

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 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 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 ($P<0.0$) in the efficiency of the diagnostic methods. Low intensity (+) was higher in 542 (36.87%) and statistically different within the states ($P<0.0$). 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 ($P<0.0$) 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* 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 (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, 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 (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

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

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

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

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

Where TP = True positive, FN = False negative

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

Where TN = True negative, FP = False positive

Data Analysis

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

Results

Prevalence of *Plasmodium falciparum* in the study area

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

Nigeria. *Plasmodium falciparum* was the only species found in causing malaria in the population with a high prevalence of 56.5% using MIC and a low prevalence (18.6%) using RDT (Table 1).

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**

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

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

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 $P \geq 0.05$

Based on age, 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 51.6% (127/241) among age group of 6-15 years by microscopy. Differences in prevalence by the RDT was significant ($P < 0.0$) but not significant by microscopy ($P > 0.1$) (Table 3).

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 ($P < 0.0$) (Table 4)

Table 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**			

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%). Significant difference ($P \leq 0.01$) was observed between the
 138 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 $P \leq 0.01$.

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 *Plasmodium 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 who 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 highly significantly high of *P. falciparum* prevalence than the rest of the age groups, may be attributed to low immunity in 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).

In spite of the fact that highest prevalence of *P. falciparum* in Kebbi State, high intensity of infection (++++) 9.6% was observed in Katsina state. 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 highest in males than in 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 24.8×10^3 parasites/microlitre of blood among the males than the females with 4.9×10^3 parasites/microlitre of blood.

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

The sensitivity of the RDT was found to be affected by 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%). This finding was in line with the work of Vander Jact *et al.*, (31), who showed that while the microscopy of a thick blood film has a remarkable sensitivity capable of detecting parasitaemia as low as 0.0001% (5 parasites/ μ l of blood or one parasite/100 thick film fields – 1 +), the best antigen detection assays described have a maximum sensitivity of 0.01 – 0.001% (5000, 500 parasites/ μ l of blood – 3 +) parasitaemia and are 5- 10 times inferior to good quality microscopy. A similar study carried out to compare microscopy and RDT sensitivity, only those samples with 4+ and 3+ parasite intensity showed positive results with the RDTs (32).

Conclusions

Plasmodium 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.

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