Original Article

A Comparison of Peripheral Blood Smear Microscopy and Detection of Histidine-Rich Protein 2 in Blood in the Diagnosis of Malaria in Pregnancy

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Received: 26-Nov-2021;
Revision: 20-Jun-2022;
Accepted: 02-Jul-2022;
Published: 22-Sep-2022

ABSTRACT

Background: Detection of malaria in pregnancy poses a huge challenge in Sub-Saharan Africa. Accurate diagnosis enables timely and appropriate clinical management. **Aims:** This study aimed to compare the accuracy of peripheral venous blood smear microscopy (PVBSM) and a rapid diagnostic test detecting histidine-rich protein 2 in the blood (RDT-HRP2) with placental histology as the control in the diagnosis of malaria in pregnancy in Nigerian women. **Materials and Methods:** This was a comparative, cross-sectional study conducted between January and July 2017. Asymptomatic pregnant women who presented to the labor ward and who gave informed consent had peripheral venous blood samples as well as placental tissue obtained following delivery. The blood samples obtained were tested for malaria parasites using PVBSM and RDT-HRP2 in blood, while the placenta was subjected to histology. **Results:** A total of 326 patients participated in the study. The prevalence of malaria in pregnancy was 13.8%, 17.8%, and 32.8% using PVBSM, RDT-HRP2, and placental histology. The sensitivity, specificity, and accuracy of PVBSM were 40.2%, 99.1%, and 79.8% and the corresponding values for RDT-HRP2 were 49.5%, 97.7%, and 81.9%. The positive predictive value was 95.6% for PVBSM and 91.4% for RDT-HRP2 and the negative predictive value was 77.2% for PVBSM and 79.9% for RDT- HRP2. **Conclusions:** The diagnostic performances of both tests were comparable; however, RDT-HRP2 had a higher sensitivity and accuracy than PVBSM for the diagnosis of malaria in pregnancy.

KEYWORDS: *Malaria, microscopy, Nigeria, placenta histology, RDT*

INTRODUCTION

Malaria in pregnancy is frequently under-diagnosed with grave consequences partly due to the use of suboptimal diagnostic modalities.[1] The greatest burden of malaria in pregnancy is in sub-Saharan African nations including Nigeria, where it is one of the leading causes of maternal and perinatal mortality and morbidity either directly or indirectly.[2-5] The prevalence of malaria in pregnancy in Malaria endemic regions varies from 10% to 65%.[6] *Plasmodium falciparum (P. falciparum)* is implicated in the vast majority of severe diseases and complications among pregnant women because of its ability to adhere to vascular endothelium and sequester deep in vascular beds in the placenta.[7] Pregnant women in the malaria-endemic areas may experience adverse outcomes from malaria such as anemia in pregnancy, while their newborns may have low birth weight from anemia, intra-uterine growth restriction, and prematurity.[2]

The major challenge peculiar to the diagnosis of malaria is that *P. Falciparum* parasites may be present

Access this article online

Quick Response Code:

Website: www.njcponline.com

DOI: 10.4103/njcp.njcp_1962_21

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How to cite this article: Mofon CE, Ebeigbe PN, Ijomone EE. A comparison of peripheral blood smear microscopy and detection of histidine-rich protein 2 in blood in the diagnosis of malaria in pregnancy. Niger J Clin Pract 2022;25:1501-6.

in the placenta but absent or undetectable in peripheral blood.[8] This phenomenon is attributable to the accumulation of infected erythrocytes in the intervillous spaces of the placenta through specific adhesion to chondroitin sulfate A.[9] Even infections with low parasite densities undetected by standard microscopy can have severe detrimental effects on pregnant woman and her fetus.[10] Accurate diagnosis of malaria in pregnancy is thus essential for appropriate clinical management. This will ensure timely intervention as well as rational prescription of anti-malarial drugs, preventing indiscriminate drug use that might result in the development of resistance.

The WHO advocates that all suspected cases of malaria should have a parasitological diagnosis before treatment.[11] Placental histology has been referred to as the gold standard for its ability to detect sequestered parasites when the yield in peripheral blood is low. It, however, has limited clinical application, being relevant mainly in the research domain.[12] Blood smear microscopy is the most widely used method for malaria diagnosis in the tropics.[8] It involves preparation and microscopic examination of blood film stained with Giemsa, Wright's, or Field's stain.[13] Thick and thin blood film can be prepared. The thick blood film has a better sensitivity of the two techniques and is much better than the thin film for detection of low levels of parasitemia and reappearance of circulating parasites during infection, recrudescence, or relapse.[13] Rapid diagnostic tests (RDTs) detecting plasmodium-specific antigens have emerged in recent times as a viable alternative to microscopy that could extend diagnosis to low-resource areas.[10] These antigens include histidine-rich protein 2 (HRP2) which is specific to *P. falciparum* and has been shown to be present in the plasma of persons infected with falciparum malaria. *P. falciparum* histidine-rich protein (PfHRP2) is water-soluble, heat-stable protein synthesized only by *P. Falciparum* with a positive correlation between blood concentration and parasite biomass.[13] PfHRP2 is released upon schizont rupture and is thus found in the supernatants of cultured parasites and in the blood of parasite-infected individuals.[14] This enables the detection of PfHRP2 when sequestered parasites cannot be detected by microscopy.

Currently, microscopy is the most commonly used method of diagnosis but in most low-resource settings, the dearth of trained personnel and equipment makes diagnosis difficult. The RDTs are becoming the preferred diagnostic tool for malaria in pregnancy owing to the ease of use; little expertise required for use and interpretation, availability as strips, and non-dependence on electricity. Many studies have suggested that RDT-HRP2 holds the potential to replace peripheral venous blood smear microscopy (PVBSM) in the routine diagnosis of malaria in pregnancy. The objective of this study was to compare PVBSM and RDT-HRP2. The aim was to achieve this by determination of the prevalence, positive predictive value, negative predictive value, sensitivity, and specificity as well as the diagnostic accuracy of PVBSM and RDT-HRP 2 using Placenta histology as a control.

Materials and Methods

This was a comparative cross-sectional study carried out from January to July 2017. The study population consisted of 326 women who were consecutively recruited at presentation to the Labor Ward. Patients with evidence of chronic illness and pregnancy complications such as diabetes mellitus and hypertension were excluded from the study. Patients who presented with features of malaria in pregnancy were also excluded from the study.

Participants' socio-demographic characteristics including parity and gestational age were obtained using structured data proforma. Two samples were obtained from consenting patients at delivery: peripheral venous blood and placenta histology samples. About 5 ml of peripheral venous blood were obtained by venipuncture and stored in the ethylenediamine-tetraacetic acid vacutainer bottle. This blood was added directly to the kit for assessing HRP2 immediately after collection, while the remaining was used for the preparation of the thick blood film. Immediately (within the first 30 min) after delivery, two biopsy specimens of placental tissue (2 by m) were excised from the maternal surface of the placenta at opposite sites halfway between the umbilical cord insertion and placenta edge and then placed into 10% neutral buffered formalin in a universal specimen bottle.

The microscopic examination of the blood smear was carried out following standard procedure[15] by two laboratory scientists. The commercially available Carestart™ Malaria HRP RDT kit was used for this study. This was carried out according to the manufacturer's instructions. After fixation, the placental samples were transferred to the pathology laboratory. The fixed placental biopsy specimen was grossed into 2 to 3 mm sections, processed using standard procedures, and embedded in paraffin wax. The final tissue sections were stained with hematoxylin and eosin for the detection of active malaria. The histology slides were examined locally under standard light microscopy by systematically counting 500 intervillous cells. Placental malaria infection was classified using the following

Table 1: Social-demographic characteristics of participants

Variable	Frequency (*n*=326)	Percentage
Age (years)		
<20	15	4.6
20-24	57	17.5
25-29	113	34.7
30-34	96	29.4
≥35	45	13.8
Booking status		
Booked state	242	74.2
Unbooked state	84	25.8
Parity		
Nullipara	90	27.6
Primipara	94	28.8
Multipara	100	30.7
Grandmultipara	42	12.9
Level of education		
Tertiary	97	29.8
Secondary	140	42.9
Primary	70	23.3
No formal education	13	4.0

Table 2: Prevalence of malaria parasitemia/ parasitization

Test	Result	Frequency	Percentage
PVBSM	Positive	45	13.8
HRP2	Positive	58	17.8
Placenta Histology	Active acute infection	80	24.5
	Active chronic infection	27	8.3
	Past Infection	50	15.3
	No Infection	169	51.8

Table 3: Measures of diagnostic accuracy for both PVBSM and RDT-HRP2

Measures	PVBSM	HRP2
True positive	43	53
False positive	2	5
True negative	217	214
False negative	64	54
False positive rate	4.4%	8.6%
False negative rate	22.8%	20.1%
Positive predictive value	95.6%	91.4%
Negative predictive rate	77.2%	79.9%
Sensitivity	40.2%	49.5%
Specificity	99.1%	97.7%
Accuracy	79.8%	81.9%

definitions: No infection is the absence of any evidence of parasites or pigment, active acute infection is the presence of parasites in the maternal erythrocytes in the intervillous space but no/minimal pigment in fibrin/cells within fibrin, active chronic infection is the presence of parasites in maternal erythrocytes in intervillous space, and pigment in erythrocytes and circulating monocytes within intervillous space and pigment in fibrin or cells within fibrin and/or chronic villous syncytiotrophoblast/ stroma, while the past infection is the absence of parasites and pigment confined to fibrin or cells within fibrin.[15] In this study, the presence of active acute and chronic infection was taken as evidence of plasmodium placental parasitization.

Ethical approval

Ethical approval was obtained from the Hospital Research and Ethics Committee. Informed consent was obtained from all participants before recruitment into the study.

Statistical analysis

The data collected on the data proforma was collated, coded, and analyzed using the Statistical Package for the Social Sciences (SPSS) version 21.

The measures of diagnostic accuracy assessed were as follows: number of true positive samples (TP), number of true negative samples (TN), number of false positive samples (FP), number of false negative samples (FN), positive predictive value, negative predictive value, false positive rate, false negative rate, sensitivity, specificity, and accuracy.

To maintain quality and reduce the effects of inter observer variation on the results of the study, the PVBSM was carried out by two laboratory scientists who were blinded to the result of the placental histology and RDT-HRP2. The histopathologist was also blinded to the result of the PVBSM and RDT-HRP2.

Results

A total of 326 parturients who presented to the labor ward of the two study centers participated in the study and all the samples taken (100%) were adequate and analyzed. The age of participants ranged from 16 to 31 years with a mean age of 28.6 years ± 5.26 as shown in Table 1. The largest proportions of participants were booked (74.2%) and were multiparas (30.7%).

Table 2 shows the prevalence of malaria parasitemia using PVBSM was 13.8%, while the prevalence using RDT-HRP2 was 17.8%. A total of 32.8% of the participants had evidence of active (acute and chronic) placental parasitization, while over half of the participants had no evidence of placental parasitization.

Discussion

The prevalence of malaria parasitemia in this study was 13.8% using PVBSM and 17.8% using RDT-HRP2, while a prevalence of 32.8% was detected by placental

histology. This shows a marked underestimation of the burden of malaria in pregnancy by both PVBSM and HRP-RDT2 with better performance by RDT-HRP2. The placental malaria prevalence of this study is similar to that of Bako *et al*.[16] in Maiduguri who reported a placental malaria prevalence of 33.9%. Our findings however showed some variance from the findings of Ezebialu *et al*.[17] in Awka, Nigeria, who reported a higher prevalence of placental malaria (69.6%) and 59.2% with PVBSM. Unlike our finding, Bako *et al*. reported a prevalence of 30.7% using PVBSM malaria. Reports of a significantly higher level of placental malaria compared to PVBSM and RDT-HRP2 have also been documented by researchers in Cameroun and Malawi.[18,19]

The differences between the findings of this study and previous studies reflect variations in the study populations in terms of symptomatology, predisposition to malaria infection, use of malaria preventive practices, and use of chemoprophylaxis and treatment of malaria infection in the environment. The use of intermittent preventive treatment of malaria in pregnancy with sulphadoxine-pyrimethamine is routine practice in our facilities and possibly contributed significantly to our findings of lower incidence of malaria compared to other reported works. The high prevalence of placental malaria compared to other malaria screening tests may also be attributed to the fact that parasites may be present in the placenta but absent or undetectable in peripheral blood.[10]

Our findings of a higher malaria prevalence with RDT-HRP2 compared to PVBSM have also been reported by Kyabayinze in Mbale, Uganda, who found a clinical malaria prevalence of 38% using microscopy and 54% with RDTs among febrile pregnant women at ANC.[15]

The sensitivity of PVBSM in this study was 40.19% while that of RDT-HRP2 was 49.53% as shown in Table 3. These findings are similar to those of Mayor *et al*. in Mozambique who reported a sensitivity of 48% for RDT-HRP2 and 35.7% for PVBSM.[10] Kyabayinze *et al*.[15] in Uganda also reported a higher sensitivity of 80.9% with HRP-RDT2 and 76.2% for PVBSM in symptomatic patients. Kyabayinze *et al*. conducted their cross-sectional study on symptomatic patients with a temperature greater than 37.2°C and that may be responsible for the considerably high sensitivities reported. The results of this study however differ from that of Dhorda *et al*.[20] also in Uganda who reported a higher sensitivity of 36.4% for PVBSM compared to 31.8% for HRP-RDT. The higher sensitivity of RDT-HRP2 compared to PVBSM reported by this study may be attributed to the fact that HRP2 secreted by the parasites continues to circulate at a concentration that corresponds to the parasite biomass, while the parasite may be adherent to the placental vascular bed and thus not detected by microscopy.

Sadly, the results of this study indicate that a little above 50% of parturients with placental malaria will be missed by both screening tests. This puts the parturients with missed diagnosis at significant risk of complications such as maternal anemia, low birth weight, and preterm delivery.[21] RDT-HRP2 performed better than PVBSM microscopy detecting 9 extra cases in every 100 parturients tested. This becomes quite significant when larger populations are screened and would result in higher detection and treatment rates and thus fewer complications of malaria in pregnancy.

The specificity of PVBSM in this study was 99.9% and that of RDT-HRP2 is 97.71%. This indicates that both tests have a similar ability to correctly identify parturients who do not have malaria in pregnancy. This means that both PVBSM and RDT-HRP2 are very useful in the exclusion of malaria as it would rarely be positive in the absence of disease. This is especially important in cases of malaria in early pregnancy where drug safety is a major concern. Both tests will be useful in excluding malaria and reducing unnecessary drug treatment.

The positive predictive value of PVBSM is 95.56% and 91.38% for RDT-HRP2. Our findings are at variance with the findings of Minja *et al*.[22] in Tanzania who reported that PVBSM had a positive predictive value of 65.9% and 65.4% to 75% for RDT-HRP2. Their study was carried out in an area that has been transformed from a hyper-endemic area to a meso/holo endemic region and the low prevalence of malaria in pregnancy may explain their findings.[22] The high positive predictive value reflects the high diagnostic power of these two screening tests and is related to the high specificity of the tests. The positive predictive value of any screening test is known to be directly proportional to the disease prevalence in the population.[23] Malaria is endemic in the location for this study and this also explains the high positive predictive value of the screening tests.

The negative predictive value was 77.22% for PVBSM and 79.85% for RDT-HRP2. This is lower than the findings of Dhorda[20] in an area of low malaria prevalence who reported a negative predictive value of 94.5% for PVBSM and 91.9% to 100% for RDT-HRP2. The contrasting negative predictive values in both studies are a reflection of the difference in the prevalence of disease in the study population. Our finding of lower negative predictive value can be attributed to the high

prevalence of malaria in the study environment. It is established that with an increasing prevalence of disease, the positive predictive value increases, while the negative predictive value decreases.[24] The negative predictive value indicates the probability that a patient with a negative test does not have malaria.[22] This value is also a reflection of the diagnostic power of a test and is related to the sensitivity of the test as the more sensitive a test is, the better the negative predictive value.[23]

The accuracy of PVBSM in the diagnosis of malaria in pregnancy was 79.75%, while that of RDT-HRP2 is 81.90%. This is a measure of the effectiveness of both diagnostic tests and indicates the number of correctly classified subjects.[24] The accuracy reflects the proximity of the results to the true value and as shown above is higher for RDT-HRP2 than for PVBSM. Clinically, this means that RDT-HRP2 gives a higher absolute number of correctly classified results compared to microscopy and thus in an area of high malaria prevalence like ours, will detect more true positive or true negative cases of malaria than PVBSM.

This study is not without limitations. The study was based on asymptomatic participants. Since in clinical, malaria testing is conducted on ill subjects, the reliability of extrapolating the findings in this study to clinically ill subjects is not clear. The acclaimed superiority of RDT-HRP2 over PVBSM for *falciparum* malaria diagnosis is not derived from inferential statistical testing; and a similar study, designed to allow such testing, should be more informing.

Conclusion

PVBSM and RDT-HRP2 have comparable specificity, false negative rate, positive, and negative predictive values, while RDT-HRP2 had a higher false positive rate than PVBSM. RDT-HRP2 had a higher sensitivity and accuracy than PVBSM. RDT-HRP2 thus detected a higher absolute number of correctly classified results than PVBSM and thus performed better than PVBSM in the diagnosis of malaria in pregnancy. Based on the findings above, it is recommended that RDT-HRP2 can be widely introduced in the diagnosis of malaria in pregnancy due to its higher sensitivity and accuracy, cost effectiveness, and ease of use compared to peripheral blood microscopy which is the current standard diagnostic modality.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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