among the study subjects, as is the
101 most common species in found Nigeria (13, 9). However the finding was slightly higher than the
102 93% prevalence of *falciparum* found by Christopher and Alexandra (14), in a similar study in
103 Southwestern Nigeria.

The overall prevalence of *P.falciparum* as determined by microscopy in the study area was 56.6% (831/1470). The highest prevalence was observed in Kebbi state 76.0% (228/300) while the lowest 44.0% (132/300) was observed in Kano state. While the prevalence of *P. falciparum* malaria determined by Rapid Diagnosis Test (RDT) was 18.6%. (273). Highest in Katsina state 31.2% (94/300) and the lowest 13.67% (41/300) was obtained in Kano state. High significant difference ($P < .00$) was observed between the states as well as between the two methods of diagnosis. These results are shown on Table 1.

For a severe disease like malaria the *P. falciparum* prevalence of 56.6% obtained was high and suggesting hyperendemicity (8). Generally malaria prevalence varies across Nigeria, from lower prevalence of 36.1% and 36.6% in Abia state (Southeast) and Plateau (North central) respectively (15), to a high prevalence of 72% among pregnant women in Oyo state, Nigeria (16). Also a prevalence of 76% was also reported by Aribodor *et al.* (17) in Anambra State, South East Nigeria, continued to show that Nigeria is known for high prevalence of malaria and it is a leading cause of morbidity and mortality in the country (18;19).

The prevalence by RDT was very low 18.6%, showing the inefficiency of the RDT in capturing the endemicity of the disease among the study populations, although some studies have shown high efficacy of the RDTs (20; 21), but it is an established fact that different brands have different sensitivity and specificity and heat stability, especially under field conditions (22). The brand name (care start), used in this study was the only brand used officially by all the visited health facilities at the time of the research. Therefore the fear of under diagnosis of malaria in the study area is possible especially where the confirmation is not supplemented with microscopy.

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Table1.Overall 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**

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RDT- Rapid Diagnostic Test, MIC- Microscopy, ** - Highly significant at $P \leq 0.00$)

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Based on gender the *P.falciparum* was more prevalent among the males' 19.4% (129/665) by

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RDT method and by microscope 58.0% (386/665). However there was no significant difference

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between the two methods and the sexes $P \geq 0.05$ (Table2). *P. falciparum* was more prevalent

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among the males 58.0% but no statistical difference ($P \geq 0.05$), this shows that both males and

females stand an equal chance of being infected by the *Plasmodium falciparum*. This is in agreement with the work of Ibekwe (23), in similar studies in Southeastern Nigeria. Furthermore a study by Gilles and Warren (24) showed that there is no scientific evidence to prove that higher prevalence is influenced by gender.

Table 2: Prevalence of *Plasmodium falciparum* by gender

Sex	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

ns – not significant at $P \geq 0.05$

Age wise, the highest *P. falciparum* prevalence was observed among age group 1-5 years 27.0% (105/389) and 59.6% (232/389) using RDT and MIC methods of diagnosis respectively. The least prevalence 9.5% (23/241) was in the age group of above 40 years of age by RDT and

143 51.6% (127/241) among age group of 6-15 years by microscopy. Differences in prevalence by
144 the RDT was significant ($P < .00$) but not significant by microscopy ($P > .11$) (Table 3).

145 The age group of 1-5 years showed a highly significant difference ($p < .01$) of *P. falciparum*
146 prevalence; a contributing factor to this, may be due to low immunity of the children during
147 childhood, as has been shown by WHO, (9). This results are also in agreement with those of (25,
148 17). Also Adefioye et al (26) reported a very low prevalence of 0.7% among children less than
149 five years attending a local health facility in Osogbo Southwest Nigeria.

150 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

ns – not significant at $P \geq 0.05$, * *- significant at $P \leq 0.01$.

Intensity of *Plasmodium falciparum*

The highest *P. falciparum* infection intensity (+++) 9.6% (29/301) was seen in Katsina state, lowest in Kebbi state 0.66% (2/300), followed by moderate intensity (++) 27.9% (84/301) in Katsina state and lowest in Kano state 10.0% (30/300), while the highest lowest intensity (+)58.0% (174/300) was observed in Kebbi state and lowest 24.92% 75/301). These differences were highly significant ($P < .00$) (Table 4.4)

A significantly high difference ($P < 0.00$) in the *P. falciparum* infection intensity (+++) 9.6% was observed in Katsina state, even though Kebbi state was the most malaria prevalent state 76.0%. This shows that the disease is most prevalent in Kebbi state but intense in Katsina state and suggests that high infection prevalence does not necessary mean high infection intensity. The positivity of the RDT was found to be directly proportional to the parasite intensity of the samples as has been the case of katsina state and Kebbi state. Although highest RDT positive result was seen in Katsina state samples (31.2%) and Kebbi state was highest in terms of prevalence level as per microscopy, the highest parasite intensity value was quite low (0.7%) as compared to katsina state value (9.6%).

Table 4.4: Intensity of *Plasmodium falciparum*

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**			

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168 Sensitivity and specificity of RDT and MIC methods of diagnosis

169 In terms of specificity and sensitivity of the RDT and MIC methods of diagnosis employed, RDT
 170 was found to be more specific (68.21%) and least sensitive (23.04%) while MIC was more
 171 sensitive and least specific (53.38%). Significant difference ($P \leq 0.01$) was observed between the
 172 sensitivity and specificity of the two methods (Table 4.7).

173 This finding was in line with the work of Chansuda *et al.*, (27), showed that while the microscopy
 174 of a thick blood film has a remarkable sensitivity capable of detecting parasitaemia as low as
 175 0.0001% (5 parasites/ μ l of blood or one parasite/100 thick film fields – 1 +), the best antigen
 176 detection assays described have a maximum sensitivity of 0.01 – 0.001% (5000, 500 parasites/ μ l
 177 of blood – 3 +) parasitaemia and are 5- 10 times inferior to good quality microscopy. A similar
 178 study by Peletiri and Ibecheozor (28) reported that of the 26 593 (32.9%) malaria positive cases
 179 encountered, 506 (1.9%) had parasite density of > 5000 parasites/ μ l of blood (4+); 970 (3.6%)
 180 had between 500 – 5000 parasites/ μ l of blood (3+); 3744 (14.1%) had between 50 – 500
 181 parasites/ μ l of blood (2+); while 21 373 (80.4%) had between 5-50 parasites/ μ l of blood (1+
 182 patients having between 5 – 396 parasites/ μ l of blood (1+, 2+) in them would give a negative
 183 malaria result.

184 Table 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 $P \leq 0.01$.		

188 Conclusion

189 The prevalence of *P.falciparum* in the study area is high (56.6%) and was influenced by age but
 190 not by gender. The sensitivity of RDT was found to be low, which could lead to under diagnosis,
 191 misdiagnosis of malaria and mismanagement of non-malarial fever. However the discordance

192 between microscopy and RDT requires further investigation under different conditions and
193 transmission settings.

194 References

- 195 1. Nevill CG .Malaria in sub-Saharan Africa. *Social Sciences Medicine*.1990.31:667–669.
- 196 2. Kebede S, Aseffa A, Medhin, G, Berhe N, & Velavan TP. Re-evaluation of microscopy
197 confirmed *Plasmodium falciparum* and *Plasmodium vivax* malaria by nested PCR
198 detection in southern Ethiopia. *Malaria Journal*, 2014. 13:48.
- 199 3. O’Meara WP, Mangeni JN, Steketee R & Greenwood B. Changes in the burden of
200 malaria in sub-Saharan Africa. *Lancet Infectious Diseases* . 2010;10:545–555.
- 201 4. World Health Organization . Assessment and monitoring of antimalarial drug
202 efficacy for the treatment of uncomplicated *falciparum* malaria 2014.
- 203 5. Federal Ministry of Health (FMOH). National Antimalarial Treatment Policy. FMOH,
204 National malaria and Vector Control Division, 2005: Abuja, Nigeria
- 205 6. Oliver M, Develoux M, Abari A C, & Loutan L. Presumptive diagnosis of malaria results
206 in a significant risk of mistreatment of children in urban Sahel. *Transactions of the Royal*
207 *Society of Tropical Medicine & Hygiene*.1991: **85**:729–730.
- 208 7. Amexo M, Tolhurst R, Barnish,G, Bates, I: Malaria misdiagnosis: effects on the poor and
209 vulnerable. *Lancet*. 2004, 364. 6736(04)17446-1.
- 210 8. WHO(1992),*Control of tropical diseases: WHO malaria report* 4-14
211
- 212 9. WHO (2013). *Guidelines for the treatment of malaria*. 2nd Ed. Geneva, World Health
213 Organization

10. WHO. Expert Committee on Malaria, 20th Report, *World Health Organisation Technical Report Series*, 2000. Geneva 892.
11. Cheesbrough M. District laboratory practice manual in Tropical Countries. 2000. pt 2. Cambridge University press.
12. WHO: *Guidelines for the treatment of malaria* second edition. 2010 [<http://www.who.int/malaria/publications/atoz/9789241547925/en/index.html>].Geneva: WHO
13. WHO. Expert committee on malaria: Twentieth report Geneva Switzerland 2012.
14. Christopher I and Alexandra BO. Plasmodium species among the inhabitants of two community, Southwestern Nigeria. *American-Eurasian J Scie Res*. 2012. 7(3): 118-122.
15. 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.
16. Adedotun AA, Salawu TO, Morenikeji OA, and Odaibo AB. Plasmodial infection and haematological parameters in febrile patients in a hospital in Oyo town south-western Nigeria.2013. 5(3) 144-148 .DOI 10.5897/JPHE12.078
17. 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.
18. 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.

19. 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.
20. Oguonu T, and Okafor HU. Comparism of clinical, microscopic and Rapid Diagnosis Test methods in *Plasmodium falciparum* malaria in Enugu, Nigeria. *Niger. Postgrad. Med. J*. 2007; 14(4):285-9.
21. Zaccheaus AJ, UKoh KE, Buseri FI, Awurtu TJ. Field evaluation of SD Biolin Rapid Malaria Diagnostic Test Among asymptomatic Malaria Infected Children in Port Harcourt, Nigeria. *Res J of Para*. 2007,2: 39-44.
22. Chiodini PL, Bowers K, Jargensen P, Barnwell JO, Grady KK, Luchavez J et al. The heat stability of *Plasmodium* lactate dehydrngenase based and histidine rich protein 2 based malaria rapid diagnostic test. *Trans. R. Soc. Trop. Med. Hyg* 2006;101:331-337
23. 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.
24. Gilles HM ,& Warrell DA .Diagnostic Methods in Malaria. In Bruce-Chwatt's *Essential Malariology*. 1993. (Eds) London 78-95.
25. Ukpai OM, Ajoku EI. Prevalence of malaria in Okigwe and Owerri areas of Imo state. *Nig. J. Parasitol*. 2002.22(1 & 2): 43-48.
26. 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.

- 257 27. Chansuda W, Maize JB, Sinuon M, Awalludin S and Walther HW. A review of
258 malaria Diagnostic Tools: Microscopy and Rapid Diagnostic Test (RDT). Am J.
259 Trop. Med. Hyg. 2007. 7 (Suppl 6) 119-127.
- 260 28. Peletiri IC, & Ibecheozor NK. Malaria Status amongst some Patients at an Elite Hospital
261 in Abuja, Nigeria. *International Journal of Malaria Research Revision*, 2013. 1: 12-21.