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# Comparative Analysis of Microscopy and Rapid Diagnostic Test (RDT) for the Laboratory Diagnosis of Malaria among Pregnant Women Attending Braithwaite Memorial Specialist Hospital, Port Harcourt

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**ABSTRACT**

*Rapid Diagnostic Test (RDT) for the laboratory diagnosis of Malaria was compared with Microscopy (Gold Standard/ Reference Method) using 120 pregnant women aged 22-39 attending Braithwaite Memorial Specialist hospital, Port Harcourt. The prevalence of malaria among the subjects was 63>33%. Slide positivity was 76(63.33%) while RDT positivity was 44(36.67%). Rapid Diagnostic Test method in detection of Plasmodium falciparum as compared to microscopy gave results of 48.9% sensitivity, 100% specificity, positive predictive value of 100% and negative predictive value of 39.5%. Accuracy was 0.6 and J index (Reliability) 0.5. A Chi square value of 17.07 (95% confidence interval) was obtained which showed a significant difference ($p<0.05$) between the two test methods. Although the use of Rapid Diagnostic tests is rapid, convenient, less demanding and recommended for use in malaria endemic areas, microscopy remains the Gold standard for laboratory diagnosis of malaria.*

**Keywords:** *Rapid Diagnostic Test, Malaria, plasmodium falciparum*

## INTRODUCTION

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 (WHO, 2009). It is a long term disease which has evaded eradication and continues to cause diseased condition, some leading to death mostly in young children, immunocompromised individuals, the aged, poverty stricken population and pregnant women (since natural defense mechanisms are reduced during pregnancy (WHO, 2009).

The Eradication of malaria especially in endemic area have 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 (Hamoudi and Sacks, 1999). Malaria poses itself with different symptoms ranging from fever to chills, headache, excessive sweating, pain, shivering. These symptoms interlace with symptoms of other disease condition therefore treatment cannot be based on symptoms but on actual diagnosis of the plasmodium specie (Berkley et al., 2005; Chandramohan et al., 2002; Hamer et al., 2007).

Diagnosis of Plasmodium specie is generally done using the Microscopical method i.e. peripheral blood smear examination of the erythrocyte for intracellular malarial parasites using Romanowsky stain and test procedure is also known as "Gold standard" (Andreji et al., 2003). Accurate diagnosis of malaria is necessary to prevent morbidity and mortality while avoiding unnecessary use of antimalarial agents, therefore new rapid tests methods are being developed (Murray et al., 2006). Due to the need for rapid and accurate detection of malaria parasites in the treatment and eradication of malaria, Malaria Rapid Test kits have been developed. 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 (Bell et al., 2006; Richter et al., 2004).

As malaria is one of the major health threats to humans and requires urgent need for rapid diagnosis and treatment for its eradication. This study was carried out to determine the efficacy, the accuracy (sensitivity and specificity) and the reliability of Rapid Diagnostic Test Kit in plasmodium detection as compared to microscopy in pregnant women attending antenatal clinic at BMS Hospital.

## STUDY AREA

Port Harcourt is the capital city of Rivers State Nigeria. The city is located between latitude 50. North and Longitude70 East on the Bonny Rivers, about twenty nautical miles from Atlantic ocean in the fresh water swamps of the Niger Delta Region.

The pregnant women recently treated for malaria infection and those on malarial drugs were excluded from this study and the age of the subjects ranged between 22 and 39 years informed consent of subject was ensured. This involved the subjects (participants) filling questionnaires.

**STUDY SUBJECTS**

The study was performed on 120 pregnant women (different trimesters) attending antenatal clinic at Braithwaite Memorial Specialist Hospital (BMSH) Port Harcourt and analysed at the Diagnostic laboratory of Medical laboratory Department, Rivers state University of Science and technology.

**BLOOD COLLECTION**:

3mls of venous blood was collected asceptitically into Ethylenediamine tetra acetic acid (EDTA) bottles and well mixed to prevent clotting.

**LABORATORY PROCEDURES**

**MICROSCOPY**

**Preparation of blood films:** Thin and thick films were made on the same slide and stained with geimsa technique

**PRINCIPLE**: Geimsa is a type of romanowsky stain containing methylene blue (a basic dye), eosin (an acidic dye) and polychrome methylene azure. The methylene blue stains the acidic cell component giving it a bluish purple colour while the eosin stains the basic components giving it a pinkish red colour. Chromatin of the parasite appears dark Red, Cytoplasm of parasite appear blue, Red cells appear grey to pale mauve, while the reticulocytes appears grey blue

**MICROSCOPY**: Blood films were examined microscopically using 40x and 100x oil immersion objective. No malaria parasite was recorded after viewing at least 200 fields and parasites not found.

The results were recorded in parasite density (parasite/µL) by the following formula:

No of malaria parasites x 8,000 = parasite/µL

No of white blood cell counted

Parasites were counted simultaneously with a white blood cell count of 200

**RAPID DIAGNOSTIC TEST (IMMUNOCHROMATOGRAPHIC METHOD)**

The rapid test kit used is for the rapid qualitative determination of malaria *p. falciparium* specific lacate dehydrogenase (pLDH) in human blood. It contains a membrane strip which is pre-coated with two monoclonal antibodies as two separate lines across a test strip. One monoclonal antibody is specific to p. falciparum. Histidine rich protein (HRP-2) and the other is pan specific to lactate dehydrogenase of *P. falciparum, P. vivax, P. malariae* and *P. ovale*. Test kit used is Accure one step malaria Pf/Pv antigen Test.

**PRINCIPLE OF TEST KIT**:

The test is based on immunochromatographic antigen-detection. It involves the capture of dye labelled antibodies to produce a visible band on a strip of nitro-cellulose. The dye-labelled antibody binds to a corresponding parasite antigen and a

resultant antigen- labelled antibody complex is formed and shown on the strip by presence of a visible line.

**METHOD**:

The test kit was placed on a flat surface aseptically.5µl of whole blood was placed in sample well followed by adding 2 drops of assay buffer to the sample well. Test result was read after 10minutes while results were documented.

**Interpretation of test result**

*P falciparium* positive reaction, the presence of 2 colour bands (control and 1) indicates a positive result.

*P. vivax* or other plasmodium species positive reaction the presence of 2 colour bands (control and 2) indicates a positive result.

*P. falciparium* and *P. vivax* positive reaction, the presence of 3 colour bands indicates a positive result.

The presence of only one band at control within the cassette indicates negative result while the test was considered invalid due to absence of band at control (c)

**STATISTICAL ANALYSIS**

Samples were analyzed based on their occupational, literacy and educational status. The results were subjected to sensitivity, specify, J index, accuracy, positive predictive value, negative predictive value and chi square and t test were performed to determine the differences among the methods.

**RESULTS**

A total of 76 (63.3%) were positive with malaria infection while 44 (36.7%) were negative as shown in **table 1 below.**

Table 1:Malaria in Pregnant women attending BMSH

| | Positive | Negative |
|---|---|---|
| Malaria | 76 (63.3%) | 44 (36.7%) |

Also the educational level showed illiterates, semi illiterates and literates have malaria positivity rate of 2 (1.67%), 32 (16.67) and 22(35%) respectively.

Occupational status **(table 2)** showed that workers had the highest level of malaria infection at 46(38.33%), housewives with 18(15%) and students was thewith 12(10%).

**Table 2**: Prevalence of Malaria in pregnant women based on education and occupation

| | Educational level | | | Occupational status | | |
|---|---|---|---|---|---|---|
| | Illiterates | Semi Illiterates | Literates | housewives | students | workers |
| No (%) | 2 (1.7%) | 44(36.7%) | 74 (61.7%) | 30(25%) | 20(16.7%) | 70(58.3%) |
| Positive (%) | 2 (1.67%) | 32 (16.67) | 22(35%) | 18(15%) | 12(10%) | 46(38.33%) |
| Negative (%) | | | | | | |

Result showed a data of 42(35%) for 1st trimester, 76 (63.33%) second trimester and 2 (1.67%) for 3rd trimester and positivity rate of 28(23.33%), 46 (38.33%) and 2(1.67%) respectively (Tabe 3).

**Table 3:** Comparison of trimester with Malaria Infection

| Trimester | No of Subjects (%) | Positive (%) | Negative (%) |
|---|---|---|---|
| 1st | 43 (35) | 28(23.33) | 14(11.67) |
| 2nd | 76(63.33) | 46(38.33) | 30 (25) |
| 3rd | 78(65) | 2(2.67) | 2(1.67) |
| Total | 120(100) | 76(63.33) | 44(36.67) |

Pregnant women between 21-30 years had the highest prevalence of malaria infection 44 (36.6%) followed by pregnant women between ages 31-40 years 30(25%) and pregnant women of the age range 11-20 years had the lowest malaria infection prevalence 2(1.67%) as shown in table 4 below

**Table 4:** Comparison of Age with Malaria Infection

| Age | No of Subjects (%) | Positive (%) | Negative (%) |
|---|---|---|---|
| 11-20 | 2(1.67) | 2(1.67) | 0(0.00) |
| 21-30 | 78(65) | 44(36.67) | 34 (28.33) |
| 31-40 | 40(33.33) | 30 (25) | 10 (8.33) |
| Total | 120(100) | 76(63.33) | 44(36.67) |

Comparison of rapid diagnostic tests with thick film microscopy gave data values sensitivity 48.9% specificity 100% positive predictive value 100%, negative predictive value 39.5% accuracy 0.6, reliability 0.5 and prevalence of malaria as 63.3% as shown in **table 5.**

**Table 5:** comparative analysis of the RDT and microscopy

| Parameter | THICK | THIN |
|---|---|---|
| SENSITIVITY | 48.9 | 52.7 |
| SPECIFICITY% | 100 | 83.33 |
| **positive predictive value** (%) | 100 | 82.6 |
| **negative predictive valve (**%) | 39.5 | 54 |
| ACCURACY | 0.62 | 0.64 |
| J INDEX**(Reliability)** | 0.5 | 0.4 |

**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 comparing 2 diagnostic methods, rapid diagnostic test and microscopy which was used as the reference method.

The prevalence rate of malaria in this study was found to be 63.33% and it was observed that working class pregnant women had a prevalence of 70(58.3%) and with the highest malaria infection level, 46(38.33%) while students and housewives had a prevalence of 20(16.7%) and 30(25%) respectively and with an infection level of 12(10%) and 18(15%). This result showed that most pregnant women attending BMSH antenatal clinic are working class women.

Results also showed that literate pregnant women had the highest prevalence rate of 76(61.66%) followed by semi illiterate 44(36.67%) and illiterate 2 (1.67%). This may be due to increased information and knowledge by this group of people of the need for attending antennal clinic during pregnancy. Also literate women had the highest negative value 32 (26.67%) when compared with their positive value of 42(61.66%) and this may also be due to their acquisition of information about preventive measures against malaria infections.

In this study rapid diagnostic test sensitivity was 48.9% while specificity was 100% which are similar to observations of Tekola et al, (2008) and Tagbo et al., (2007) with sensitivity of 47.5% and 42.31% and specificity of 98.5% and 93.65% respectively. This moderately low sensitivity could be due to different variables such as drug treatment, deletion of HRP 2 genes in certain parasites (Baker et al., 2005), cross reaction with other antibodies such as rheumatoid factor, degradation of rapid diagnostic tests (Tagbo et al., 2007). The degradation varies from one test to another due to varying condition such as temperature of environment, storage condition, transportation etc.

The specificity of 100% is indicative of the ability of rapid diagnostic test to accurately detect a person who is free from malaria infection and a negative result from this diagnostic method in a patient with associated symptoms should prompt health personnel to quickly change presumptive diagnosis to another source. Negative predictive value of 9.5% positive predictive value of 100%, accuracy 0.6 and reliability of 0.5 were obtained.

The chi square test showed a significant difference in the two test methods ($p<0.05$). This showed that the two diagnostic test methods differ in their analysis suggesting that whenever rapid diagnostic tests are used, microscopy the 'Gold standard' should always be referred to. 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

The result from this study may vary with some previous studies because trials do not share common guidelines, epidemiological characteristics of the study population and genetic composition differ. Also factors such as different reference standard even among those using

Geimsa microscopy, microsopist skills vary, products of different brands and lots number which differ in quality or damage due to storage or temperature during transportation are used making comparative assessment a little bit difficult.

## CONCLUSION

In conclusion, rapid diagnostic tests are simple, rapid and more convenient with a great promising future for diagnosis of malaria. They could be used as added value (valuable tools) in the overall diagnosis of malaria infections and ultimately help in the world's combat against this infectious disease. However the test method should be subjected to further improvements to counter its limitation. Most importantly due to the advantages of rapid diagnostic tests they should be in endemic areas, in remote areas where microscopy is difficult to support for early detection and treatment of the infection. However microscopy remains the Gold Standard for detection of malaria parasites and should be referred to as much as possible in combating this infectious disease not minding its disadvantages such as time and cost. Finally based on the fact that both methods have peculiar advantages and disadvantages accurate diagnosis should not be based on one method only but a combination of both.

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