International Journal of TROPICAL DISEASE & Health
23(2): 1-8, 2017; Article no.IJTDH.28884
ISSN: 2278–1005, NLM ID: 101632866

SCIENCEDOMAIN *international*
www.sciencedomain.org

Investigating the Prevalence and Intensity of *Plasmodium falciparum* Infection and Two Methods of Malaria Diagnosis in North-Western Nigeria

M. H. Ruqayyah[1*], I. H. Nock[2], I. S. Ndams[2] and B. D. J. George[2]

[1]Federal University Dutse, Jigawa State, Nigeria.
[2]Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author MHR designed the study and data collection. Author ISN did the statistical analysis. Authors IHN and BDJG managed the literature searches. Authors MHR and IHN wrote the first draft. Subsequent drafts were written by authors MHR and ISN. Authors IHN and BDJG reviewed the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJTDH/2017/28884
Editor(s):
(1) Wei Wang, Jiangsu Institute of Parasitic Diseases, China.
(2) Jorge Paredes Vieyra, Universidad Autonoma De Baja California, Campus Tijuana, Mexico.
(3) Giuseppe Murdaca, Clinical Immunology Unit, Department of Internal Medicine, University of Genoa, Italy.
(4) Shankar Srinivasan, Department of Health Informatics, University of Medicine & Dentistry of New Jersey, USA.
Reviewers:
(1) Daniel Griffin, Columbia University, USA.
(2) Stefan Nilsson, University of Gothenburg, Gothenburg, Sweden.
(4) Abdulraheem Ibraheem Shola, University of Ilorin, Nigeria.
(4) Kumkum Srivastava, CSIR-CDRI, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/18850

Original Research Article

Received 11th August 2016
Accepted 22nd April 2017
Published 29th April 2017

ABSTRACT

Aims: The study was undertaken to estimate the prevalence and intensity of *Plasmodium falciparum* infection, 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 conducted in public health facilities from five out of the seven states of north –western Nigeria between April and August, 2013.
The states are Kano, Kaduna, Katsina, Kebbi and Jigawa states, respectively.

**Corresponding author: Email: ruqayyahmohd@gmail.com, ruqayyahmohd@yahoo.co.uk;*

Methodology: A total of one thousand four hundred and seventy (1,470) blood samples were collected. Patients were asked to sign consent form after which 2-5 ml of blood was drawn (venepuncture) into EDTA sample bottles. Rapid one step malaria HRP2 Rapid test was carried as described by (Cheesbrough, 2016), to determine presence of *Plasmodium falciparum* and stained in Giemsa and examined by thick and thin blood smears under light microscope (X 40 objective) in oil immersion.
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<0.05) in the efficiency of the diagnostic methods. Low intensity (+) was higher in 542 (36.87%) and different significantly within the states (P<0.05).The RDT showed high specificity of 68.21% while a higher sensitivity of47.68% was observed from the microscopy results which differed significantly (P<0.05) when the two methods were compared.
Conclusion: The results of the study established that *P. falciparum* malaria was endemic in the states evaluated with certain level of intensity. Microscopy was the most specific method of *falciparum* malaria diagnosis. However discordance between microscopy and RDT requires further investigations.

Keywords: Plasmodium falciparum; microscopy; Rapid Diagnostic Test (RDT); Northwest; Nigeria.

1. 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.

2. MATERIALS AND METHODS

2.1 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 [10].

2.2 Ethical Consideration

Scientific and Ethical clearance was obtained from Ministries of Health/Hospital Management Boards (MOH/HMB) of Kano, Kaduna, Katsina, Kebbi, and Jigawa state before commencement of the research.

2.3 Participation Consent

Written/informed consent was obtained from patients prior to recruitment into the study.

Consent for the children was provided by their parents/guardians.

2.4 Blood Collection

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

2.5 Screening Blood Samples for *P. falciparum*

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

2.6 Microscopic Examination

A thin and thick smear of each blood sample was prepared for the microscopy. The blood films were allowed to dry thoroughly, especially the thick film and Giemsa stained as described by WHO [11]. Light microscope was used to examine the blood films. A drop of oil immersion was placed on each of the films and viewed using X100 power objective of the microscope [12]. The number of asexual parasites per 200 white blood cells (WBCs) was counted and intensity was computed assuming a mean WBC count of 8,000/ µl. A slide was defined as negative if no asexual forms were observed 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.

2.7 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.

Sensitivity = TP / (TP + FN)

Where,

TP = True positive, FN = False negative

Specificity = TN / (FP + TN)

Where,

TN = True negative, FP = False positive

2.8 Data Analysis

Chi-square was used to determine the relationship between prevalence and the states, gender, age, and parasite intensity. Significance was determined at $P < 0.05$.Paired T-test was used to determine significant difference between Rapid Diagnostic Tests (RDT) and microscopy, and also the specificity and sensitivity of the two methods.

3. RESULTS

3.1 Prevalence of *Plasmodium falciparum* in the Study Area

Prevalence of *Plasmodium falciparum*, which causes the most serious form of malaria especially in sub Saharan Africa, was studied among the populations of North Western Nigeria. *Plasmodium falciparum* was the only species found to cause malaria within the populations with a high prevalence of 56.5% using Microscopy (MIC) and a low prevalence (18.6%) using Rapid Diagnostic Test (RDT) as shown in Table 1.

The prevalence of *P. falciparum* was found to be higher in the male clients with 129(19.4%) positive by RDT and 386 (58.0%) by microscopy. However, there was no significant difference between the two methods of diagnosis and the gender $P > 0.05$ (Table 2).

Based on age, the highest prevalence of *P. falciparum* was recorded among the age group 1-5 years, with 105 (27.0%) clients and 232 (59.6%) positive using RDT and MIC methods of diagnosis respectively. The least prevalence 23 (9.5%) was recorded among clients above 40 years of age by RDT and 127(51.6%) among age group of 6-15 years by microscopy. Differences in prevalence by the RDT was significant ($P<0.05$) but not significant by microscopy ($P > 0.05$) as shown in Table 3.

3.2 Intensity of *Plasmodium falciparum* Infection

The highest *P. falciparum* infection intensity (+++) 29(9.6%) was recorded in Katsina state, lowest in Kebbi state 2 (0.66%), followed by moderate intensity (++) 84(27.9%) in Katsina state and lowest in Kano state 30(10.0%), while the lowest intensity (+) 174(58.0%) was higher in Kebbi State and lowest 75(24.92%) in Katsina state. These differences were highly significant (P<0.05) (Table 4).

Table 1. 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	<0.001**	<0.001**
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<0.001*

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

ns – not significant at P>0.05

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	<0.001**	0.111ns

*ns – not significant at P>0.05, * *- significant at P<0.01*

Table 4. Intensity of *Plasmodium falciparrum*

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)**
Chi square	91.777			
Df	8			
P value	0.000**			

3.3 Sensitivity and Specificity of RDT and MIC Methods of Diagnosis

In terms of specificity and sensitivity of the RDT and MIC methods of diagnosis employed, 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%). Significantly high difference (P≤0.05) was observed between the sensitivity and specificity of the two methods (Table 5).

Table 5. Sensitivity and specificity of RDT and microscopy methods of 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*

4. DISCUSSION

Plasmodium falciparum was the only species found to infect 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 *falciparum* malaria prevalence had been reported to vary across Nigeria. Lower prevalence 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 [17] and [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 chances of bitten by mosquitoes that and become infected regardless of the gender. The result is similar to what was reported by [22], in a similar study undertaken in southeastern Nigeria where males were not 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 higher prevalence of *P. falciparum malaria* 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 to 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 the study of [25] who obtained a higher parasite load of 24.8 x 103 parasites/microlitre of

blood in males than in females with 4.9 x 103 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 and acquire immunity becoming relatively protected against the 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 years 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 affected the intensity of the samples as has been the case of Katsina and Kebbi states. Although highest RDT positive result was obtained 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/µl 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/µl 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].

5. 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.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

ACKNOWLEDGEMENT

Authors wished to acknowledge the efforts of Mr Muhammad Dakata and Dr Abdullahi Sulaiman in carrying out the microscopy, and all laboratory staff of the respective hospitals where sampling was carried out.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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, Abuja, Nigeria; 2005.
6. Oliver M, Develoux M, Abari AC, 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(9448): 1896-8.
9. World Health Organization. Control of tropical diseases: WHO malaria Report 4-14; 1992.
10. World Health Organization. Guidelines for the treatment of malaria. 2nd Ed. Geneva, World Health Organization; 2013.
11. World Health Organization Expert Committee on Malaria, 20th Report, WHO Technical Report Series. Geneva. 2000; 892.
12. Cheesbrough M. District laboratory practice manual in tropical countries. Cambridge University Press. 2000;pt2.
13. World Health Organization: Guidelines for the treatment of malaria second edition; 2010.
Available:http://www.who.int/malaria/publications/atoz/9789241547925/en/index.html
Geneva.
14. World Health Organization. Expert Committee on Malaria: Twentieth report Geneva Switzerland; 2012.
15. World Health Organization. World Malaria Report. Geneva, Switzerland: Fact Sheet; 2013.
16. Noland GS, Graves PM, Sallau A, Eigege A, Emukah E, Patterson AE. Malaria prevalence, anemia and baseline intervention coverage prior to mass net distribution in Abia and Plateau States, Nigeria. BMC Infectious Diseases. 2010; 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 KB, Denisa EI, Modeste M, Eric K, Pierre-Blaise M, Elie M, Maryvonne K. Prevalence of *Plasmodium falciparum* infection in pregnant women in Gabon. Malaria Journal. 2003;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 (First year 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. (Eds.) London. 1993;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 VC, 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, Hill AV. The immunology of malaria infection. Current Opinion Immunology. 2000;12(4):437-441.
27. Oyeyi TI, Muhammad RH, Muhammad AD. Slide positivity rate of malaria among patients attending two hospitals in Kano Metropolis. Bayero Journal of Pure and Applied Sciences. 2009;2(2):194–196.
28. Abdullahi K, Abubakar U, Adamu T, Daneji AI, Aliyu RU, Jiya N, Ibraheem MTO, Nata'ala SU. Malaria in Sokoto, North Western Nigeria. African Journal of Biotechnology. 2009;8(24):7101-7105.
29. Rupashree S, Igboanua IG, Sanjay S, Rajendra BS, Nike TI, Ukatu VE. High prevalence of asymptomatic malaria in apparently healthy school children in Aliero, Kebbi State, Nigeria. Journal of Vector Borne Disease. 2014;(51):128–132.
30. Millicent LU, Uyaiabasi GN. Prevalence of malaria in patients attending the General

Hospital Makarfi, Makarfi Kaduna State, North-Western Nigeria. American Journal of Infectious Diseases and Microbiology. 2015;3(1):1-5.

31. VanderJagt TA, Ikeh EI, Ujah IOA, Belmonte J, Glew RH, 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.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/18850