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Original Article

A Comparative Study of Peripheral Blood Smear, Centrifuged Buffy Coat smear and Antigen Detection Test in Diagnosis of Malaria in Paediatric Population

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ABSTRACT

Rapid diagnosis is pre-requisite for institution of effective treatment and reducing mortality and morbidity of malaria. The study was taken up to compare the efficiency of various methods available, i.e. peripheral blood smear (PBS), Centrifuged Buffy Coat (CBC) and Antigen card test (RDT). In the present study, thick smear was compared with CBC and Antigen card test for the diagnosis of malaria. A total of 100 samples were collected from patients presenting with classic symptoms of malaria. For traditional microscopy; thick smear were prepared and stained with Leishman's stain, taking thick smear as a gold standard. CBC and Antigen detection were done using commercially available kits. Malaria was diagnosed in 60, 62 and 65 patient by thick smear, CBC and antigen card test respectively. In antigen card test the sensitivity 91.6%, specificity 75%, Positive predictive value (PPV) 84.62% and Negative predictive value (NPV) 85.71% were observed. Although the antigen card test is superior than thin smear and CBC. Antigen card test has its advantages in terms of speed, sensitivity and specificity especially in an endemic area. Therefore we recommended antigen card test which was simple, reliable and effective for the diagnosis of malaria in remote and rural areas of our country.

Keywords: Malaria, thick smear, thin smear, CBC, Antigen card test.

1. INTRODUCTION

Even in this era of newly emerging deadly diseases malaria remains the most serious parasitic disease worldwide especially in the tropical and sub tropical countries. It is a serious, some time fatal, parasitic disease posing a major public health problem in India [1].The earliest symptoms of malaria are very non-specific and variable which poses

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difficulty in clinical diagnosis. Being associated with most serious complications, diagnosis of malaria constitutes a medical emergency. Since prompt treatment can grossly reduce mortality and morbidity associated with malaria, specific of rapid diagnosis of this disease becomes imperative and main emphasis of current WHO malaria control strategy.

At peripheral level of health centers, treatment is generally given following a diagnosis based on clinical symptoms. Chloroquine and other low-cost drugs were highly effective and cost-effective but the emergence of chloroquine resistant malaria has made it urgent to ensure that treatment is based on rapid and reliable diagnosis of disease.

During the last 100 years, malaria has been diagnosed by microscopic examination of Giemsa-stained thick and thin blood films [2, 3] and today this approach is the gold standard for malaria diagnosis that is recommended by the World Health Organization

(WHO). However, microscopy performed by poorly trained personnel that live in the rural areas of endemic malaria has alow sensitivity [4, 5].

However, Over the years many new tests have been developed in an attempt to improve the diagnosis of malaria, but conventional method by smear microscopy remains the gold standard against which all other tests have been evaluated. Centrifuged buffy coat (CBC) technique and Rapid Diagnosis Technique (RDT) for detection of malaria antigen and enzymes are two among those newly emerged tests. All these tests vary in their sensitivity and specificity.

Keeping in mind the seriousness of the condition and the current availability of diagnostic facilities across India we decided to conduct a comparative study of the peripheral blood smear, CBC and antigen card test.

2. MATERIALS AND METHODS

This prospective study was conducted in the department of pathology Dr.B.C.Roy PGIPS Kolkata. The study was conducted from May 2015 to April 2016.This study was done in 100 cases of patients presenting pyrexia with chills, rigor and other suggestive symptoms of malaria.

Participants: Patients of either sex and paediatric age groups (upto 12yrs.) with a clinical suspicion of malaria were included in the study. Informed consent was taken from all patients who participated in the study. A single sample was collected in K2 EDTA Vacutainer.

Thick and thin blood smears were prepared as per the standard method. The smears were stained with Leishman's stain [6] and microscopically examined for malarial

parasites under oil immersion objective. A total of 200 to 300 microscopic fields were examined before the film was declared negative.

Second, CBCs were prepared with the blood collected in a wide bored EDTA vacutainer, centrifuging at 2500 rpm for 15 minutes. The supernatant plasma is discarded, the buffy coat and equal thickness of RBCs layer just below the buffy coat was picked, smeared and stained by standard Field's Staining method; 200 oil immersion fields were examined before considering the smear as negative. Level of parasitaemia was calculated if PBS was negative.

Third, Antigen detection was performed using commercially available card, Malascan by Zephyr Biomedicals as per manufacturer's instruction. The cards detects the Histidine-rich protein 2 antigen (HRP II) of P.falciparum and the lactate dehydrogenase of Plasmodium.

3. RESULTS & ANALYSIS

Samples were classified as true-positive (TP), true-negative (TN), false-positive (FP) or false-negative (FN) by comparison with a reference standard. Sensitivity (TP / TP + FN) and specificity (TN /TN + FP), as well as positive (TP / TP+ FP) and negative (TN /TN + FN) predictive values, for the test were then calculated.

Out of a total of 100 samples of clinically suspected malaria tested by CBC, PBS and antigen detection methods, 86 (86%), 84 (84%) and 88(88%) were positive. for malaria respectively. (Table 1)

Table 1: Comparison of PBS, QBC and Antigen detection SD bioline

Diagnostic Test Cases positive for malaria	
Study group = 100	
QBC	62 (62%)
Blood smear examination	60 (60%)
Antigen detection	65 (65%)

Table-2: comparative study of peripheral smear and rapid diagnostic test (pLDH):

Rapid antigen detection test

Rapid antigen detection test	Microscopy positive	Microscopy negative	Total
positive	55	10	65
negative	05	30	35
Total	60	40	100

Above table shows that by taking thick smear as gold standard, sensitivity, specificity, positive and negative predictive values of *Rapid Diagnostic Test* were 91.67 %, 75%, 84.62% & 85.71% respectively **(Table 2)**.

Table 3: comparative study of peripheral smear and centrifuged buffy coat test (CBC)

Centrifuged buffy coat test	Microscopy positive	Microscopy negative	Total
Positive	54	08	62
Negative	06	32	38
Total	60	40	100

Above table shows that by taking thick smear as gold standard, sensitivity, specificity, positive and negative predictive values of *CBC* were 90%, 80%, 87.1% & 84.21%, respectively**(**Table 3).

Table 4: Sensitivity(SN), Specificity(SP), Positive predictive value (PPV) and negative predictive value (NNV) of CBC and RDT in comparison with peripheral blood smear

	SN	SP	PPV	NPV
CBC	90%	80%	87.10%	84.21%
RDT	91.67%	75%	84.62%	85.71%

4. DISCUSSION

Rapid detection and effective treatment is a prerequisite for reducing the morbidity and mortality in malaria cases. Leishman or Giemsa stained blood smears are considered to be the 'Gold standard' in diagnosis. However, the interpretation of thick smear is laborious and results depend on the quality of microscope, staining technique with which blood film is prepared and also the concentration [7, 8] and motivation of microscopist. This is time [9] consuming and therefore delays diagnosis. However the advantages of Leishman stained smearis that a permanent record of the smear can be kept, it's low cost and species identification is done without much difficulty in most of the cases. The present study was done to demonstrate the performance of adding centrifugation to conventional PBS.

In this study, rapid diagnostic tests were found more sensitive as compare to CBC with good negative predictive value (84.21%). However, in 5 cases the rapid diagnostic test result was false negative, two of these shows grade 1 parasitemia. This may be due to insufficient enzyme production which occurs during early malarial infection or the patient blood samples contained parasites at concentration below the RDT's detection level. [10] Occasional false negative results may becaused by deletion or mutation of the HRP-2 gene [11].

False positive RDT results occur in a 10 cases. In 9 of these, *P. falciparum* seen and only in 1, non-*falciparum*detected. This may be explained by the fact that *P. falciparum* can sometimes sequester and may not be present in circulating blood [12].

RDT is a valuable complement to microscopy because it helps expand the coverage of parasite-based diagnosis to the periphery and minimize exclusively clinical diagnosis. RDTs offer a more promising strategy to deal with increasing costs of therapy driven by drug resistance. Today's multi-million dollar investment in anti-malarial drug development should be accompanied by a parallel commitment to improve diagnostic tools and their availability to those living in malarious areas.

RDTs are useful and easy tools for field surveys because they are easily read by the field workers without supervision and require no training or instruments. In situations where adequate laboratory back up is not available, antigen detection test can be employed. However, RDTs may not be able to replace the peripheral smear examination as the most comprehensive and cost-effective test for malaria.

5. CONCLUSION

There were some limitations in the present study, sample size was small and it was a hospital based study, involving only the paediatric population so can not represent whole population. There is need to perform such studies on larger and community based population

In conclusion, CBC method provides a reliable, quick, easily mastered method for diagnosis of malaria. QBC method is useful in laboratories which screen large number of samples and in endemic areas where parasite level is low. The CBC system can also be used in the diagnosis of other parasitic diseases from blood like filariasis and kala-azar.

However, the Antigentest (RDT) was found to be more user friendly and interpretation was more objective as compared to smear and CBC.

Taking all factors into consideration we suggest using at least a combination of antigen detection and smear should be done to detect the maximum number of cases and combining the advantages of both methods. This will help in the early diagnosis of malaria along with calculation of the parasitic index. The antigen detection can be used as a primary screening tool followed by microscopy in all positive cases.

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