PARASITE-BASED DIAGNOSIS OF MALARIA IN PREGNANT WOMEN IN A TERTIARY HOSPITAL IN SOUTHWEST NIGERIA

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ABSTRACT

Background: **Malaria in pregnancy has significant adverse consequences for the mother, foetus and baby. Presumptive diagnosis continues despite recommendation for parasite-based diagnosis. We performed Paracheck-*Pf*™, an HRP-II based malaria Rapid diagnostic test (Paracheck-*Pf*RDT) and microscopy among pregnant women in a prospective, cross sectional study, at the University College Hospital in Ibadan, Nigeria.**

Methods: **The study was conducted between 2009-2011. Consecutive pregnant women presumptively diagnosed as having malaria >18 years were enrolled after obtaining written informed consent. Demographic information, symptoms and clinical measurements were obtained. Capillary blood was obtained by finger prick for thick blood smear and RDT evaluation. Summary statistics included mean (standard deviation) for quantitative variables and percentages for categorical variables. Chi-square, analysis of variance (ANOVA), the odds ratio (OR) and 95% confidence intervals (CI) were computed with p-value less than 0.05 considered statistically significant.**

Results: **Of the 746 pregnant women aged 30.9 ± 4.6 years enrolled, 243 (32.7%) were primigravida. The mean gestational age was 23.3 ± 9.2 weeks with about 81% in the second and third trimester. The prevalence of malaria parasitaemia by microscopy and Paracheck-*Pf*™ were 22.8% and 24.5% respectively. The geometric mean parasite density was 2,091/µL (range 40-156,975/µL). HIV positivity rate was 8.1% and 16.1% of patients were anaemic (PCV <30%). Women with axillary temperature >37.4^{0}C were significantly more likely to have malaria parasitaemia [p<0.0001] by microscopy. Sensitivity and specificity of Paracheck overall were 69.9% and 88.2% respectively while those at of parasite densities ≥200/µL were 84.8% and 88.7% respectively. Positive and negative predictive values were 66.9% and over 90% respectively.**

Conclusion: **RDTs are a reasonable alternative in view of the need for parasite-based diagnosis of malaria.**

Keywords: Malaria, Pregnancy, Microscopy, Paracheck-rapid diagnostic test

INTRODUCTION

Malaria in pregnancy is a major public health challenge with significant adverse consequences for the pregnant woman, her foetus, and the new-born child.[1] The adverse consequences of malaria during pregnancy include maternal anaemia, placental malaria, congenital malaria, low birth weight (LBW), preterm delivery, intrauterine growth restriction and increased infant and maternal mortality.[2-4] Pregnant women are the adult population in malaria endemic areas who bear the brunt of malaria infection and its adverse consequences. Malaria parasite infected red blood cells are characteristically sequestered in the placenta of infected pregnant women. These parasitized erythrocytes are believed to express unique variant surface antigens (VSA) that can bind chondroitin sulphate A which enable their sequestration in the placenta. Pregnant women develop antibodies to VSA with each succeeding pregnancy with the result that susceptibility to malaria reduces with subsequent pregnancies. Primigravida or secundigravidae are thus more prone to having malaria and its deleterious consequences during pregnancy.

About 30 million pregnant women are at risk of malaria in sub-Saharan Africa every year especially among primigravid, secundigravid and young women below 20 years of age.[5] Other risk factors which increase susceptibility include gestational age, HIV status, host and parasite genetics as well as intensity and stability of malaria transmission in the environment.[6-9] Malaria preventive measures if well implemented do modulate the prevalence and severity of episodes of infection.[6-9]

Malaria has been diagnosed presumptively in malaria endemic areas for a long time. This practice has continued despite the WHO recommendation that malaria diagnosis be confirmed by laboratory tests to identify the parasite or its antigen.[10,11] In presumptive diagnosis of malaria during pregnancy, the patient is diagnosed based on the presence of symptoms like fever (elevated body temperature), vomiting, severe headache, aches and pains, chills and rigors, diarrhoea and abdominal pain. These symptoms however are not specific to malaria and may have resulted from other diseases.[10]

The WHO recommends microscopy of Giemsa-stained blood smear or malaria rapid diagnostic test (RDT) for parasite-based diagnosis of malaria. Microscopy of Giemsa-stained blood smears which remains the gold standard when carried out by competent persons identifies, quantifies and speciates malaria parasites. In addition, microscopy detects parasite recurrence during follow up. However, there are many challenges for the implementation of good quality microscopy especially in malaria endemic areas. Some of the challenges are shortage of resources such as well-trained microscopists, high quality reagents, and regular electricity to power microscopes that are often in short supply. In addition, malaria microscopy is tedious and time consuming as the standard recommendation is that 200 microscopic fields must be examined before a blood smear is pronounced free of malaria parasites. Malaria RDTs on the other hand do not require extensive training or equipment, are easy to read and the results are available within 15 to 20 minutes.[12,13] These characteristics make malaria RDTs more practicable for deployment in malaria endemic regions where the burden of malaria is high and the requirements for quality assured microscopy are often not available. Malaria RDTs are immuno-chromatographic antigen-based single-use tests which detect circulating parasite antigen. Targeted antigens include *P. falciparum* histidine rich protein II (HRP-2), plasmodium lactate dehydrogenase (pLDH) and aldolase. HRP-2 is specific for *P. falciparum* while aldolase can detect all plasmodium species. pLDH on the other hand could be *P. falciparum* specific or pan malarial specific.

In sub-Saharan Africa, where there is stable and intense malaria transmission, most infections in adults, including pregnant women are asymptomatic as they have developed partial immunity against malaria. However, pregnant women are more prone to malaria infection and have increased parasite densities due to immune tolerance in pregnancy[11,12] but may not be overtly symptomatic. Thus, a high index of suspicion is essential if most cases of malaria during pregnancy is not to be missed. In an effort to control malaria in pregnancy, the WHO has recommended that pregnant women should receive intermittent preventive therapy (IPT) using sulfadoxine-pyrimethamine at specified intervals after quickening.[13,14] However, the WHO also recommends that every suspected malaria case should have parasitological diagnosis before treatment[15] as presumptive diagnosis of malaria generally leads to over-diagnosis and misdiagnosis of malaria. Presumptive diagnosis also leads to insufficient investigation of alternative causes of the presenting complaints which can have adverse consequences for the pregnant woman and her unborn child/children.[16-18] It also causes inappropriate and irrational use of antimalarial drugs which may lead to avoidable adverse drug reactions and emergence of antimalarial resistant strains of the parasite.[19]

We report here the performance of Paracheck-*Pf*™, an HRP-II based malaria RDT (Paracheck-Pf RDT) and expert microscopy for parasite-based diagnosis of malaria among pregnant women presumptively diagnosed as having malaria in the antenatal and emergency obstetrics care setting of the University College Hospital in Ibadan, southwest Nigeria where malaria transmission is intense.

METHODS

Study location

The study was conducted at the University College Hospital, Ibadan, Oyo State, Nigeria. Ibadan is located within the tropical rain forest belt of southwest Nigeria. Malaria transmission is intense all year round in southwest Nigeria with peak transmission during the rainy season months of May to October and a nadir during the dry season months of November to April. The University College Hospital is a 900-bed teaching hospital which acts as a referral centre for southwest Nigeria. There are about 90 beds in three lying-in wards, one labour ward (with about 20 beds) and an antenatal clinic which runs four days a week with 24 hours emergency cover seven days a week. The hospital also has a Prevention of Mother-to-Child Transmission (PMTCT) unit as part of an adult ARV clinic. The clinic initially supported by the HARVARD partnered President's Emergency Plan for AIDS Relief (PEPFAR) is now supported by AIDS Prevention

Initiative in Nigeria (APIN) and the government of Nigeria.

Study Population

The study population comprised of consenting pregnant women aged 18 years and above who presented at the antenatal and emergency obstetrics care setting of the University College Hospital, Ibadan, Nigeria between October 2009 and January 2011 and were presumptively diagnosed as malaria cases.

Study Design

This study was a prospective, cross sectional study, using convenience sampling method. Consecutive pregnant women presumptively diagnosed as having malaria by the obstetrics and gynaecology doctor were enrolled at the ante natal and emergency obstetric clinics after provision of written informed consent.

Data Collection and Laboratory Methods

At enrolment, information was collected using an interviewer-administered questionnaire. The questionnaire captured the enrollee's socio-economic and demographic characteristics, obstetric history, and medication history especially antimalarial drug therapy within two weeks of enrolment as well as IPTp-SP dosing. The history of blood transfusion within the same time frame and presenting symptoms and signs of current illness were also recorded. Clinical measurements recorded included weight, height, pulse rate, temperature and blood pressure of each study participant.

Capillary blood was obtained, through a finger prick using aseptic procedure, for preparation of thick blood smear as well as malaria RDT and packed cell volume (PCV) evaluation. The blood smears were air dried, stained with freshly prepared 10% Giemsa stain at pH 7.2 for 15 min using standard procedure.[20] Dried stained blood smears were viewed under a light microscope at x1000 magnification for identification and quantification of asexual stages of malaria parasites.[6, 8] A blood smear was considered positive if asexual stages of *Plasmodium specie* were identified on the thick smear, and negative if no parasite was seen after examining 100 high power fields. Parasite density was determined using standard protocol as previously described by Trape.[21] Two experienced microscopists read the slides and the mean of the two counts was recorded as the final parasite density for each study participant. For quality assurance 10% of the blood smears were randomly selected and were re-read by a different microscopist blinded to the earlier result.

Paracheck-P*f*™ (a histidine-rich protein-2-based malaria rapid diagnostic test, Orchid Biomedical Systems, Goa India) was used according to manufacturer's instruction within the duration of its shelf life. Appearance of the test and control band signified a positive result, appearance of only the control band was considered a negative result while appearance of only the test band was classified an invalid result.

To determine the packed cell volume, capillary samples were spun in a Hawksley™ micro-haematocrit centrifuge for 5 minutes at 5000g and read using a Hawksley microhematocrit reader. HIV diagnosis was offered as part of PMTCT services in the antenatal clinic. Briefly, after pre-test counselling, blood from the same finger prick was tested using rapid immunodiagnostic test kits (Determine®, Abbot). This was followed by collection of five millimetres of venous blood from reactive patients for confirmation by western Blot techniques in the hospital's HIV reference laboratory.

Blood smear results were made available at enrolment and all smear positive patients were treated according to standard of care which is 6-dose artemether-lumefantrine (Coartem™; Novartis Pharma Switzerland).

Ethical Consideration

Ethical approval for the study was obtained from the University of Ibadan/University College Hospital Ethics Committee. A signed informed consent was also obtained from each study participant.

Data Analysis

Data entry and analysis were performed using Statistical Package for Social Sciences (SPSS) version 15 (IBM-SPSS Inc., IL, USA). Descriptive statistics such as mean and standard deviation were used to summarize quantitative variables while categorical variables were summarized with proportions and percentages. Frequency tables were obtained for relevant variables. The chi-square test was used to investigate associations between two qualitative variables. Analysis of variance (ANOVA) was used to compare the mean values of more than two groups. For significant associations, the odds ratio (OR) and 95% confidence intervals (CI) were computed. A p-value less than 0.05 was considered statistically significant.

RESULTS

Seven hundred and forty-six pregnant women were enrolled between October 2009 and January 2011 at the University College Hospital, Ibadan. The enrolees were aged between 18 to 44 years with an average age of 30.9 ± 4.6 years.

Table 1: Characteristics of pregnant women suspected of having malaria between October 2009 and January 2011.

Characteristic	No. (%)
Gestational age in trimester at presentation [N (%)])	
1st trimester (up to 13 weeks)	138 (18.5)
2nd trimester (14 week-26 weeks)	305(40.9)
3rd trimester (after 26 weeks)	303 (40.60)
Gravidity (%)	
Primigravidae	243 (32.7)
Secundigravidae	209 (28.1)
Multigravida	292 (39.2)
Prevalence of malaria	
Microscopy	170/746(22.8)
Paracheck™ (Malaria RDT)	151/617 (24.5)
Geometric parasite density	
Mean	2,091/μL
Range	40-156,975/μL
Anaemia (PCV) <33% **(%)**	
No	319/591 (54.0)
Present	272/591 (46.0)
Anaemia (PCV) <30%	
No	496/591 (83.9)
Present	95/591 (16.1)
HIV status	
Positive	58/719 (8.1)
Negative	661/719 (91.9)

PCV <30% is the figure normally used in malaria endemic areas, PCV <33% is the World Health Organisation (WHO) definition for anaemia.

Most (83.2%) study participants had attained one form of post- secondary school education or another, 83.9% were gainfully employed and about 91% were in a monogamous marriage. The mean gestational age of the enrolees was 23.3±9.2 weeks with about 81% (608) in the second and third trimester. Two hundred and forty-three (32.7%) enrolees were primigravida. Further socio-demographic details are shown in Table 1. The prevalence of malaria parasitaemia by microscopy and Paracheck-*Pf*™ was 22.8% (170/746) and 24.5%

Table 2: Presenting clinical history of pregnant women suspected of having malaria at the antenatal clinic of UCH.

Characteristic	No (%)
IPTp use in index pregnancy for women in 2nd/ 3rd trimester	
Yes	243/608 (40.0)
No	365/608 (60.0)
Previous attack of malaria in index pregnancy	
Yes	263/744 (35.3)
No	481/744 (64.7)
Diagnostic method of previous attack of malaria in index pregnancy	
Clinical	109/245 (44.5)
Microscopy	136/245 (55.5)
Who prescribed antimalarial	
Medical doctor	218/249 (87.6)
Self	17/249 (6.8)
Other health workers	14/249 (5.6)
Antimalarial drug used	
Yes	184 (24.8)
No	559 (75.2)
ACT	87/259 (33.6)
None ACT	189/245 (77.1)

Table 3: Presenting symptoms of pregnant women suspected of having malaria between October 2009 and January 2011.

Symptom (n)	MP by microscopy			OR	(95% CI of OR)		p-value
	Positive	Negative	X^2 p- value		Lower	Upper	
Chills and rigors (745)							
Yes	69	103	<0.0001	2.208	1.455	3.352	.0001
No	101	471					
Vomiting (746)							
Yes	71	150	<0.0001	1.600	1.084	2.363	.018
No	99	426					
Fever (745)							
Yes	95	203	<0.0001	1.545	1.052	2.268	**.027**
No	74	373					
Headache (746)							
Yes	124	327	<0.0001	1.538	1.026	2.304	.037
No	46	249					
Abdominal pains (743)							
Yes	42	155	0.543	.750	.490	1.148	.185
No	128	418					
Cannot sleep (745)							
Yes	41	91	0.011	1.256	.791	1.994	.335
No	128	485					
Loss of appetite (744)							
Yes	75	177	0.001	1.197	.806	1.779	.372
No	95	397					
Cough (743)							
Yes	18	56	0.733	.774	.422	1.418	.407
No	151	518					
Irritability (741)							
Yes	26	52	0.017	1.158	.693	1.934	.576
No	142	521					
Aches and pains (746)							
Yes	86	239	0.036	1.066	.728	1.559	.744
No	84	337					
Diarrhoea (743)							
Yes	11	35	0.863	1.070	.678	1.690	.771
No	159	538					

(151/617) respectively. The geometric mean parasite density was 2,091/μL (range 40-156,975/μL). HIV positivity rate was 8.1 % (58/719) and 16.1% (95/591) were anaemic (PCV < 30).

History of malaria in index pregnancy and antimalarial drug use pattern

About two-fifths (40.0%, ;243/608) of the pregnant women in their second and third trimesters had received at least one dose of IPT-SP by the time of presentation while over one third (263/744; 35.3%) admitted having had a previous attack of malaria in the index pregnancy before enrolment. There was no significant association between IPT use and malaria infection (chi square = 0.664, p = 0.415). All but two of the enrolees who admitted to having had malaria during the index pregnancy reported only one previous episode. Almost half [44.5% (109/245)] of those who claimed to have had at least one episode of malaria during the index pregnancy were diagnosed presumptively while the remainder (136; 55.5%) had microscopic diagnosis. Majority of the enrolees who reported previous episodes of malaria in the index pregnancy [87.6% (218/249)] received their prescriptions for antimalarials from medical doctors, 17 (6.8 %) were self/family prescription while other cadres of health personnel prescribed the remaining 14 (5.6%). About a third of them received ACTs (87/259; 33.6%) while the vast majority (189/245; 77.1%) received non-ACT antimalarial drugs. The non-ACT

antimalaria drugs received are as follows: amodiaquine (84; 44.4%), chloroquine (27; 14.3%), sulfadoxine-pyrimethamine (17; 9.0%) and artesunate monotherapy (11; 5.8%), others (40; 21.2%).

Symptoms and their association with malaria parasitaemia

Sixty of 688 (8.7%) had axillary temperature ≥37.4^{0}C. Women with a temperature >37.4^{0}C were significantly more likely to have malaria parasitaemia [p<0.0001] by microscopy. HIV positivity rate was 8.1 % (58/719) among the pregnant women and was not significantly correlated with malaria parasitaemia. A quarter, 25.3% (24/95) of the anaemic patients were positive for malaria by microscopy, anaemia was not significantly correlated with malaria parasite (p = 0.285).

Table 4: Comparison of RDT and microscopy results of pregnant women suspected of having malaria between October 2009 and January 2011.

		Microscopy			P value
		Positive	Negative	Total	
RDT	Positive	95 (15.6%)	56 (9.2%)	151 (24.8%)	**<0.0001**
	Negative	41 (6.7%)	417 (68.5)	458 (75.2%)	
	Total	136 (22.3%)	473 (77.7%)	609	

There was no correlation between the presence of malaria parasite and parity (p= 0.690), HIV status (p=0.509), anaemia (p= 0.056) or IPT use (p = 0.416). Fever or a history of fever, vomiting, chills and rigor and headache were significantly associated with malaria parasitaemia (Table 3). Pregnant women presenting with chills and rigors were 2 times more likely to have malaria fever than those presenting with other non-specific symptoms.

Malaria parasite diagnosis by microscopy and Paracheck-Pf™

There was a significant difference between the prevalence of malaria parasitemia by microscopy and Paracheck™ (p<0.0001) (Table 5). The overall sensitivity and specificity of Paracheck were 69.9% and 88.2% respectively while for parasite densities ≥200/μL it was 84.8% and 88.7% respectively. Positive predictive value was 66.9% while the negative predictive values for the two cut off parasite densities (overall and ≥200/μL) were 91.1% and 96.3% respectively.

DISCUSSION

Malaria during pregnancy in areas with stable transmission is often asymptomatic, and also associated with non-specific symptoms.[12] Less than a quarter of the pregnant women with malaria-like symptoms in this study were confirmed positive by microscopy. This is close to the prevalence of 19.1% obtained from a study in the same hospital among HIV positive patients suspected of having malaria.[21] These values maybe attributed to the immunocompromised states of pregnancy and HIV infection which increases the susceptibility to malaria infection.[22, 23] The prevalence reported in this study however lies within that obtained from different studies among asymptomatic pregnant women across the country (7.7% to 42.3%)[7,24, 25] and also within the malaria risk range of 6.46% to 43.33% for the country as reported by Adigun *et al.* in their analysis of the 2010 Nigeria Malaria Indicator Cluster Survey.[26] This wide range is due to the different vegetation and rainfall index and thus the transmission intensity across Nigeria.[26]

The HIV positivity rate of 8.1% was higher than the value reported in the general Nigeria population. This can be attributed to the presence of an HIV treatment centre in the hospital and as such, pregnant HIV women receiving anti-retroviral treatment at the facility will also receive ante natal care at the UCH. Considering the significant overlap in the social and geographical distribution of HIV and malaria in sub-Saharan Africa and the synergistic effects of both, there should be a substantial number of co-infections of malaria and HIV.[11, 23, 27] And so a significantly higher prevalence of malaria parasitaemia was expected although this was not the finding in this study. The fact that HIV positive women in our cohort were receiving ARV may be responsible for this. About one-quarter of the anaemic patients were positive for malaria. This anaemia may be due to nutritional anaemia, worm infestation, haemodilution and chronic inflammatory processes coexisting with the pregnancy.[28] However, studies among asymptomatic pregnant women reported a significant correlation between anaemia and malaria in pregnancy.[29-31] This difference may be due to the different study populations – symptomatic pregnant women in this study and asymptomatic pregnant women in other studies.

IPT-SP has been shown to be effective in preventing maternal malaria and improve pregnancy outcome.[32,33] It is however noteworthy that less than half of the

pregnant women enrolled in our study and who were in their second and third trimesters had received at least one dose of IPT-SP. There was no significant association between IPT use and malaria infection. This is consistent with a report from Burkina Fasso that have shown that use of IPTp-SP does not reduce the risk of malaria incidence during pregnancy.[6] This maybe due to increasing prevalence of drug resistant parasites to SP.[34,35]

Although National guidelines recommend laboratory diagnosis of malaria in order to confirm the presence of malaria, and/ or treatment failure,[36] It is important to note that almost half of those who reported to have had a previous attack of malaria fever in the index pregnancy were diagnosed presumptively by clinicians. This is not surprising considering that this study was carried out between 2009 and 2011 before Nigeria adopted the parasite- based diagnosis policy in 2011. From this study, pregnant women presenting with fever or a history of fever, headache, vomiting, chills and rigors were likely to have malaria parasitaemia. Those with chills and rigors were twice more likely to be at risk of malaria parasitaemia. However, these symptoms are non-specific to malaria infection[12] and should not be used for presumptive diagnosis.[19] as this may lead to misdiagnosis and over-diagnosis of malaria. Moreover, not investigating and treating other sources of fever can increase the morbidity and mortality risk of the pregnant woman, her fetus and newborn.[17] There is therefore a need for parasite based diagnosis among symptomatic pregnant women in areas with stable malaria transmission as malaria during pregnancy presents with non-specific clinical features (symptoms & signs).[12] This fact is also evidenced by this study where about three quarters of the symptomatic pregnant women were free of patent parasitaemia. Malaria in pregnancy in endemic areas therefore demands proper diagnosis, to ensure effective treatment and proper use of antimalarial drugs.

The performance of Paracheck-*Pf*™ in this study was good and reliable especially at parasite densities >200/ iL. Although the performance was similar to the finding by Ojurongbe *et al.* (2013),[37] who reported a sensitivity of 62.3%, specificity of 87.4%, positive predictive value of 67.7% and negative predictive value of 84.5% in children, there was however a remarkable difference in the Negative Predictive Value of 91.1%. The high NPV is particularly useful in excluding malaria as a diagnosis. The slow clearance of HRP-2 is well known to lead to false positive results which leads to a reduction in specificity of HRP-2 -based malaria RDTs. In conclusion, parasite-based diagnosis is important to confirm malaria in pregnancy as malaria has non-specific symptoms and can coexist with other illnesses, treatment failure and complications. In view of the challenges associated with providing microscopy, RDTs are a reasonable alternative. While symptoms such as fever, chills/rigors, vomiting were found to be associated with malaria parasitaemia, efforts must be made to rule out other possible diseases. This will prevent misdiagnosis and over-diagnosis of malaria while ensuring effective treatment and proper use of antimalarial drugs.

Authorship and Contributions

BAA, COF conceived and designed the study, supervised data collection, and were involved in data analysis, FO data entry, analysis, interpretation and drafting of the research paper. ADA participated in the data analysis, interpretation, critical revision and preparation of the final draft of the research paper. All authors read and approved the final manuscript.

Conflict of Interest: Nil

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