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Comparative Study of Malaria Diagnosis: Rapid Diagnostic Test (RDTs) Against Microscopy among Pregnant Women Attending Antenatal Clinic in Specialist Hospital, Sokoto

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Malaria is an infectious disease caused by a protozoan parasite of the genus *Plasmodium.* It was estimated that 219 million cases of malaria occur in 87 countries, with an estimated death of 435,000 in 2017 among pregnant women. Other species include *P. ovale*, *P. vivax,* and *P. malariae* is a blood parasite of human and is one of the major public health burdens in developing countries, particularly in Sub-Saharan Africa. It is estimated that about 3.5 billion people globally and 450 million people are thought to be ill as a result of such infections, the majority being children.
Aims: This study was aimed at comparing RDTs against microscopy in the detection of malaria parasite among pregnant women. Samples were collected and analyzed following cross-sectional comparative study design. It was conducted between Septembers to November 2019.
Study Design: This was a cross-sectional, comparative study

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Place and Duration of Study: This study was conducted among patients attending Specialist Hospital Sokoto, Sokoto State, between March and November, 2019.
Methodology: A total of 106 participants were enrolled for the study. Standard parasitological examination was carried out on blood samples using microscopy followed by Rapid Diagnostic test (RDTs).
Results: Finding revealed, in this study, CareStart kit had sensitivity of 77.7%, specificity of 100s%. It is expected that any RDT used for malaria diagnosis should have a high sensitivity of 95% and specificity 97% (WHO, 2003); this is in contrast with the RDTs results in this study.
The false negative (FN) Carestart and SD-Bioline kits in this study were 10% and 21%, respectively using microscopy as the gold standard due to lack of sensitivity of RDTs at low parsitaemia compared to microscopy.
The false positive rate in this study for the Carestart and SD-Bioline kits are 0% and 0% respectively. Out of 106 patients screened, 35% and 24% tested positive for *Plasmodiumfalciparum* using Carestart and SD-Bioline RDTs respectively, while 45% were positive to malaria by microscopic examination. There was high prevalence of malaria parasite among age group 18-23 which is 42.2%.
Conclusion: It can be concluded that using microscopy is more time consuming compared to RDTs due to the fact that the time taken to read the results for RDTs is within five minutes and that of microscopy is higher compared to RDTs. Over all prevalence of malaria by microscopy was 42.5% while the prevalence of malaria by Carestart and SD-bioline was 33% and 22.6% respectively. Conventional microscopy remains the gold standard compared to RDTs according to this study with the level of its sensitivity and specificity which is higher than RDTs.

Keywords: Comparative study; malaria Infection; RDTs; microscopy; pregnant women; Sokoto State; Nigeria.

1. INTRODUCTION

Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected female *Anopheles* mosquitoes. It is preventable and curable. In 2017, there were an estimated 219 million cases of malaria in 87 countries. The estimated number of malaria deaths stood at 435 000 in 2017.The WHO African Region carries a disproportionately high share of the global malaria burden. In 2017, the region was home to 92% of malaria cases and 93% of malaria deaths [1].

Malaria is an infectious disease caused by a protozoan parasite of the genus *Plasmodium* (*Plasmodium falciparum, Plasmodium malaria, Plasmodium ovale, Plasmodium vivax*) within the red blood cells. The disease is transmitted by the female anopheles Mosquito. Malaria is one of the most deadly infectious diseases and is a leading cause of death and illness worldwide especially in the tropics and subtropics [2]. There are about five known plasmodium species that can cause malaria in humans; *Plasmodium falciparum, Plasmodium ovale, Plasmodium malariae, Plasmodium vivax and Plasmodium knowlesi.* The most ubiquitous and deadly among them is *Plasmodium falciparum* [3].

According to Ugochukwu [3] the eradication of malaria especially in endemic area has posed problems in terms of diagnosis; accurate and prompt diagnosis, technical manpower, availability of reagent for test procedure. Diagnosis of the disease is more difficult in endemic area in that these areas have financial challenges and transmission of infection is quick due to poor living conditions.

Diagnosis of *Plasmodium* species is generally done by microscopic method using peripheral blood smear examination to detect intracellular malarial parasites using Romanowsky stain and test procedure is also known as “Gold standard” [1,4].

Several methods to diagnose malaria exist, each with a certain degree of accuracy. They include: Light microscopy using stains such as Giemsa, Leishman and rapid Fields stain. These are relatively cheap, readily available stains used on thick and thin blood films and remain the gold standard in the diagnosis of malaria. Acridine orange stain is expensive and unsuitable for routine diagnosis of malaria. Molecular method using Polymerase Chain Reaction (PCR) is highly sensitive but expensive. Immunological tests such as indirect Fluorescent Antibody tests (IFAT), Indirect Haemaglutination tests (IHA) and Enzyme Linked Immunosorbent Assay (ELISA) are antibody detection methods that are less sensitive and therefore not suitable for routine

diagnosis of malaria. Antigen detecting immunological methods are less expensive, more sensitive and rapid in application. This has been variously studied for its effectiveness in the diagnosis of malaria in different settings [5]. Accurate diagnosis of malaria is necessary to prevent morbidity and mortality while avoiding unnecessary use of anti-malarial agents, therefore new rapid tests methods have been developed [6]. Bell et al., [7] reported that the need for rapid and accurate detection of malaria parasites in the treatment and eradication of malaria led to the invention of Malaria Rapid Test kits. These malaria rapid diagnostic tests are based on detection of specific antigens produced by malaria parasites. These rapid test kits are mostly used in endemic areas where microscopy is not available. Microscopes are limited in number even in advanced countries. Therefore, this study was aimed at comparing RDTs against microscopy in the detection of malaria parasite among pregnant women.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in Specialist Hospital Sokoto (SHS). It is situated in Sokoto South Local Government Area of Sokoto State. Sokoto State is located in North-Western part of Nigeria and shares borders with Niger-Republic to the North, Kebbi State to the South-West and Zamfara State to the East [8].The city lies between longitude 05°42`` to 22`` East and latitude 12° 15 to 29`` North and covers a total land area of about 32,000 Square kilometres and a population of 4,602,298 million based on 2013 projection (UNFPA, 2013). Sokoto has an annual rainfall ranging from 500mm to 1300mm and the rainy season is from June to October. The dry season starts from October to February every year and is characterized by harmattan wind blowing Sahara dust over the land [8].

2.2 STUDY POPULATION

The study was hospital based and blood samples were collected from 106 pregnant women attending antenatal care in Specialist Hospital Sokoto (SHS).

2.2.1 Inclusion criteria

Pregnant women with symptoms suggestive of malaria presenting to the hospital and referred to the laboratory for malaria parasite examination was recruited for the study.

2.2.2 Exclusion criteria

Pregnant women who are on malaria drugs, those with other symptoms not suggestive of malaria and non pregnant women with symptoms suggestive of malaria were excluded from the study.

2.2.3 Sample size determination

The sample size was calculated using the standard formula for calculating minimum sample size [9].

$$R = \frac{Z^2Pq}{d^2}$$

Where;

n = Minimum sample size

Z= Standard normal deviation at 95% level of confidence = 1.96

P= prevalence rate (6.10%). [10].

q= Compliment of p i.e. 1-p = 1-0.06 = 0.939

d= Tolerance margin of error = 95% i.e. (100-95%) = 5% = 0.05

Therefore $n = \frac{(1.96)^2 \times 0.06 \times 0.939}{(0.05)^2} = 88.02 \approx 88$

Adding 20% attrition rate to the calculated sample size [11].Using attrition rate of 20 %,

$= \frac{20}{100} \times 88$

= 17.6

Total minimum sample size = 88+17.6 = 105.6 = 106

2.3 Study Design

This was a cross-sectional comparative study design. It was conducted at Specialist Hospital Sokoto. A structured-interviewer administered questionnaire was used to obtain the participants socio-demograhic and clinical information, while blood samples were taken using standard protocols.

2.4 Sampling Techniques

2.4.1 Subject selection

Subjects that satisfied the inclusion criteria were contacted through a good approach. Their written informed consent was sought for inclusion into the study. Both the physical assessment and laboratory examination was explained to them in their native language.

2.4.2 Blood sample collection and storage

From each selected subject, blood specimen was collected aseptically by venipuncture and dispensed into an EDTA container and labelled with the subjects' unique identification number. Blood samples collected were stored at 4ºC in a refrigerator until the time for analysis.

2.5 Laboratory Analytical Method

2.5.1 Microscopy method (conventional)

Procedure

From the collected blood sample, thick blood film was made by adding few drops of EDTA anticoagulated blood on a clean grease-free glass slide, then spread out into a coin size using the tip of a spreader to cover about 15×15mm and was allowed to air dried. It was then stained with 10% Giemsa stain for 10 minutes then rinsed with buffered distilled water and also allowed to air dried and examined using a light microscope at oil immersion objectives (×100) for the presence of malaria parasite using WHO grading system i.e.

i. 1-10 parasites per hundred (100) high power field (HPF) = (+)

ii. 11-100 parasites per hundred (100) high power field (HPF) = (++)

iii. 1-10 parasites per every high power field (HPF) = (+++)

iv. More than 10 parasites per every high power field (HPF) = (++++)

v. If no parasite is found after examination of hundred (100) high power fields (HPF) = Negative [12].

Parasitaemia was estimated on all positive slides using the above-described method.

2.6 Immunochromatographic Rapid Assay Method

All samples to be used for conventional method were also tested for the presence of malaria parasite using Care Start and SD- Bioline kits following the manufacturer's instruction.

Principle

This is an in-vitro immunochromatograhic test that detects circulating *P. falciparum* Histidine-Rich Protein 2 (HRP2) antigen in whole blood. When blood is added into the sample well, followed by addition of buffer in the well, the buffer enables the blood to flow along the strip embedded in the cassette. If malaria parasite antigen (HRP2) is present, a control and a positive test bands are formed. In the absence of the antigen, only the control band is seen [3].

Procedure

About 5µl of the subject's blood was picked using the pipette from the kit and was dispensed on the sample region of the test cassette. Then, 60µl of buffer solution was added into the buffer well in the cassette. The result was read after 20minutes following the manufacturer's instruction.

2.7 Interpretation of Result

(a) Positive: the appearance of two colour bands (control "C" and test "T" regions) was indicated a positive result.

(b) Negative: the appearance of a colour band on control "C" region was indicated a negative result.

(c) Invalid: the absence of a colour band on the control "C" region was indicated an invalid result

2.8 Evaluation of RDT Using Microscopy as Gold Standard

The gold standard: is the best single test (or a combination of tests) that is considered the current prefered method of diagnosing a particular disease. Microscopy was used as the gold standard in this study.

Sensitivity: is the ability of a test to correctly classify as 'diseased'. This was calculated as: TP/TP + FN × 100.

Specificity: is the ability of a test to correctly classify as ‘disease- free’. This was calculated as: TN/TN + FP × 100.

Positive predictive value: it is the percentage of patient with a positive test who actually have the disease. This was calculated as: TP/TP + FP × 100.

Negative predictive value: it is the percentage of patient with a negative test who do not have the disease. This was calculated as: TN/TN + FN × 100.

(TP= true positive, FP= false positive, TN= true negative and FN= false negative) [13].

2.9 Statistical Analysis

Statistical Package for Social Science (SPSS) Software Version 20 was used to analyze the data generated. The results were expressed as Mean±SD. and Pearson’s Chi-squared test was used to find statistical association between the variables. *P-value* ≤ 0.05 was considered statistically significant.

3. RESULTS

Out of 106 stained thick blood film examined,45 (42.5%) was positive for malaria infection using microscopic technique, then 35 (33.0%) patients were positive for malaria with carestart RDT kit, while only 24 (22.6%) patients tested positive for malaria parasite infection with SD-Bioline kit as shown in Table 1.
The Table 2 shows the results of sensitivity and specificity of Carestart using microscopy as gold standard in malaria parasites evaluation. The sensitivity of Carestart (RDT) was 77.7% while its specificity value was 100%.

True positive rate of carestart was 35 while false positive rate was 0. False negative rate was 10 while true negative rate was 61.

Table 3 shows the results of sensitivity and specificity of SD-Bioline RDT method for detection of malaria parasite in pregnant women using microscopic examination as the gold standard.The sensitivity of SD-Bioline (RDT) was 53.3% while its specificity value was 100%. The true positive rate of the SD-Bioline was 24 while false positive value was 0. The false negative rate of SD-Bioline was 21 while true negative rate was 61.

4. DISCUSSION

Accurate diagnosis is the basic step to malaria treatment and must be endured in order to be effective in the global fight against malaria infection therefore this study was carried out to further look into the diagnostic test of malaria by

Table 1. Comparison of malaria parasite using rapid diagnostic test against convectional microscopy among pregnant women. n=106

Screening test	Microscopy		Carestart		SD Bioline	
	n	%	n	%	n	%
Positive	45	42.5	35	33.0	24	22.6
Negative	61	57.5	71	67.0	82	77.4
Total	106		106		106	

Table 2. Sensitivity and Specificity of carestart against microscopy

Screening test	Infection	No Infection	Total
Positive	True Positive=a	False Positive=b	a+b
35	35	0	35
Negative	False Negative=c	True Negative=d	Total
71	10	61	71
Total	a+c	b+d	a+b+c+d
106	45	61	106

*Sensitivity = a(a+c)*100 = 35/35+10×100/1 = 35/45×100/1 = 77.7%*
*Specificity = d(d+b)*100 = 61/61+0×100/1 = 61/61×100/1 = 100%*

Table 3. Sensitivity and Specificity of SD-Bioline against microscopy

Screening test	Infection	No Infection	Total
Positive	True Positive=a	False Positive=b	a+b
24	24	0	24
Negative	False Negative=c	True Negative=d	Total
82	21	61	82
Total	a+c	b+d	a+b+c+d
106	45	61	106

*Sensitivity = a(a+c)*100 = 24/24+21×100/1 = 24/45×100/1 = 53.3%*
*Specificity = d(d+b)*100 = 61/61+0×100/1 = 61/61×100/1 = 100%*

comparing 2 diagnostic methods, rapid diagnostic test and microscopy which was used as the reference method. The symptoms and complications of malaria during pregnancy differ with the intensity of malaria transmission and the level of immunity the pregnant women have acquired [14].

The prevalence of malaria in this study using microscopy as the gold standard was found to be 42.5%. And according to the pregnant women age, it was observed that pregnant women aged 18-23 had a prevalence of 15 (42.9%) and with the highest malaria infection level, 9 (37.5) while the pregnant women aged 30-35 years and 36-41 years had a prevalence of 18 (17.0%) and 12 (11.3%), respectively and with an infection level of 2 (8.3%) and 5 (20.8%). This result showed that most pregnant women attending Sokoto Specialist Hospitalare infected with malaria parasites. Both Immunochromatographic dipsticks offer the possibility of more rapid, non-microscopic technique for malaria diagnosis, thereby saving on training and time. These tests are easy to perform and require little training to interpret the results but also had limitations like false positive and false negative results.

In this study, the prevalence of malaria parasite by microscopic technique was 42.5%, while Carestart kit was 33% and SD-Bioline 22.6%. The observed high prevalence of malaria by microscopic method may be attributed to precision of the method compared to RDTs, in the same vein RDTs detect parasite antigens which may be error prone probably because of low sensitivity of RDTs at low parasitaemia.

Rapid diagnostic test SD-Bioline test sensitivity was (53.3%) why specificity was 100% which are similar to observations of Bell et al, [15] and Murray et al.,[16] with sensitivity of 47.5%.

In this study, CareStart kit had sensitivity of 77.7%, specificity of 100s%. It is expected that any RDT used for malaria diagnosis should have a high sensitivity of 95% and specificity 97% [14]; this is in contrast with the RDTs results in this study.

The false negative (FN) results of Carestart and SD-Bioline kits in this study were 10% and 21%, respectively using microscopy as the gold standard, similar to study of Jamshaid and Nabila, [17] due to lack of sensitivity of RDTs at low parasitaemia compare to microscopy.

The false positive rate in this study for the Carestart and SD-Bioline kits are 0% and 0% respectively.

This study further proves that rapid test kits are good for diagnosis however should not be absolutely relied upon as the only basis for diagnosis.

5. CONCLUSION

The prevalence of malaria by microscopy was 42.5% while the prevalence of malaria by Carestart and SD-bioline was 33% and 22.6%, respectively. It is expected that any RDT used for malaria diagnosis should have a high sensitivity of 95% and specificity 97%; this is in contrast with the RDTs results in this study.

The false negative (FN) results of Carestart and SD-Bioline kits in this study were 10% and 21% respectively using microscopy as the gold standard. Conventional microscopy remains the gold standard compared to RDTs according to this study with the level of its sensitivity and specificity which is higher than RDTs.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely

no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

Subjects to be recruited into the study were requested to give a written inform consent prior to the sample collection by filling a standard informed consent form.

ETHICAL APPROVAL

The ethical approval for the study was obtained from the Ethical Committee of Specialist Hospital Sokoto.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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