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Evaluation of Rapid Diagnostic Kits (Card Test) in Comparison With Routine Smear Examination in The Diagnosis of Malaria

KEYWORDS

Dr. Vijaysathishkumar	* Dr.Amudhavalli
Associate Professor, Dept of Pathology ,Chengalpet medical college	Assistant Professor, Dept of Pathology, Chengalpet medical college. * Corresponding Author
Dr.Ravi	**Dr.Aishwarya**
Professor, Dept of Pathology, Chengalpet medical college.	Final year undergraduate student, Dept of Pathology, Chengalpet medical college.

ABSTRACT *Malaria, one of the most prevalent protozoan disease causes about 1.5million deaths worldwide. Rapid diagnosis is a prerequisite for effective treatment and reducing the mortality and morbidity of falciparum malaria. This study aims at comparing gold standard leishman stained peripheral smear examination with rapid diagnostic tests. 1ml of blood is collected from 100patients having features suspicious of malaria and subjected to Leishman stained smear examination and card tests. Out of the 100 samples, 16 were positive by smear examination and 20 positive by card test. 2cases were positive for p.falciparum by both methods. Of the 20 card test positive cases, 11 were negative by smear method. In this study the sensitivity of card test is 56.25% and specificity is 87%. Though microscopy is the gold standard and cost effective method, this card test proves useful for rapid diagnosis and preventing unnecessary Artemesinin Combination therapy in true negative patients.*

INTRODUCTION:

Malaria is one of the most prevalent protozoan diseases of red blood cells, caused by any one of the 4 Plasmodium species: P.falciparum, P.vivax, P.ovale, P.malariae.It is transmitted by the bite of a blood feeding female anopheline mosquito. Over 500 million people suffer from malaria infection annually which leads to a global death rate over 1.5 million/year(1). In India about 9.75 million people get infected with malaria each year with 2,00,000 deaths/year(2). Though there are numerous tests available for detection of malaria, a single most prompt and effective test is not available. Conventional Leishman/Giemsa stained smear examination remains the gold standard in diagnosis of malaria but these traditional methods of microscopy are time consuming and need manpower. So new techniques like Rapid Kits, QBC are introduced and developed to overcome these limitations. This study deals with comparison of routine thin smear examination with rapid kit (PfHRP2/p LDH) in patients with clinically suspected signs and symptoms suggestive of malaria. These tests are most effective in diagnosing malaria in non-endemic regions so that anti-malarial prescription can be avoided in patients diagnosed as malaria negativewhich prevent drug resistant strains to develop.

AIMS AND OBJECTIVES:

To study and compare the sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio and negative likelihood ratio of the rapid diagnostic kit (PfHRP2 & p LDH test) with Peripheral thin smear examination stained by Leishman stain which is the gold standard.

To determine the most appropriate diagnostic tool for diagnosis of malarial parasites in terms of cost, technical expertise, laboratory facilities, rapidity, sensitivity and specificity for quicker treatment to avoid morbidity, mortality, further transmission of the disease and development of drug resistance.

MATERIALS AND METHODS:

This Cross sectional study is conducted at Chengalpet Government Medical College hospital, Chengalpet for a period of 2 months from July to August 2014. Patients with clinical features, suspected of having malaria were included. Patients treated for malaria in last 2 weeks and patients with confirmed diagnosis of infections like typhoid, pneumonia, urinary tract infection, septicemia, dengue or leptospirosis were excluded from the study. Patient's name, age, sex, OP/IP number and signature/thumb impression was obtained in the informed consent form. 1ml of blood is collected by venipuncture aseptically in EDTA bulbs. Thin blood films were prepared and stained with Leishman stain and the samples were also subjected to antigen detection using Pf/Pan test (PfHRP2 and parasite LDH tests) which is a dual antigen test.

METHODOLOGY AND PROCEDURE:

ROUTINE SMEAR EXAMINATION (THIN BLOOD FILMS):

Laboratory diagnosis of malaria involves microscopic examination of stained blood films using Leishman stain. 1 drop of venous blood is placed on one end of slide and a spreader slide is used to smear blood over the entire length of slide. The slide is left to air dry after which blood is fixed to slide by immersing it briefly in methanol. After fixation, the slide is stained with Leishman stain. After staining, the slide is viewed under a microscope using oil immersion lens. 100 of such slides were prepared and viewed under the microscope. 100 different fields in each smear were examined carefully for the presence of malarial parasites and identification of species. Thin smear is used for determination of species and morphological details of the parasite.

RAPID DIAGNOSTIC TESTS (RDTs):

PfHRP2 test and p LDH test (SD Bioline)-Dual antigen test:

This rapid diagnostic test (P.falciparum HRP2/Pan p LDH) is a three-band test consisting of a control line and 2 test

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lines targeting PfHRP2 and p LDH respectively. These tests are based on the capture of parasite antigens from peripheral blood using either monoclonal or polyclonal antibodies against the parasite antigen target. They target histidine-rich protein2 of P.falciparum and parasite specific lactate dehydrogenase. These RDTs do not require laboratory facilities, electricity or any special equipment.

Card is used as the test format. The anticoagulated blood specimen (2 to 50 microliter) is mixed with a buffer solution that contains a hemolyzing compound and a specific antibody that is labelled with a visually detectable marker like colloidal gold. If the target antigen is present in the blood, a labelled antigen-antibody complex is formed and it migrates up the test strip to be captured by the predeposited capture antibodies specific against the antigens and against the labelled antibody. A washing buffer is then added to remove hemoglobin & permit visualization of any coloured lines formed by immobilized antigen-antibody complexes.

The Pf/Pan test strip has 3 lines. 1st for control, 2nd for non-falciparum antigens (PMA/ pan specific p LDH) and the 3rd line for PfHRP2 (Histidine Rich Protein2 of P.falciparum) antigen. Change of colour on control line is necessary to validate the test & its non-appearance, with or without colour changes in test lines, invalidates the test. With colour change only on control & without colour change on other lines is interpreted as negative. Colour change on 1st two lines(control and Pan) indicates positive non-falciparum infection. Colour change on 1st and 3rd lines indicates (control and Pf) Plasmodium falciparum infection. Colour change on all the 3 lines indicates falciparum infection either as monoinfection or mixed infection with non-falciparum species. In pLDH test, in presence of vivax infection, genus specific line is much darker than species specific line due to presence of all stages of parasites in blood.

OBSERVATIONS AND RESULTS:

Using Gold standard: Routine smear examination using Leishman stained thin film, 16 patients with malaria and 84 patients without malaria were detected. This was followed by performing rapid diagnostic test and the results were interpreted.

The results are represented in table format below:

Table1. Comparison of conventional Leishman stained thin smear examination with RDT for malaria diagnosis

	Leishman stained thin smear(gold standard)	Pf/Pan test
No. of negatives	84	80
No. of P.falciparum positives	2	2
No. of P.vivax positives	14	18
No. of mixed infection positives	0	0
Total no. of patients	100	100

Table.2: Statistical data

Tests	Microscopy (thin smear) positive	Microscopy (thin smear) negative
RDT positive	9	11
RDT negative	7	73

Table.3: Comparison of sensitivity and specificity and other data of RDTs with Leishman stained thin smear microscopy

S. No.	Parameters	Thin smear microscopy	Pf/Pan RDT test
1	Sensitivity	75-95%	56.25%
2	Specificity	90%	87%
3	Cost	Rs.5/test	Rs.45/test
4	Technical expertise	Highly skilled	Minimal skills required
5	Equipment	Laboratory, microscope, electricity	Kit only, no special equipment
6	Time for results	25-45 minutes	15-20 minutes

Other statistical data of RDT:

Sensitivity(true positive rate)- 56.25%

Specificity(true negative rate)- 87%

Positive Predictive Value-PPV(probability of malaria in a positive test)- 45%

Negative Predictive Value-NPV(non-probability of malaria in a negative test)- 91.25%

Positive likelihood ratio(how likely the test result to be found in malaria)- 43%

Negative likelihood ratio- 51%

	Chi squares	P values
Uncorrected	15.64	0.0000765
Yates corrected	13.06	0.0003012

Table.4: P value

Figure 1. Rapid diagnostic kit (Pf/Pan) showing coloured lines in both control and Pan test strip- Plasmodium vivax positive

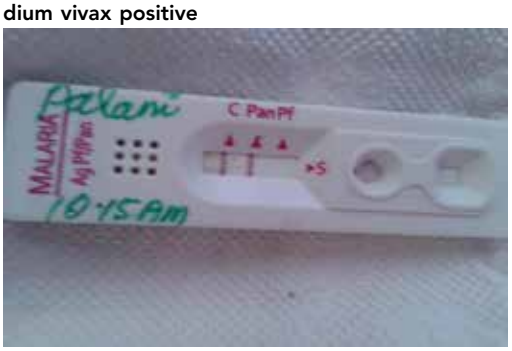

Figure 2. 100 X magnification of Leishman-stained thin smear showing schizont stage of Plasmodium vivax inside the red blood cells.

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DISCUSSION:

Malaria is the leading protozoan disease in the world with much of the cases concentrated in the tropical countries like Africa and India. The number of malaria infected cases is about 9.75 million in India(3) with mortality rate of 30,014-48,660 malarial deaths/year in India(4). The clinical diagnosis of malaria still remains a challenging task and blood smear examination is time consuming compelling the start of empirical drug regimen despite the side effects. Thus the use of rapid diagnostic kits can minimise the time taken to confirm the diagnosis and to start empirical drug regimen and also to avoid unnecessary side effects. The advantages of rapid diagnostic tests include quicker results, less technical expertise.

According to the results from the current study, the sensitivity of the rapid diagnostic kits is 56.25% which is comparatively less compared to other studies in various journals. The specificity of RDT in this study is 87% which is higher than the sensitivity, keeping the microscopy examination as the gold standard in the diagnosis of malaria.

The sensitivity of the current study is less and specificity is higher when compared to the study by Hawkens M, et al.,(4) which had sensitivity and specificity of the three-band RDT was 88% and 82% respectively. The conclusion of this journal was that the use of a three-band HRP2/p LDH combination RDT increased diagnostic specificity for P.falciparum in Ugandan children and distinguished acute infection from recently treated infection. The PPV of our study was only 45% which is very less compared to 84% in the other study which shows that true positive rate in our study area was very less which indicates the non-endemicity of malaria.

According to the journal of Grigg MJ, et al.,(5) Study on combining parasite LDH and HRP2 based rapid tests to improve specificity for diagnosis of malaria due to P.knowlesi and other Plasmodium species in Sabah, Malaysia gave results for CareStart RDT as P.vivax sensitivity of 83% and specificity of 71%. OptiMAL-IT RDT gave P.vivax sensitivity of 60% and specificity of 97%. The results of this study is similar to present study which indicates that there might be cross-reactions between different antigens of Plasmodium species like knowlesi and hence combined RDT tests can be used to detect P.knowlesi infection.

The sensitivity of the current study is less compared to one research analysis by Djalle D et al.,(6)performance of Paracheck-Pf, SD Bioline malaria Ag-Pf and SD Bioline malaria Ag-Pf/pan for diagnosis of falciparum malaria in Central African Republic was done which showed Sensitivities of Paracheck-Pf, SD Bioline Ag-Pf, SD Bioline Ag-pan were 85.7%, 85.4% and 88.2% respectively. SD Bioline kits performed slightly better than Paracheck.

In the study by Singh et al.,(7) 5 commercially available RDTs were tested simultaneously in parallel with peripheral blood smears in outbreak-affected areas. Parascreen RDT gave a sensitivity and specificity of 94.7% and 69.9% respectively for P.falciparum and 84.2% and 96.5% respectively for non-falciparum infection. Comparing with present study, RDT performed well in detecting true negatives in non-endemic area for malaria. The negative predictive value of RDT in our study is 91.25% which indicates non-probability of the disease in a negative test result.

In the current study, those patients tested as RDT negative can be avoided of giving unnecessary antimalarial treatment and so drug resistant strains can be reduced. This is similar to another research study by Williams et al,(8) over-prescriptions of antimalarial drugs were decreased for the patients tested as RDT negative over the study period.

In the study by Kyabianze et al,(9) ICT(Immunochromatographic test) was an accurate diagnostic tool where microscopy is unavailable. The persistent antigenecity and low specificity continues to be a posing limitation in HRP2 based RDTs. Comparing with the current study, comparatively high specificity which overcomes the limitations in misdiagnosing the patients as false negatives by using the three-band PfHRP2/p LDH test served as a reliable tool for diagnosis and treatment of patients suspected of having malaria.

In the current study, 80 patients were tested as RDT negative and therefore highlights the enormous potential of RDT in improving appropriate prescription of anti-malarials in pharmacies and preventing unnecessary use of artemesinin combination therapy(ACT). This is similar to the study by Ikwuobe JO, et al.,(10) having a RDT test negative reduced the chance of purchasing an anti-malarial by 42% compared to not having a test.

In the study by Aydin-Schmidt B et al.,(11) the LDH-based RDT was superior to HRP2 based for monitoring of treatment outcome and detection of recurrent infections after ACT in a moderately high transmission setting. In the current study, the three-band RDT used was p LDH specific and sensitivity was also good for detection of P.vivax infection.

Malaria is a mosquito-borne disease and they breed predominantly in stagnant water. Since this study was conducted in the period of July 2014-August 2014 which was not a rainy season, the incidence of malaria was significantly reduced in Chengalpet localities. Moreover, Malaria Control Programmes have emerged and awareness among people in protecting themselves from mosquito-exposure has increased which has lead to the reduction in the incidence of malaria. Even then false negative cases tested by RDT(due to deletion or genetic heterogeneity of PfHRP2 gene expression) must be analysed and confirmed with microscopic examination which remains still the gold standard in malaria diagnosis.

CONCLUSION:

The reduced sensitivity and increased specificity of RDTs in laboratory diagnosis of malaria keeping the routine thin film microscopic examination as the reference gold standard indicates the reduction in incidence of malaria among the people in Chengalpet, TamilNadu which in turn implies the fact of effective use of Control Programmes and proper management of malaria in the study area. The prevalence of malaria is just 9% which is very less compared to other surrounding regions near Chengalpet. The development of various Malaria Control Programmes by the Central and State Governments has triggered people to keep the surrounding areas free from mosquito breeding and appropriate draining of stagnant rain water.

In the current study, RDTs had more specificity than sensitivity which will help preventing unnecessary Artemesinin Combination Therapy in true negative patients. The logistic, economic and technical factors limit rapid access to microscopic confirmation of malaria in many tropical countries including India. So taking into account of all factors fpr diagnosis of malaria, antigen detection using three

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band RDTs is considered as the most reliable and appropriate diagnostic tool for present condition prevailing in India for malaria.

Though the cost of each rapid kit is Rs.45/kit, high specificity in detecting the true negatives make it a reliable tool for diagnosis of malaria. Although no test can replace the conventional method of Peripheral Blood Smear examination, these newer diagnostic tests can be used as supplement to microscopic examination, where diagnosis is required urgently and laboratory facilities are not in vicinity. These RDTs do not require any special equipment like electricity, microscope, laboratory facilities. This study can pave way for diagnosis of malaria in both endemic as well as non-endemic areas for control and management of malaria transmission

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