Journal of Infection and Public Health (2016) 9, 471—477

http://www.elsevier.com/locate/jiph

Reliability of rapid diagnostic tests in diagnosing pregnancy and infant-associated malaria in Nigeria

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Received 27 August 2015; received in revised form 31 October 2015; accepted 10 November 2015

KEYWORDS
Malaria RDT;
Infants;
Pregnant women;
Nigeria

Summary
Background: The effective management of maternal and infant malaria requires rational and prompt diagnosis. This study aims to determine the diagnostic efficiency of malaria RDT in infants and pregnant women.
Methods: The study was conducted on infants ($n = 200$), pregnant women ($n = 80$) and non-pregnant women ($n = 100$) who were recruited from two hospitals in Lagos, Nigeria. *Plasmodium falciparum* infections were assessed in the febrile subjects by microscopic examinations of blood smears and by RDT.
Results: The lowest (44.3%) and the highest (83.3%) sensitivity (SS) values were recorded in the infants and pregnant women, respectively. Other diagnostic parameters, including the specificity (SP, 97.5%), positive predictive value (PPV, 92.1%) and negative predictive value (NPV, 72.8%), in the infants were greater than the values recorded in non-pregnant (SP = 77.5%, PPV = 83.9%, NPV = 70.5%) and pregnant women populations (SP = 65.6%, PPV = 78.4%, NPV = 72.4%). The diagnostic efficiency of malaria RDT exhibited higher sensitivity in women in early gestational stages (1st trimester = 78.6% and 2nd trimester = 88.0%) compared with those in the 3rd trimester (71.4%). The sensitivity of malaria RDT (100.0%) was significantly higher in the multigravid women than in the primigravida (78.6%) and secundigravida women (77.8%, $P < 0.05$). The sensitivity of the RDT significantly increased with the intensity of the malarial parasites ($P < 0.05$).
Conclusion: Malaria is endemic in the study populations. Malaria RDT can serve as a first-line of diagnosis for pregnant women in early gestational stages and

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http://dx.doi.org/10.1016/j.jiph.2015.11.012

multigravid women and can aid the differential diagnoses of other diseases due to its high specificity in infants.

Introduction

Malaria is a disease that is caused by apicomplexan parasites and threatens the lives of millions in sub-Saharan Africa. It has been estimated that approximately 125 million pregnant women reside in malaria-endemic areas, and 32 million of the women in these regions were at risk for malaria toward the end of last decade [1,2]. The hallmark of pregnancy-associated malaria is the presentation of placentally sequestered malaria parasite infection of the erythrocytes mediated by VAR2CSA (a member of the *P. falciparum* erythrocyte membrane protein 1 (PfEMP-1) family) [3,4]. In addition to placental parasitemia, other adverse effects of maternal malaria include maternal anemia, infant anemia and low birth weight, which can pose significant threats associated with maternal, infant and fetal death [5,6]. The disease is also being implicated as one of the major childhood killers in Africa [7]. In cases of cerebral malaria, this disease causes a high mortality rate, and 5—30% of surviving children are left with a neurological disability [8].

Microscopic examinations of stained blood smears have always been the reference standard for malaria diagnosis in resource-poor endemic areas of sub-Saharan Africa. However, this method is often compromised by poor infrastructure [9], which has prompted the need for individuals with expertise in microscopy. Other problems associated with the microscopic diagnosis of malaria parasites include poor sensitivity [10], sub-standard reagents, poor microscope maintenance, and the time-consuming nature of the process [11].

The effective management of malaria in more susceptible pregnant women and infant populations requires quick and efficient diagnoses. The World Health Organization has further reiterated the role of timely, the accurate and accessible detection of malaria parasites in reducing the malaria burden [12]. There has been wide-spread advocacy for the use of rapid diagnostic test kits (RDTs) for malaria parasites for the management of malaria in endemic areas, and some studies conducted in these areas have reported that the sensitivities of RDTs are better than that of microscopy [13,14]. Therefore, RDTs should be made a supplementary diagnostic tool to aid evidence-based decision-making in malaria treatment [15]. This diagnostic approach is simple, heat-stable and can detect malaria parasites at low levels of parasitemia [16]. One shortcoming on the use of malaria parasite RDTs that can detect histidine-rich protein 2 (HRP-2) is the problem of high false positive rates due to the detection of the HRP-2 antigen circulating in the blood more than two weeks after the infected erythrocytes have been cleared from the blood stream [17]. Moreover, false-negative results of rapid diagnostic tests for *P. falciparum* have been reported to be associated with the deletion of the histidine-rich repeat region of the hrp2 gene [18].

This study assessed the performance of malaria parasite RDTs in pregnant women and infants, which are two malaria-susceptible population groups in Nigerian urban cities.

Methods

Study area and subjects

The study subjects were recruited from two hospitals in Lagos State, Nigeria between May and August of 2014. Lagos is a mega-city in the southwestern part of Nigeria. Lagos is one of the most densely populated cities in Nigeria. The poor drainage systems characteristic of many areas in the city and the large expanse of land covered with water are the strongest predisposing factors in the city. The subjects included non-randomly recruited febrile infants, pregnant and non-pregnant women who presented themselves at the hospitals for medical consultation.

Sample size determination

The sample size was determined based on a malaria prevalence of 50.0%, which is the prevalence level that has been suggested to provide a reasonable sample size for epidemiological studies [19]. Due to resource limitations, 0.08 (8%) precision was used. The minimum sample sizes computed for the infants and adult women (pregnant and non-pregnant) were 150. The totals numbers of infants and adult women participant who were recruited were 200 and 180, respectively.

Malaria parasite diagnosis by microscopy

The study included febrile infants and women (pregnant and non-pregnant) who attended outpatient clinics for malaria parasite examinations in Ifako Ijaye General Hospital and Reddington Hospital, Lagos, Nigeria. Thick and thin blood smears prepared from venous blood were made on clean grease-free glass slides. The thick blood smears were stained (without fixing) with 10% Giemsa solution for 30 min. The thin smears were fixed in absolute methanol for approximately 2 min and then stained with 10% Giemsa solution for 30 min [20]. The stains were washed in running tap water, left to air-dry in a slanted position and then examined on a microscope under oil immersion for malaria parasites by trained medical laboratory personnel. Both the thick and thin blood films were examined over a minimum of 200 high-power fields before a patient was declared as either being negative or positive for malaria parasites. This approach enabled the detection of parasites at very low parasitemia levels ($\leq$40 parasites/μL blood) and thus increasing the chance of detecting more malaria-positive individuals.

Malaria parasites diagnosis by RDT

The FirstResponse® RDT (Premier Medical Corporation Limited, Kachigam, Daman, India) was employed for the rapid diagnosis of malaria parasites in the blood samples obtained from the subjects. This kit employs an immunochromatographic principle. The cards are coated with a monoclonal antibody that binds with the specific histidine-rich protein-2 (HRP-2) that is associated with *Plasmodium falciparum* infection. Five microliters (5 μL) of anticoagulated blood was applied to the RDT card followed by addition of two drops of buffer to form a blood-buffer mixture. The RDT result was read within 10—15 min, and the results were recorded immediately. The tests were interpreted as follows: the presence of one colored band (the C control line) within the results window indicated a negative result, and two colored bands (test line 1 and the C line) indicated *P. falciparum*. The non-appearance of a colored line in the control region with or without a colored line in the test region indicated an invalid result. For invalid results, the tests were repeated twice before a conclusion was reached.

Informed consent and ethical consideration

Oral informed consent was obtained from the adult women, and consent was obtained from the mothers of the recruited infants. Ethical approval (OOUTH/DA.326/899) was obtained from Olabisi Onabanjo University Teaching Hospital, Ogun State, Nigeria.

Inclusion criteria

All volunteering subjects and regular residents of the area were included in the study. Individuals with signs of uncomplicated malaria, such as fever ($\geq$40 °C), headache, rigor, chills and joint pain, were included in the study.

Quality control

The study research team was well trained and was continuously supervised. The RDTs were purchased centrally and stored between 4 and 30 °C according to the manufacturer's storage temperature specifications. All malaria parasite results were confirmed by another microscopist, and the assessors were blinded to the RDT results.

Statistical analysis

The data were carefully entered into an Excel spread sheet (version 2007) and transferred to GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA) for analyses. Chi square analysis was used to determine the significant differences in the malaria prevalences across the groups. Fisher's exact test was used to determine the significance differences in the proportions of the true and false RDT diagnostic results in the different population groups. Comparisons between the diagnostic performances of the RDTs in the three categories of participants were achieved by determining areas under the receiver operating characteristic (ROC) curves. *p*-values less than 0.05 were considered statistically significant.

Results

The average ages of the infants and the women were 1.8 $\pm$ 1.3 and 29.9 $\pm$ 8.6 years, respectively. The characteristics of the population and the malaria parasite infection statuses are presented in Table 1. The prevalences of *Plasmodium falciparum* according to RDT and microscopy were 19.0/39.5%, 56.0/61.0% and 60.0/61.3% in the infants and the non-pregnant and pregnant women, respectively. The geometric mean intensities (GMI) of infection were 2.8, 709.9 and 637.8 parasites/μL blood in the infants and non-pregnant and pregnant

Table 1 Population characteristics and infection statuses by microscopy and RDT.

Population		No. examined	No. positive Mic (RDT)	Prevalence (%) Mic (RDT)	GMI (parasite/μL)	*p*-Value
Infants (sex)	Male	102	41 (19)	40.2 (18.6)	2.9	0.417
	Female	98	38 (19)	38.8 (19.4)	2.7	
		200	79 (38)	39.5 (19.0)	2.8	
Non pregnant (age)	18–22	24	17 (14)	70.8 (58.3)	728.8	0.511
	23–27	20	16 (13)	80.0 (65.0)	561.9	
	28–32	21	10 (9)	47.6 (42.9)	748.8	
	33+	35	18 (20)	51.4 (57.1)	721.0	
		100	61 (56)	61.0 (56.0)	709.9	
Pregnant women						
Age	18–22	3	2 (2)	66.7 (66.7)	1414.3	0.063
	23–27	29	15 (14)	51.7 (48.3)	662.8	
	28–32	32	20 (20)	62.5 (62.5)	320.7	
	33+	16	12 (12)	75.0 (75.0)	1580.4	
		80	49 (48)	61.3 (60.0)	637.8	
Trimester	1st	22	14 (13)	63.6 (59.1)	625.3	0.870
	2nd	42	25 (27)	59.5 (64.3)	654.6	
	3rd	16	10 (8)	62.5 (50.0)	611.8	0.812
Parity	Primi	25	14 (14)	56.0 (56.0)	553.2	
	Secund	30	18 (19)	60.0 (63.3)	558.7	
	Multi	25	17 (17)	64.0 (64.0)	838.2	

Note: Mic, microscopy; RDT, rapid diagnostic test; GMI, geometric mean intensity.

Table 2 Mean parasitaema and the number of participants with true/false negative and true/false positive RDT's results.

Categories	Parasitaemia (per μL blood)	True positive (%)	False positive (%)	True Negative (%)	False negative (%)	Total	*p*-Value
Infants	3.3 ± 1.8	35 (17.5)	3 (1.5)	118 (59.0)	44 (22.0)	200	<0.0001
Non pregnant women	1756 ± 3339	47 (47.0)	9 (9.0)	31 (31.0)	13 (13.0)	100	<0.0001
Pregnant women	1490 ± 1964	40 (50.0)	11 (13.8)	21 (26.3)	8 (10.0)	80	<0.0001

women, respectively (Table 1). The highest proportion (50.0%) of true positive results was recorded in the pregnant woman population, and the lowest was recorded in the infants (17.5%). Conversely, the highest (59.0%) and lowest (26.0%) proportions of true negatives RDT results were recorded in the infants and pregnant women, respectively (Table 2). The highest false positive (13.0%) and lowest false negative rates (10.0%) were observed in the pregnant woman population.

The results regarding the diagnostic performances of the malaria RDTs are presented in Table 3. The lowest (44.3%) and highest (83.3%) malaria parasite RDT sensitivities (SSs) were recorded in the infants and pregnant women, respectively. Other diagnostic parameters, including the SP (97.5%), PPV (92.1%) and NPV (72.8%), were higher in the infants than in the non-pregnant (SP = 77.5%, PPV = 83.9%, and NPV = 70.5%) and pregnant woman populations (SP = 65.6%, PPV = 78.4%, and NPV = 72.4%; Table 3). The malaria RDT exhibited higher sensitivity in the women in the early gestational stages (1st trimester = 78.6% and 2nd trimester = 88.0%) compared with those in the 3rd trimester (71.4%). However, the specificity of the RDT was higher in the 1st trimester women (71.4%) than in the 2nd (62.5%) and 3rd trimester women (64.7%; Table 4). The sensitivity of the malaria RDT (100.0%) was significantly higher in the multigravid women than in the primigravida (78.6%) and secundigravida women (77.8%; $p < 0.05$). With the exceptions of specificity (66.7%), PPV (84.2%) and NPV (100.0%), the other diagnostic parameters were higher in the multigravida women than in the primigravida women (PPV = 78.6% and NPV = 72.7%)

Table 3 Diagnostic performance of RDT in different groups using ROC of area under curve.

Diagnostic parameters	Infants (%)	Non pregnant women (%)	Pregnant women (%)
Sensitivity (SS)	44.3	78.3	83.3
Specificity (SP)	97.5	77.5	65.6
Positive predictive value (PPV)	92.1	83.9	78.4
Negative predictive value (NPV)	72.8	70.5	72.4
Overall accuracy	76.5	78.0	76.3
Area under curve (AUG)	0.716	0.768	0.745

Note: ROC; receiver operating characteristics.

Table 4 Diagnostic performance of RDT related to pregnancy characteristics.

Diagnostic parameters	Trimester (%)			Parity (%)		
	1st	2nd	3rd	Primi	Secund	Multi
Sensitivity	78.6	88.0	71.4	78.6	77.8	100.0
Specificity	62.5	64.7	71.4	72.7	58.3	66.7
Positive predictive value	78.6	78.6	71.4	78.6	73.7	84.2
Negative predictive value	62.5	78.6	71.4	72.7	63.6	100
Overall accuracy	72.7	78.6	71.4	76.0	70.0	88.0

Note: Primi, primigravidae; Secund, secundigravidae; Multi, multigravidae.

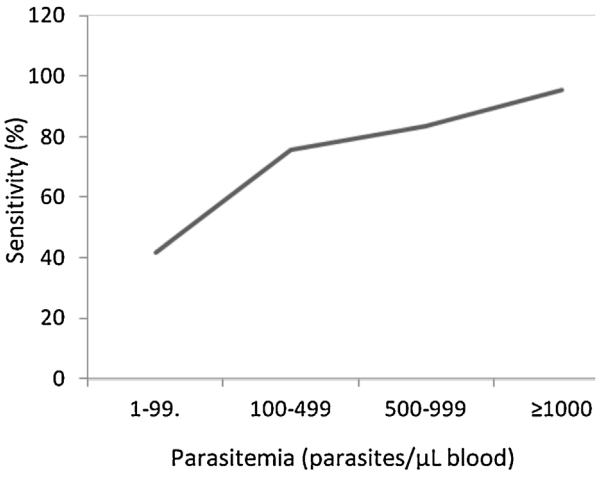

Fig. 1 Relationship between sensitivity of malaria RDT and parasite density.

and the secundigravida women (PPV = 73.7% and NPV = 63.6%; Table 4). The sensitivity of the RDT increased significantly with the parasitemia level ($p < 0.05$, Fig. 1).

Discussion

There was a high prevalence of malaria in the subjects. The prevalence of malaria in infants in this study was higher than the previous reported values for Nigeria and elsewhere in Africa [21—23]. The prevalence of malaria in pregnant women in our study was also higher than those reported in most studies conducted in Nigeria and across Africa [15,24—26].

The false negative results ranged from 10% to 22%, which is worrisome because a high proportion of the population who are infected could be left untreated if the RDT is the sole diagnostic method used to guide malaria treatment decisions. This high proportion of false negative results could be associated with low parasitemia levels as evidenced in the infant population. Similar observation have been reported in similar studies in which RDTs produced high false negative rates in patients with malaria parasite intensities below 50 parasites/μL of blood [27,28]. Other possible reason could be occurrence of mutant parasites that escape the antigenic determinant of the malaria test kit [29,30]. The false positive rate, which was as high as 13% in the pregnant women, has serious implications for control in low-resource areas. The false positive results could be due to the ability of the RDT to pick up the remnant parasite antigens in patients who were possibly on treatment and whose parasitemia had been cleared [31]. Studies have also demonstrated that false positive results could be the consequence of the sequestration of infected red blood cells in the placenta and tissue capillaries, which results in the absence of parasites in the peripheral blood but yields positive RDT results [14,32].

The sensitivities of the malarial RDT were low (≤59%) and moderate (60—89%) in infants and

adult women (pregnant and non-pregnant women), respectively. The very poor sensitivity in infants is attributable to the high proportion of false negative results due to low levels of parasitemia as discussed earlier. The 83.3% sensitivity of the RDT in the diagnosis of *P. falciparum* in the peripheral blood of febrile pregnant women recorded in this study is similar to the 83.3% reported among pregnant women in eastern Sudan [26] but significantly higher than the value recorded (31.8%) in a Ugandan febrile pregnant woman population [10]. Our results revealed a superior sensitivity (78.3%) in the non-pregnant woman population compared with a similar population group (69.0%) in Kassala in eastern Sudan [33]. The higher RDT sensitivity in pregnant women compared with the non-pregnant women recorded in this study is desirable because the placental sequestration of malaria parasites often hampers accurate microscopic malaria diagnosis during pregnancy. The specificity of the malaria RDT in infants (97.0%) was acceptable, but these values were moderate (77.5%) or low (65.6%) in the pregnant and non-pregnant women, respectively. These values in the non-pregnant and pregnant women are lower than previously reported values in sub-Saharan Africa [10,25]. The higher sensitivities of the RDTs recorded in the first and second trimesters are consistent with the higher parasite intensities that were recorded in these groups. The increase in the sensitivity of the RDT with the *P. falciparum* parasitemia level observed in this study is similar to a previous report in Nigeria [34]. The maximum sensitivity (100%) of the RDT recorded in the multigravid women is desirable because it indicated that this RDT could serve as a good alternative to microscopy in this group. The probable greater persistence of HRP-2 antigens in women of lower gravidity might have resulted in lower sensitivity due to the higher rates of false positive results [35]. One limitation of this study was the failure to perform confirmatory tests for malaria parasite diagnoses using the much more sensitive polymerase chain reaction method. Moreover, a larger sample size might have provided more reliable RDT diagnostic results.

This study demonstrated a high prevalence of malaria in the subject populations. The sensitivity of the malaria RDT was to a large extent dependent on the parasitemia level. Therefore, better diagnostic performances of the kit could have been achieved in infants with high malaria parasite parasitemia levels. This RDT could help aid the differential diagnoses of other diseases due to its high specificity, although it may not be recommended for malaria diagnosis in infants particularly when good microscopes and expert microscopists are available. This diagnostic approach can serve as first-line diagnosis in pregnant women particularly those in the early gestational stages and multigravid women.

Funding

No funding sources.

Competing interests

We declare that we have no conflicts of interest.

Ethical approval

Not required.

Acknowledgments

We wish to thank the staff of the two hospitals from which the subjects were recruited and the volunteer subjects for their co-operation and patience.

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