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Accuracy of Malaria Diagnosis by RDT using Microscopy as gold standard amongst Pregnant Women Attending Antenatal Clinic in Eket

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Abstract

The global impact of malaria has spurred interest in developing effective diagnosis strategies not only for resource on limited areas where malaria is a burden on society, but also in developed countries where malaria diagnostic expertise is lacking. Malaria diagnosis involves identifying malaria parasite or antigens product in patients' blood. Although this may seem simple, the diagnostic accuracy is subject to many factors, including expertise, sensitivity and effectiveness of diagnostic tools utilized. This study was conducted to ascertain whether the use of microscopy and Rapid Diagnostic test (RDT) methods influences accuracy of malaria diagnosis on pregnant women attending antenatal clinic in Eket. Forty (40) consenting pregnant women were recruited in the study. Blood samples collected through venous procedure were analyzed microscopically and SD Bioline Malarial test kits. Socio-demographic data showed 50% were participants between the age of <18 – 34 years and 40% were ≥35years. Fourteen (35%) of the participants had tertiary education, 25% had primary and secondary education each and 15% had no formal education. Thirty (75%) samples were positive to the Ab-Ag RDT test with 10 (25%) samples being negative, while 24 (60%) samples were positive to microscopy with 16 (40%). RDT had a 75% of sensitivity, 25% specificity, 60% PPV and 40% NPV. This study revealed that the detection rate of malaria parasite by RDT is higher than microscopy and this could be because of some human factor during microscopy processes.

Keywords: Microscopy; Rapid diagnostic test (RDT); Accuracy; Malaria; Pregnant

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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 [1]. 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 [2]. 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 [3].

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 [4-6].

Diagnosis of Plasmodium specie is generally done using the microscopic method. 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 [7]. 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. This study will therefore compare the accuracy of two malaria diagnostic techniques utilized for pregnant women in Eket.

Materials and Methods

The study was conducted in Immanuel Hospital and Assurance Medical Centers Eket. A total of 40 pregnant women (20) twenty each from the two antenatal clinics were recruited for the study. The inclusion criteria for this study were study participants with the body temperature 30^0C for less than 10 days biometric data including age and duration of pregnancy was recorded.

Microscopy

Blood samples of subjects were collected through various procedures and stored in Ethylene Diamante Tetra Acetate (EDTA) bottle to prevent coagulation. From the blood samples, blood films (thick and thin) were prepared (Fig 2), stained by covering the slides with diluted Giemsa for 30 minutes and then washed in buffered water pH 7.2. The films were dried in a vertical position and examined microscopically for malaria parasites using 100 high power (100 x objectives) fields and result was recorded (Fig 3). Controls were made from used films. For quality assurance, two trained parasitologists viewed each blood film before declaring slide positive or negative.

Rapid diagnostic techniques

The Bioline malaria Ag (HRPA/PHDH) RDT kit produced by standard diagnostic incorporated was used, and the usage was according to the manufacturers instruction where 0.05ml of whole blood was dropped into sample well. 2 drops of assay buffer was added into Assay buffer well immediately. The result was read after 20 minutes (Fig 1).

Data analysis

The data obtained for rapid diagnostic technique (RDT) was compared with microscopy to evaluated sensitivity, specificity, positive predictive value and negative predictive value.

Results

Bioinformation of respondents

The age, educational level and gravidity status of the pregnant women at the two health centers selected for the study is as shown in the Table 1.

Malaria diagnosis using RDT and microscopy

Table 2 shows that 30 (75%) samples were positive to the Ab-Ag RDT test with 10 (25%) samples being negative, while 24 (60%) samples were positive to microscopy with 16 (40%).

Performance of RDT in Diagnosis of Malaria

From the study we have observe that RDT has a 75% of sensitivity, 25% specificity, 60% PPV and 40% NPV. Hence RDT detects 75% of individuals suffering from malaria, 25% of malaria free individuals, 60% of individuals that tested positive and actually have malaria, 40% of individuals that tested negative and don't actually have malaria (Table 3). Hence all patients who test positive on RDT have the disease thereby indicating the presence of the *Plasmodium falciparum* parasite.

Discussion

Microscopy is the most widely used tool for diagnosis of malaria and in capable hands it is very sensitive for parasitaemia [8] and it can give important information to the clinician about the species, parasites stages and parasite density. However, good quality of microscopy is difficult to implement and maintain. It is labor intensive and requires highly skilled personnel and regular quality control [9]. The use of malaria RDTs which appears to be the most rapid method to detect the presence of malaria parasite and requires minimum or no training at all, is recommended by WHO when reliable microscopy is not available.

Table 1: Age, educational level and gravity of respondents

Parameter	Frequency	Percentage
Age		
<18	4	10%
≥ 18 to 34	20	50%
>35	16	40%
Educational Level		
No formal education	6	15%
Primary	10	25%
Secondary	10	25%
Tertiary	14	35%
Gravidity Status		
Primigravida	14	35%
Multigravida	26	65%

Table 2: Result of microscopy and RDT diagnosis of the respondent's blood samples

Malaria Test Results	Microscopy	RDT
Positive	24 (60%)	30 (75%)
Negative	16 (40%)	10 (25%)

Table 3: Performance of RDT discriminated by Microscopy (standard) in Diagnosis of Malaria

RDT	Microscopy +ve	Microscopy -ve	Sen	Spec	PPV	NPV
Positive	18	12	75%	25%	60%	40%
Negative	6	4				

In the present study, prevalence rate obtained through RDT (75%) was significantly higher than in microscopy as gold standard (60%). The observation agreed with *Tekola et al* [10] who revealed a high prevalence (80%) by RDT and low prevalence (48.9%) by microscopy. However, these results are not in conformance with a study by Oyeyemi *et al* [11] which had high prevalence (66.8%) by microscopy and low prevalence (36.8%) by RDT.

In the present study, RDT reported a sensitivity of 75% and specificity of 25% when compared with microscopy as gold standard. This is lower than the World Health Organization (WHO) recommended minimal standard of 95% sensitivity for *Plasmodium falciparum* densities of 100/μL and a specificity of 95% for an acceptable Rapid Diagnostic Test for malaria

[12]. Also, this low RDT sensitivity makes it disadvantageous as it will impair control intervention since fractions of the infected population will be left untreated, especially if RDT is the only available diagnostic tool. However, the diagnostic performance of RDTs can be affected by several factors such as storage temperature, humidity, and end users' performance. The sensitivity of RDT reported in this study is higher than other studies in Nigeria by Ojurongbe *et al* [13], Osei-Yeboah *et al* [14] and Oyeyemi *et al* [11] which had 62.3%, 62.5% and 42.5% respectively for RDT. However, this study's sensitivity was lower than previous reports in other parts of the world; 100, 96, 97 and 79% had been reported in USA, Zambia, Zanzibar and Ghana, respectively [15-18]. The specificity of the present study is lower than 87.1, 88, 92.73, 93 and 100% reported by Oyeyemi *et al* [11], Msellem *et al* [16], Osei-Yeboah *et al* [14], Buchachart *et al* [19] and Dougnon *et al* [20] respectively. The sensitivity reported in this study is yet to attain the 95% recommended World Health Organization value [21]. This low sensitivity is disadvantageous as it will impair control intervention since a fraction of the infected population will be left untreated especially if RDT is the only available diagnostic tool. However, the high specificity will improve the cost effectiveness of malaria diagnosis since the RDT is unlikely to missout the non-infected individuals.

While microscopy still remain the gold standard for detection of malaria parasites which is similar to Andrej *et al* [22] who suggested that both methods have peculiar advantages and disadvantages, accurate diagnosis should not be based on one method only but a combination of both.

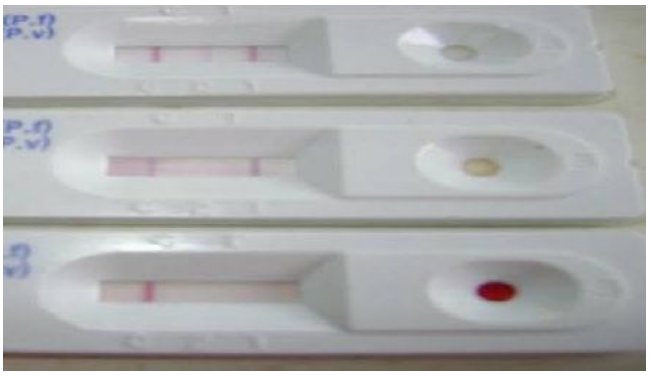

Fig 1: RDT test showing positive, negative and neutral results

Fig 2: Slide showing thick and thin film for microscopy test

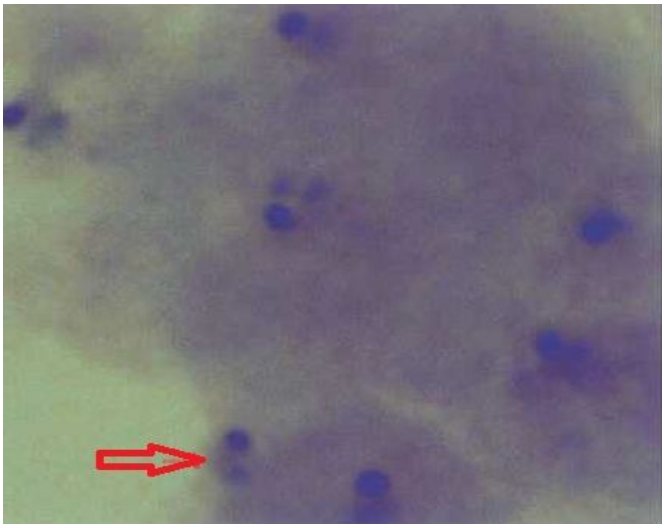

Fig 3: Thick film microscopic view showing *P. falciparum* ring

of both [9-23].

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 by 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 or 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 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.

Recommendations

- Comparison of microscopic results with rapid diagnostic test kits should be done
- The study using both methods should be carried out to ascertain the specificity of each kind
- The sample size should be increased in further studies

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