A COMPARATIVE STUDY OF MALARIA RAPID DIAGNOSTIC TEST (RDT) WITH CONVENTIONAL MICROSCOPIC METHOD IN KADUNA STATE, NIGERIA

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ABSTRACT

Laboratory confirmation of malaria infection requires the availability of rapid, sensitive and specific test at an affordable cost. Conventional methods of laboratory diagnosis for malaria use the microscopic examination of Giemsa and Leishman stained blood films, which require technical expertise and availability of a good quality microscope (binocular with in-built illumination) which is time consuming and of limited sensitivity in the detection of low parasitaemias. It is in view of the above that this work was conducted to compare the Malaria Rapid Diagnostic Test (RDT) with conventional microscopic method. A comparative study of Malaria Rapid Diagnostic Test (RDT) with conventional microscopic method was carried out among symptomatic out-patients in General Hospital Kachia, Gwamna Awan and Sabon Gari-Zaria respectively. Giemsa-stained thick film was used for the microscopy, while the Standard Diagnostic (SD) kit was used for the Rapid Diagnostic Test. A Total of 300 blood samples were collected from the study area (Kaduna State) which was divided into 3 zones: Southern, Central and Northern senatorial zones. One hundred (100) blood samples were collected from each zone. Using the thick film technique, the prevalence rate of 44%, 31% and 41% were recorded for the Southern, Central and Northern Senatorial zones respectively, while a total prevalence of 39% was observed for Kaduna State as a whole. On the other hand, the RDT (SD) had a malaria prevalence rate of 46%, 34% and 40% for Southern, Central and Northern Senatorial zones respectively with a total malaria prevalence of 40% for the whole state. Hence, there was no statistical significant difference between the microscopy technique and the RDT (SD) technique (P = 0.023). The sensitivity as well as the specificity was 97% and 98% respectively for the RDT (SD) method. This confirmed the RDT (SD) technique to be valid, reliable and accurate technique when compared to microscopy which is regarded as the gold standard for the detection of malaria parasites to be used.

Keywords: *Comparative Study, Malaria, Rapid Diagnostic Test, Microscopy, Kaduna State*

INTRODUCTION

Malaria is one of the world's most prevalent parasitic diseases and ranks third in the world among other major infectious diseases in terms of rates of morbidity and mortality, after HIV/AIDS and TB (Beadle *et al.*, 1994).

In Africa, a child dies from malaria every 30 seconds (WHO/UNICEF 2005). The majority of malaria infections in the region are caused by Plasmodium falciparum, the most virulent of the four human malaria parasites. The Anopheles gambiae mosquito is the most widely spread insect vector of malaria and most difficult to control (WHO/UNICEF, 2003).

Malaria has a great negative effect on the African economy because it causes severe illness and death. The symptoms include fever, chills, headache, muscle ached, vomiting, and malaise. Complications such as cerebral malaria, aneamia, algid malaria, dysenteric malaria, black water fever and kidney failure are associated with Plasmodium falciparum infection (Gerhard, 1989; WHO, 1996; Oyerinde, 1999). The severe forms of the disease can result in death or life-long neurologic impairment especially in subjects with little protective immunity (CDC, 2006).

In Nigeria, malaria is characterized by a stable and perennial transmission in all parts of the country though transmission is higher in the wet than in the dry season. It is the commonest cause of hospital attendance in all age groups in all parts of the country and tops the mortality table (Ekanem, 1998, Federal Republic of Nigeria, 2005).

There is no effective vaccine despite many years of efforts and drug treatment is continually undermined by the development of drug resistant parasite strains and insecticide resistance of the vector (Christian and Michael, 2008).

The protozoan parasites that cause malaria are from the genus Plasmodium. Four species of Plasmodium protozoa cause malaria; namely Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae and Plasmodium ovale. The parasite is transmitted to humans principally through the bite of the female Anopheles mosquito. Malaria infections may also occure from contacting infected blood, such as from blood transfusions, placental transmission from mother to foetus and sharing of needles and syringes (Patrick et al., 2005).

The use of dipsticks and other rapid diagnostic devices for the detection of malaria antigen from peripheral blood is now an accepted method for malaria diagnosis worldwide (Lema et al., 1999; Moody and Chiodini, 2002; UNICEF, 2007).

Comparative evaluation between microscopic diagnosis as the "gold standard" and detection of malaria antigen or enzyme using rapid test has been done in many centres and the sensitivity as well as the specificity achievable by these devices is now well documented (Buchachart *et al.*, 2004).

Laboratory confirmation of malaria infection requires the availability of rapid, sensitive and specific test at an affordable cost. Conventional methods of laboratory diagnosis for malaria use the microscopic examination of Giemsa and Leishman stained blood films, which require technical expertise and availability of a good quality microscope (binocular with in-built illumination). It is time consuming and of limited sensitivity in the detection of low parasiteaemias (Lema et al., 1999; Shujatullah et al., 2016; UNICEF, 2007).

The inventors and manufacturers of the Tests of Rapid Diagnosis of malaria claim that they emerged to meet the need as reliable diagnostic adjunct to microscopy in clinical settings (Krishna and Deshpanded, 2006).

These tests include Parasight F test, Binax, BID, Combo test, OptiMAL test, KAT-Quick test and SD Malaria Antigen P.f/Pan test among others which the manufacturer claim can be used at times where there is urgent need of diagnosis to provide quick intervention so as to prevent mortality and morbidity

associated with malaria. The Rapid Diagnostic Test (RDTs) are said to be very helpful for diagnosis in areas where facilities of microscopy are not available, especially in the night when services of routine laboratories and experienced microscopists are not available (Beadle et al., 1994; Haditsch, 2004).

The observation of asexual parasites by light microscopy in Giemsa-stained thick and thin blood films is still the gold standard for the diagnosis of malaria. However, there are other improved diagnostic tools to determine the presence of the parasite in human blood samples (Collier and Longmore, 1983; Caraballo and Ache, 1996; Graig and Sharp, 1997; WHO, 2001). The World Health Organization has repeatedly emphasized the urgent need for simple and cost-effective diagnostic tests for malaria that can overcome the deficiencies of light microscopy (Dietze et al., 1995; WHO, 1996; William et al., 2006). Microscopy is time consuming, unreliable at low parasite densities and requires well-trained personnel and regular quality control to be used as a gold standard (Buchachart *et al.*, 2004).

Several rapid tests based on the immune-detection of Plasmodium antigens have been successfully developed and recommended by the WHO/USAID (2000). Some are cost-effective, require neither sophisticated laboratory facilities nor electricity and are easily performed and interpreted. Most rapid tests, however, cannot quantify parasitaemia (Buchachart et al., 2004).

Rapid diagnostic tests for malaria are now commonly used procedures for malaria diagnosis (Moody and Chiodini, 2002). New or improved devices need to be evaluated against a recognized gold standard procedure and subjected to conditions of temperature and humidity that may affect their performance (Graig and Sharp, 1997; Moody and Chiodini, 2002).

The majority of cases of malaria worldwide are treated on the basis of clinical diagnosis and microscopy. Although microscopic examination of blood smear continues to be the gold standard, it has a drawback that it is time consuming and requires an expert miscroscopist and results are poor in cases of low parasitaemia (Lema et al., 1999).

MATERIALS AND METHOD

Study Area

A cross sectional study of three geopolitical zones (i.e. Southern Kaduna Senatorial Zone, Central Kaduna Senatorial Zone and the Northern Kaduna Senatorial Zone) in Kaduna State, Nigeria was done. General Hospital Kachia, Gwamna Awan General Hospital and General Hospital Sabon Gari- Zaria were selected from the Southern, Central and Northern senatorial zones, respectively, by simple random sampling method.

SAMPLING

Ethical Consideration: Approval for the study was obtained from the Ministry of Health, Kaduna State Ethical Committee.

Sample Size: The sample size was obtained using the formula (Lema et al., 1999).

$$n = \frac{Z^2 pq}{d^2}$$

where

z = standard normal deviate 95% = 1.96

n = numbers of sample collected

p = prevalence of previous studies = 23.08% = 0.2308 (Lema et al., 1999)

q = 1 – p

d = allowable error (precision) = 5% = 0.05

$n = \frac{1.96^2 \times 0.2308 \times 0.7692}{0.05^2} = 272.8$ approximately 273

Therefore, 273 blood samples from patients were supposed to be collected but the number was added up to 300 samples. A structured questionnaire was used to obtain personal data of patients. Study Population: Three hundred patients attending the General Out-Patient Department (GOPD) of the General Hospitals in the three senatorial zones made up the study population.

Inclusion criteria include:

1. Consented patients
2. Symptomatic patients sent for laboratory test
3. Patients must have been in senatorial area for the past four (4) days and above.

Exclusion criteria include:

1. Asymptomatic individual
2. Patients not sent for malaria laboratory test
3. Volunteers
4. Subjects who declined enrolment in the study

Sample Collection

A tourniquet was used to apply pressure to the upper arms of the patients in order to make the vein visible. Cotton wool soaked in 95% alcohol was used to swab the site of collection. Using a 5mL hypodermic syringe, two (2) mL of peripheral blood was obtained by vein-puncture and transferred into Ethylene Diamine Tetra Acetic acid (EDTA) bottles. Thick blood films were prepared from each of the samples thereafter.

Where the samples were not used immediately, they were stored at 4°C in a refrigerator for not more than 72 h.

LABORATORY DIAGNOSIS

Determination of Malarial Parasitaemia

This was done by detection of malaria parasite antigen (HRP-2) and the enzyme lactate dehydrogenase (pLDH) using the Rapid Diagnostic Test (SD) Kit and microscopic examination of Giemsa Stained thick blood film (Gold Standard). The color intensity on the zone reaction windows of the cassettes was observed. A light banding appearance on the test reaction zone on the cassettes indicates low parasitaemia estimated at (+) level of parasitaemia, moderately colored band on the test reaction zone indicated (++) level of parasitaemia, an intense color band on the test reaction zone reflected a (+++) levels of parasitaemia for the Rapid diagnostic Test.

The Intended use of the SD/RDT Kit: The SD Bioline Malaria Antigen P.f/Pan Test is a one-step rapid, qualitative and differential test for the detection of HRP-2 and Pan-pLDH in

human blood sample

Procedure of the Test: Prior to test, the test device (cassette) was removed from the foil pouch and placed on a flat, dry work bench surface. In the case of finger prick, the fingertip was swabbed and pricked with sterilized lancet on the other hand with a 5µ1 capillary pipette, a whole blood sample was drawn to a black line mark and transferred into the small round sample well. 4 drops of assay diluents were added into the square assay diluents well. The result was read after 5-10 minutes.

Interpretation of Results

Negative: Observation of one line "C" in control result window

Positive:

1. P.f positive: two colour bands ("P.f" Test line and "C" control line within the result window.
2. Other Plasmodium species (Plasmodium vivax, Plasmodium malariae, Plasmodium ovale) 2 colour bands ("Pan" Test line and "C" control line) within the result window.
3. Mixed infection Plasmodium falciparum and Plasmodium vivax (or Plasmodium malariae, Plasmodium ovale): 3 colour bands ("P.f", "Pan" Test lines and "C" control line) within the result window. Many mixed infection of Plasmodium falciparum and Plasmodium vivax (or Plasmodium malariae, Plasmodium ovale) were indicated.

Invalid: no "C" line in result window. It is recommended that the specimen should be re-tested.

Limitation and Interferences

1. The test procedure precautions and interpretation of results from this test must be followed when testing.
2. This test detects Plasmodium falciparum HRP and/or Pan-specific pLDH in patient's whole blood and useful as screening procedure for malaria diagnosis.
3. The test is limited to the detection of antigen to malaria Plasmodium species. Although the test is very accurate in detecting HRP-2 specific to Plasmodium falciparum or pLDH specific to Plasmodium species (Plasmodium falciparum, P. vivax, P. malariae, and P. ovale) a low incidence of false results can occur, other clinically available tests are required if questionable results are obtained as with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have to be evaluated.

MICROSCOPIC METHOD

Thick blood films were made, stained with one in ten (1/10) diluted Giemsa stains and examined using the oil immersion objective (100X) Cheesbrough, 2005).

Preparation of Thick Blood Films

A large drop of whole blood was placed at the center of a clean grease-free microscope slide. The edge of another slide was used to spread the blood in a circular motion to about 0.5 cm in diameter. The thick blood film was air dried and protected from dust and flies.

Staining of the Thick Blood Films

The dried-unfixed films were placed on a staining rack. 1 in *10th* (10%) diluted commercially prepared Giemsa was used to stain the films and allowed to stand for 30-45 minutes. The slides were then rinsed using distilled water pH 7.2 and allowed to air dry.

Examination of the stained films:

A drop of immersion oil was applied on the Giemsa dried blood films and mounted on the microscope stage. Well-stained area was selected and examined under the x100 (i.e the oil immersion objective). The malaria parasites seen were identified and the parasite density examined.

Estimation of parasite density:

The malaria parasite number/µ1 of blood was carried out by counting the number of malaria parasite observed in 10-50 high power field (HPF) and the result was expressed using the formula below:

$$\frac{\textit{Number of parasites counted}}{\textit{Number of HPFs viewed}} \times 500$$

(Cheesbrough, 2005).

Data Analysis

All statistical analyses were done using SAS (i.e. system Analytical software) packages. Interpretation of analysis, a p-value of less than or equal to 0.05 (5%) implies significance for the variable considered (Mayor et al., 2007).

RESULTS

Three hundred (300) patients were tested for malaria parasitaemia in the study. Out of the 100 samples collected from each senatorial zone for thick film staining method, prevalence rates of 44%, 31% and 41% were observed for Southern, Central and Northern Kaduna senatorial zones respectively. A total number of 116 infected persons with a prevalence of 39% for the state were recorded (Table 1).

On the other hand, the Rapid Diagnostic Test (RDT) method using the SD cassette gave prevalence rates of 46%, 34% and 40% for Southern, Central and Northern Senatorial Zone respectively. Therefore, Kaduna state had a total malaria parasitaemia of 120 (40%) for RDT (Table 1). Hence, there was no statistical significant difference between the Microscopy method and the RDT used during the period of research investigation (P=0.023).

Table 1: Comparison of microscopy (conventional method) with Rapid Diagnostic Test (RDT) using Standard Diagnostic (SD) test cassette.

	ZONE										
	SSZ			CSZ			NSZ				
Method employed	NE	NP	P (%)	NE	NP	P (%)	NE	NP	P (%)	T+	TP (%)
Thick film	100	44	44	100	31	31	100	41	41	116	39%
RDT	100	46	46	100	34	34	100	40	40	120	40%

$x^2 = 0.287$, df = 4, p-Value = 0.023

Key
SSZ = Southern Senatorial Zone, CSZ = Central Senatorial Zone
NSZ = Northern Senatorial Zone, NE = Number Examined,
NP = Number Positive, P (%) = Prevalence, T+ = Total positive/Method, TP (%) = Total prevalence/Method.
Note: Where p-value is less or equal to 0.05, significant difference exist between methods or in the prevalence rates between groups of data information.

A total of 300 patient blood samples were tested using both the malaria test cassette (SD) and the Gold Standard (Microscopy). A comparison was made between both methods. It was observed that, the ability of the RDT cassettes to correctly give a positive result when the individuals were truly positive with malaria parasitaemia,(i.e. sensitivity) was 120 (97%). The individuals that were truly negative for Miscoscopy and RDT (i.e. specificity) were 172 (98%). (Table 2)

Table 2: The validity and accuracy of the RDT-kit, using SD cassette. (Sensitivity and Specificity)

Observation	RDT	Microscopy
True positive	120	116
False positive	04	00
True Negative	172	184
False Negative	04	00
Total	300	300

True Positive = 120, True negative = 172, False positive = 4 False negative = 4

Sensitivity of RDT (SD) Method

$$Sensitivity = \frac{TP}{TP + NF}$$

$$Sensitivity\ rate = \frac{120}{120 + 4} \times 100 = 97\%$$

Specificity of RDT (SD) Method

$$Specificity = \frac{TN}{TN + FP}$$

$$Specificity\ rate = \frac{172}{172 + 4} \times 100 = 98\%$$

Key:
TP = True Positive, TN = True Negative
FP = False Positive, FN = False Negative
RDT = Rapid Diagnostic Test, SD = Standard Diagnostic

DISCUSSION

In this study, blood sample of patients that tested positive to anti-Histidine-Rich Protein-2 (HRP-2) antigen of Plasmodium falciparum or plasmodium lactate Dehydrogenase (pLDH) antigen or had malaria parasites seen in Giemsa-stained thick blood films were regarded as positive for malaria parasitaemia.

From the result obtained, there was a total prevalence of 39% in thick blood film and 40% in Rapid Diagnostic Test (RDT) method. These results indicated that the Rapid Diagnostic Test (RDT) method is slightly more sensitive than the microscopy method. This may be due to low parasitaemia, especially in partially or improperly treated cases.

In India, Shujatullah et,al. (2006) in their comparison of different diagnostic techniques for Plasmodium falciparum malaria, reported that paraSight F-test was position in 47 patients, 46 in QBC while Microscopy was positive in 28 cases in their findings of malaria parasitaemia in early therapeutic intervention.

Also based on the comparison of the two methods, the Rapid Diagnostic Test (RDT) had sensitivity of 97% and a specificity of 98%. This finding corresponds with several studies that had demonstrated an overall high sensitivity of Histidine-Rich Protein-2 (HRP-2) antigen based diagnostic assays and their potential utility for the diagnosis of malaria in symptomatic patients which satisfies the recommendations made by W.H.O on non-microscopy Rapid Diagnostic Test. It states that for the sensitivity of RDT to be used, it should be above 95% compared to microscopy (WHO/USAID/2000). It is very vital to note that the comparative test was on the use of thick film method and the RDT and so a thin film method was used as a tie breaker where there is disagreement in result, hence this helped in the determination of false positive and false negative results for the calculation of sensitivity and specificity.

Conclusion

The result of this research shows no significance difference between the RDT (SD) and the microscopic method of diagnosis. However, the RDT sensitivity was 97%, while the specificity was 98%, therefore making it fit to be used as a reliable diagnosis kit for malaria parasite test. In view of the result obtained, the RDT (SD) kit should be complemented with microscopy when clinical signs suggest malaria. The easy and early diagnosis can lead to timely therapeutic intervention, which can prevent mortality and morbidity cause by malaria parasites.

There was no statistical significant variation in term of geographical location. However, the southern senatorial zone (SSZ) had the highest prevalence of malaria parasitaemia followed by the northern and central senatorial Zones respectively.

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