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KEY WORDS: Malaria, thick smear, thin smear, CBC, Antigen card test

IMPLEMENTATION OF NON-MICROSCOPIC METHOD FOR MALARIA DIAGNOSIS

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ABSTRACT

Rapid diagnostic tests for malaria are now a commonly used procedure for malaria diagnosis. Despite some problems related to sensitivity and applicability, malaria rapid diagnostic tests (RDTs), are currently considered the best option to overcome well trained experts in peripheral blood smear diagnosis. The present study was done to compare the non-microscopic methods like centrifuged buffy coat smear(CBC) and Antigen kit test(RDT) with the conventional peripheral blood smear for the rapid diagnosis of malaria which is essential to prevent the morbidity and mortality especially in endemic areas. A total of 100 samples were collected from patients presenting with classic symptoms of malaria. For traditional microscopy; thick and thin smears were prepared and stained with Leishman's –Giemsa stain. 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.

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. 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. Centrifuged buffy coat (CBC) technique and Rapid Diagnosis Technique (RDT) for detection of malaria antigen and enzymes are two among newly emerged non microscopic methods of diagnosis.

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 2017 to April 2018.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 Vials. Thick and thin blood smears were prepared as per the standard method. The smears were stained with Leishman's- Giemsa stain 6 and microscopically examined for malarial parasites under oil immersion objective. A total of 200 microscopic fields were examined before the film was declared negative. Second, CBCs were prepared with the blood collected in EDTA vials, centrifuging at 2500 rpm for 15 minutes using microcapillary tubes. 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 Lieshman's – Giemsa 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 Histidinerich 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 PBS , CBC and antigen detection methods(RDT), 70 (70%), 72 (72%) and 76(76%) were positive. for malaria respectively.

Table-1: comparative study of peripheral smear(PBS) and rapid diagnostic test (RDT):

RDT	PBS POSITIVE	PBS NEGATIVE	Total
POSITIVE	67	9	76
NEGATIVE	3	21	24
Total	70	30	100

Table-2: comparative study of peripheral smear(PBS) and centrifuged buffy coat smear (CBC):

CBC	PBS POSITIVE	PBS NEGATIVE	Total
POSITIVE	66	6	72
NEGATIVE	4	24	28
Total	70	30	100

Table 3: Sensitivity(SN), Specificity(SP), Positive predic tive value (PPV) and negative predictive value (NNV) of CBC and RDT in comparison with peripheral blood smear

	SN	SP	PPV	NPV
CBC	94.2	80	91.7	85.7
RDT	95.7	70	91.8	87.5

DISCUSSION

. 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.

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 (85.2%). However, in 3 cases the rapid diagnostic test result was false negative. These show 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 False positive RDT results occur in a 9 cases. In 7 of these, P. falciparum were seen and in 2 cases, non-falciparum were detected. This may be explained by the fact that P. falciparum can sometimes sequester and may not be present in circulating blood 11. 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

As peripheral blood smear examination requires high level of expertisation and time for the diagnosis of malaria the need of implementation of non microscopic methods are considered. Molecular techniques are the most sensitive and accurate methods that can detect low parasitaemia and mixed infection, but also laborious, coasty and needs experts to perform. Techniques like CBC and RDT are useful in detecting malaria cases with low parasite load which can appear negative with peripheral smear examination. The short time of diagnosis, ease of execution and the higher sensitivity than standard thick films are advantages of CBC and RDT. The Antigentest (RDT) was found to be more user friendly and interpretation was more objective as compared to smear and CBC.

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.

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.

6. REFERENCES

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