



# Comparison of Microscopic Examination and Rapid Diagnostic Tests Used To Diagnose Malaria among Pregnant Women in Kano, North –Western, Nigeria.

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## ABSTRACT

**Background and Objectives:** A study was carried out to compare the two methods of rapid diagnostic test (RDTs) and blood films examination in the diagnosis of malaria among pregnant women in Kano, Nigeria. **Materials and Methods:** A total of 300 pregnant women with clinical suspicion of malaria attending antenatal clinic (ANC) who gave informed consent were recruited to participate in the study. Blood sample were collected aseptically and dispensed into an EDTA container where RDTs and microscopic examination were performed to assess the presence of malaria parasites. Results from the rapid diagnostic kits were analyzed and compared to those obtained by general microscopy. **Results:** Results show that of the 300 samples enrolled 116 (38.7%) were found to be positive with blood films examination while in rapid diagnostic test 103 (34.3%) pregnant women were positive. Based on frequency of infection by age 21-25 years had 50 (43.1%) as the most vulnerable group, followed by 15-20 year with 34 (11.3%) 26-30 had 15 (5%) while age group of 31-35 had 12 (4.0%). Age group 36-40 had 10 (3.3%) and lastly the age group of 41-45 had least value of 2 (0.67%). **Conclusion:** The study, therefore, highlights the importance of both methods in diagnosis of malaria and it could be concluded that there was no significant difference between rapid diagnostic test and stained blood film malaria microscopy. Thus, the two malaria diagnostic tests could be routinely carried out to confirm and check each other.

**Keywords:** *Rapid Diagnostic Test, Blood Films Examination, Malaria Diagnosis, Pregnant Women*

## I. INTRODUCTION

Malaria is a deadly threat to pregnant women, but diagnostic tools often fail to accurately define either infected or uninfected women. Pregnancy associated malaria (PAM) causes severe anemia in the mother and low birth weight (LBW) in the child, and these sequel alone result in an estimated 10,000 maternal deaths and 200,000 infant deaths annually in Africa [1].

Accurate diagnosis and prompt treatment of pregnancy-associated malaria (PAM) is essential to avert adverse pregnancy outcomes [2]. Detection of sub-microscopic infections is crucial in order to not only effect prompt treatment of asymptomatic cases, but also to identify and clear potential reservoirs of transmission [ 3 ] and to reduce malaria related morbidity and mortality. Presumptive treatment of malaria based on clinical diagnosis is relatively cheap but it is unreliable due to overlapping symptoms with non-malarial infections caused by viruses or bacteria and could lead to over-diagnosis as well[ 4 ] . Wrong diagnoses may lead to presumptive medication and hence many patients may leave the health facility without the right treatment. Rational prescription of anti-malarials is not only important in saving on the cost of expensive drugs but it also prevents drug overuse that might result in the development of resistance [5]. Sub-microscopic infections during pregnancy might be associated with increased risk of adverse pregnancy outcomes including low birth weight babies and maternal anemia. Therefore, treatment of these

infections may prevent potential risks of adverse pregnancy outcome [6].

Malaria presents a diagnostic challenge to laboratories in most malaria endemic countries. The world health organization has opened a dialogue with scientists, clinician and manufacturers on the realistic possibilities for developing accurate, sensitive, and cost effective rapid diagnostic test for malaria [7]. New technology has to be compared with accepted WHO standards that make comparisons of sensitivities and specificity between different methods. Majority of malaria cases are found in countries where cost effectiveness is an important factor and case of performance and training is a major consideration [8].

Accuracy of a clinical diagnosis varies with the level of endemicity, malaria season and age group. No single clinical algorithm is a universal predictor [9]. Rapid, accurate and accessible detection of malaria parasites is important in the prevention and treatment of malaria. Malaria morbidity, mortality and transmission can be reduced if prompt diagnosis and adequate treatment is available [10]. Rapid diagnostic tests (RDTs) offer the potential to provide accurate and timely diagnosis to everyone at risk, reaching those previously unable to access good quality and qualitative microscopic services[ 11 ]. In malaria-endemic regions, the use of RDTs is very helpful for the effective use of antimalaria drugs as treatment is based on parasite diagnosis and not just fever alone in these region, a considerable proportion of these drugs have been wasted on patients with no malarial disease due to lack of prompt and accurate diagnosis[ 12 ]. Malaria microscopy is part of good



clinical practices; it should always be part of malaria case management [13].

The choice between microscopy and RDTs is not always clear-cut as the performance of both diagnostic techniques in operational conditions varies depending on transmission intensity, prevalence of infections, and parasite density [14]. Microscopy is reported to detect about 75% of malaria infections in high transmission areas, whereas in low transmission areas this method has been reported to miss up to 88% of infections [15]. Furthermore, the level of expertise of technicians, quality of the equipment, and workload may lead to inaccurate estimates of parasite density and species differentiation [16].

Clinical studies provide effective comparison between different test format, as well as the clarification on the feasibility and clinical relevance of using non microscopic method [4]. Therefore the study was aimed to compare the two methods of microscopy and RDTs in the diagnosis of malaria among pregnant women.

## II. MATERIALS AND METHODS

### Study Site and Subjects

This study was carried out at research laboratory of Aminu Kano Teaching Hospital (AKTH), Kano between June and August 2012. The subjects were 300 pregnant women aged 18 – 45 years attending antenatal clinic (ANC) who presented with signs and symptoms of malaria infection and for whom a malaria test was requested by the physician. Informed consent was obtained from each participant and the study was approved by the Ethics Review Committee of the institution.

### Sample Collection

Blood samples were collected by venipuncture into vacutainers containing EDTA. After mixing, the blood samples were used to determine the haematocrit, for the preparation of thick and thin slides for microscopy, and for the RDTs assay.

### Staining of Blood Film for Microscopy

#### Making Thick Film

A small drop of blood was placed at the center of the cream grease free slide and spreaded with the edge of another slide in a repeated coil shape to a diameter approximately 2cm. the slide was labeled and left horizontally while drying and are kept well to prevent them from dust and damage[ 13 ]. It was stained

using field stain and observe microscopically using x100 oil objective lens and result was recorded.

### Test Procedure

Test device, buffer and specimen were allowed to equilibrate at room temperature (10°C – 30°C) prior to testing. The test cassette was removed from the foil pouch by tearing at the notch and the placed on level surface.

- 5µl of whole blood were slowly added into the sample well (A)
- Then 3 drops of clearing buffer was added to the buffer well (B)
- As the test begins to work, a purple colour moving across the result window in the center of the test device.
- A result was read after 25 minutes.

### Interpretation of Results

#### Positive (+)

Rose pink bands are visible in both the control region and the test region.

#### Negative (-)

A rose pink is visible in the control region no colour bands appear in the test region.

#### Invalid

No visible band at all or there is a visible band only in the test region but not in the control region.

### Haemoglobin Estimation

The haemoglobin level was estimated using digital direct read out haemoglobin meter (DHT).

### Test Procedure

1. 200ul (0.02ml) of well mixed venous blood and 2ml of Ammonia diluting fluid was mixed in the stopper tube.
2. The performance of the meter was checked by inserting the control standard glass provided in cuvette apparture.
3. The patients sample was transfer into a clean 10mm light path cuvette. The cuvette was held by its non optical and air bubble were avoided in the sample.



4. Cuvette was placed in the cuvette holder, it was waited for an audible signal, and haemoglobin value was read from the display.
5. The sample was return to its tube and cuvettes were allowed to drain by inverting it on a paper towel.

### Statistical Analysis

The data generated was analyzed for significant difference between the two methods employed during the study using chi-square test as described [17].

### III. RESULTS

Blood samples were collected and analysed from three hundred (300) participants in which each were analyzed and compared

using two malaria diagnosis method that is rapid diagnostic test and stained blood film microscopy. The baseline characteristics of stained blood film microscopy and rapid diagnostic test and statistical analysis values using chi-square were shown in Table 1.

Table 2 showed that out of the 300 samples analyzed using stained blood film microscopy 116 (38.7%) were found to be positive, while in rapid diagnostic test 103 (34.3%) pregnant women were positive. In comparison with respect to sensitivity, stained blood film microscopy had 100% sensitivity while rapid diagnostic test microscopy had 88.8% sensitivity and there is no significant difference between the two methods employed. The frequency of malaria infection as shown in Table 3, 21-25 years of age had 51 (43.1%) as the most vulnerable group, followed by 15-20 years with 34 (11.3%) , and lastly the age group of 41-45 which had least value of 2 (0.67%).

**Table 1: Baseline Characteristics of Stained Blood Film Microscopy and Rapid Diagnostic Test with Statistical Analysis Values**

Characteristics	Stained blood film microscopy/rapid diagnostic test
Mean age group (year)	30+/-15
Fever in last 48 hours	14
Use of antimalaria IPT drugs	112
Hemoglobin level <11g/dl	109
Chi-square calculated value	2.49
Chi-square table value	3.84

**Table 2: Sensitivity, Specificity and Predictive Value of Stained Blood Film Microscopy and Rapid Diagnostic Test.**

Method	Positive	Negative	Sensitivity	Specificity	PPV	NPV
Rapid diag. test	103	197	88.8%	100	100	83
Stained B.F.M.	116	184	100%	100	100	100

**Key:** PPV = positive predictive value

NPV = negative predictive value

B.F.M = blood film microscopy

**Table 3: Age Groups in Years Compared to the Rate of Malaria Infection**

Age Group	Stained Blood Film Microscopy (%)		Rapid diagnostic test (%)	
	Positive	Negative	Positive	Negative
15 – 20	34 (10.3%)	42 (22.8)	30 (29.1)	45 (22.8)
21 – 25	51 (4.31)	78 (42.39)	47 (45.6)	81 (4.11)
26 – 30	16 (5)	27 (14.6)	12 (11.6)	30 (5.08)
31 – 35	12 (10.3)	11 (5.98)	10 (9.7)	10 (5.08)
41 – 45	1 (0.86)	9 (4.89)	1 (0.97)	12 (6.09)

#### IV. DISCUSSION

Malaria can be a life threatening disease in a vulnerable population like pregnant women if not treated. Therefore, a quick and accurate diagnosis is very important. To prevent unnecessary anti malarial treatment, it is therefore important to confirm clinical suspicions with a good laboratory test. RDTs for malaria are being increasingly adopted across endemic countries like Nigeria to strengthen parasitological diagnosis and appropriate management of all cases of fever especially in pregnant women. They are particularly valuable in areas which do not have good resources for microscopy.

There are four principal methods for diagnosing malaria. These are symptomatic, microscopy, antigen test and molecular methods. Symptomatic diagnosis is the most common, and people in poorer countries often use symptoms alone to diagnose malaria. In other areas too symptomatic diagnosis is often the initial one, followed by one of the other methods. However it should be noted that many other diseases present symptoms very similar to that of malaria, and diagnosis by symptoms alone can be misleading and even harmful. Treating for malaria where other treatment is called for leaves the actual diseases uncured and the patients in critical conditions. It is therefore imperative to follow up symptomatic disease with one of the other more accurate methods [ 18 ].

Microscopy is the most widely used tool to diagnose malaria at peripheral levels and it can give important information to the clinician like species, parasite stages and parasite density [19] . However, a good quality of microscopy is difficult to implement and maintain. It is labor intensive and requires highly skilled personnel and regular quality control. The use of

malaria RDTs is recommended by World Health Organization (WHO) when reliable microscopy is not available [18].

In this study routine microscopic examination of stained blood films which is considered as the gold standard for malaria diagnosis had a sensitivity of 100% and was able to detect more parasites than the RDT (sensitivity 88.8%). Though the specificity of microscopy and that of RDT was 100%, nevertheless, it has high sensitivity, possibility for quantification of parasitemia, and easy handling which is a good advantage.

The challenges for diagnostic laboratory in Kano State and most of the laboratories in Nigeria include defective microscope, poor consumables that flooded our chemical stores, work over load, shortage of staff are well known to both laboratory managers and their customers [12] . The sensitivity of rapid diagnostic test at level of parasitaemia and for non immune population remains a problem compared to stained blood film microscopy, the PFHRP/PMD were found to be less sensitive in detecting asymptomatic patient particularly at low parasitaemia [ 20 ]. Further the rapid diagnostic tests have been reported to give false negative result even at higher level of parasitaemia. Therefore in case of suspected severe malaria or complex health problem emergencies a positive result may not rule out malaria. However negative RDTs should always be confirmed by microscopy [21].

Rapid, accurate and accessible detection of malaria parasites is important in the prevention and treatment of malaria, malaria morbidity, mortality and transmission can be reduced if prompt diagnostic and adequate treatment is available. Rapid diagnostic tests (RDTs) offer the potential to provide accurate and the



timely diagnostic to everyone at risk, reaching those previously unable to access good, accurate, and timely diagnosis [21].

Concurrently the world health organization (WHO) has begun a dialogue with scientist clinician and manufacturers of malaria rapid diagnostic test devices, regarding the realistic possibilities for developing accurate, sensitive and cost effective rapid diagnostic test for malaria Diagnosis services, limitation for these rapid diagnostic test include in capability for the test to detect 100 parasites/ $\mu$ l from all *Plasmodium* species and the inability to perform semi quantitative measurement for monitoring drug treatment results. Also the new technologies must be compared against accepted “gold standard” method [22].

## V. CONCLUSION

Stained blood film microscopy and rapid diagnostic test each with its characteristics, strength and the limitation together present the best hope for diagnosis as a key component of successful malaria control hence rapid diagnostic test does not eliminate the need for stained blood film malaria microscopy therefore all negative rapid diagnostic test must be followed with stained blood film microscopy to confirm the result. Microscopy is the more reliable method in areas where malaria is most prevalent. RDT offers a good alternative, being an easy and rapid method that does not require an experienced laboratory technician. Moreover, when a rapid diagnostic test is used alone it should be followed with stained blood film microscopy to ascertain the degree of infection and to know the malaria specie involved for proper treatment. This is because the stained blood film microscopy reveals the degree of infection as well as the species involved. The present study demonstrates that RDTs can act as a diagnostic tool to manage malaria during pregnancy in resource poor settings with limited access to expert microscopy as they are easy to use and perform better than microscopy.

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