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EVALUATION OF MALARIA ANTIGEN TEST AND PERIPHERAL BLOOD SMEARS IN DIAGNOSIS OF MALARIA: A COMPARATIVE STUDY

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Abstract

Aim: Comparative evaluation of malaria antigen test and peripheral blood smears in diagnosis of malaria.

Methods and Material: A competitive evaluation study was carried out in the Department of Microbiology, Neta Ji Subhas Medical College and Hospital, Bihta, Patna, Bihar India from November 2019 to April 2020. All the Blood samples from suspected cases of malaria were routinely subjected to peripheral smear examination and RDT for the presence of malaria parasite.

Results: out of 400 patients 50% male and 50% female on the basis of age 60% patients 20to 40 year and 21.25 % patients above 40 year and most of the patients 82.5% from urban area and 17.5% from rural area. Of the 400 Peripheral smears studied, 125 showed positive for malarial parasite. Plasmodium Vivax(Pv) was diagnosed in 122 Cases, Plasmodium Falciparum (Pf) was identified in 2 case and one smear showed mixed infection with both Plasmodium Vivax and Plasmodium Falciparum. Rapid Diagnostic test showed 137 positive cases, of which 129 were plasmodium Vivax, 3 cases were Plasmodium Falciparum and 5 cases showed mixed infection with Falciparum and Vivax. Sensitivity, specificity, Positive Predictive Value and Negative Predictive value were 100%, 95.63%, 91.24% and 100% respectively.

Conclusions: Our evaluation shows that malaria antigen card test is a simple, reliable and rapid test for the diagnosis as well for speciation of the malaria parasite. The test can be a promising alternative to microscopy in urban and rural areas of our country.

Keywords: Malaria diagnosis, Rapid Diagnostic test, Diagnostic accuracy

Introduction

Malaria remains a major public health problem to tropical and sub tropical regions of the world.[1] In 2010, the World Health Organization (WHO) recommended parasitologic confirmation of suspected malaria cases before initiating anti-malarial treatment and many malaria endemic countries have adopted this policy.[2-4] The scale-up of antigendetecting malaria rapid diagnostic tests (RDTs) for Plasmodium species forms a vital part of the strategy to confirm malaria infection prior to treatment in resource-poor settings.[5] The immunochromatography antigen based malaria RDTs detect histidine rich protein -2 (HRP-2) antigen or Parasite lactosedehydrogenase (pLDH) enzyme. The HRP2 antigen is specific for P. Falciparum and Pan-pLDH detects all human infecting species.[6,7] Malaria confirmation can be achieved with RDTs in resource poor endemic settings where microscopy is not readily available. However, there are growing concerns about the accuracy of malaria RDTs results and their usefulness in providing informed decisions on malaria case management. Previous studies have reported the performance of HRP 2 and pLDH based RDTs for detecting Plasmodium Falciparum when compared to microscopy as the reference standard.[8] A systematic review of 48 studies describing malaria diagnostic performance indicated that although performance varied by species, parasite density and immunity, overall HRP2-detecting RDTs outperformed pLDH-based RDTs with high sensitivity and low specificity for diagnosing malaria in clinical cases in endemic areas.[9] However, HRP2-detecting RDTs are unsuitable for monitoring parasite clearance following anti-malarial treatment due to the persistence of the PfHPR2 antigen in the blood for up to four or five weeks following curative treatment of an infection.[10] The issue of persistent antigenaemia in endemic areas has been raised as a factor leading to reduced specificity of HRP2-detecting RDTs for diagnosing acute malaria and over-estimates of malaria prevalence in community surveys.[11,12] Studies have reported significant variations in RDT sensitivity and specificity [13,14] and particularly when RDTs are exposed to adverse conditions, such as higher temperature.[15] Hence the present study was undertaken with aim to carryout comparative evaluation of the malaria antigen test and peripheral blood smears in diagnosis of malaria in tertiary care setting.

Methods and Materials

A competitive evaluation study was carried out in the Department of Microbiology, Netaji Subhas Medical College and Hospital,Bihta, Patna, Bihar India from November 2019 to April 2020,after taking the approval of

the protocol review committee and institutional ethics committee.

Sample Collection: During this period, 1990 blood samples were received for malaria diagnosis from clinically suspected cases. Blood samples were collected in EDTA vacutainer tube. Peripheral smears were made on a clean glass slides with a drop of blood, air dried and stained with the help of Leishman stain. Smears were thoroughly examined under 100 x with oil immersion for the presence of malaria parasite. Of 1990 samples, 400 samples were randomly selected and Rapid Diagnostic test was performed using Antigen based Pf (HRP-II) and PV (pLDH) specific kit. Procedure was performed as per manufacturer's instructions. About 5 µl of blood was put in sample well with the help of disposable loop provided with the kit. 4-5 drops of assay diluent provided with the kit was added to second well. Results were interpreted after 10 -15 minutes. Results were interpreted as negative when only control band appeared with two negative test bands and as mixed infection when control band and two test bands appeared. It was interpreted as Plasmodium Vivax infection when PV band appeared along with control band. Plasmodium Falciparum was diagnosed when Pf band and control band appeared

Results

Table 1: demographic characteristics of the 400 patients

Parameter	N=400	%,
Gender		
Male	200	50.0
Female	200	50.0
Age		
Below 20 year	75	18.7
20-40year	240	60.0
Above 40	85	21.2
Area of Residence		
Urban	330	82.5
Rural	72	17.5

Table 2: Showing comparison of Peripheral smears and Rapid Diagnostic Tests diagnoses

Results	Peripheral smears	Rapid Diagnostic tests
Positive cases	125 /400 (31.25%)	137/400 (34.25%)
Plasmodium Vivax	122	129
Plasmodium Falciparum	02	03
Mixed infection	01	05
Negative	275/400 (68.75%)	263/400 (65.75%)
Total cases	400	400

Discussion

Accurate diagnosis and early treatment of malaria is essential to reduce mortality and morbidity due to malaria. The various modalities to diagnose malaria are conventional peripheral smear, Quantitative Buffy coat, antigen based Rapid diagnostic kits and Molecular studies (PCR). As per 2011 WHO report, the sensitivity of microscopic examination is less than 75%. It is a common practice in many parts of India to treat febrile patients with antimalarial drugs even after negative microscopic examination which has resulted in resistance to commonly used drug chloroquine. Now the concern is emergence of drug resistance to artemisinin therapy if empirical therapy is followed and this may not be cost effective also as artemesisnin is more expensive than chloroquine.[16] There are more than 60 brands of RDTS in the market based on different combination of antigen specificity. Previous studies have shown RDTs that detects Histidine Rich Protein type 2 (HRP-2) are more sensitive in diagnosing Plasmodium falciparum whereas those detecting lactate dehydrogenase (LDH) enzyme are more specific for P.Vivax diagnosis.[17] In the present study RDT with Pf (HRP 2) and PV (pLDH) specificity were used. Past studies have also proven that the cost of malaria treatment can be reduced by 24% by using RDT and 46% by microscopy against presumptive treatment.[18]

In the present study out of 400 patients 137 (34.25%) were positive and 263 (65.75%) were negative to RDT whereas 125 (31.25%) were positive and 275(68.75%) were negative on microscopic examination. Similar findings were also reported in a study conducted by Rajini Kurup.[19] Previous studies have shown sensitivity and specificity ranging from 84 to 100% for RDT.[20-22] In the present study we found 100% and 95.63% respectively. In our study peripheral smear were negative in 12 cases that showed positivity with RDT.

These peripheral smears were retrieved and studied again. In few cases parasite density was very low and occasional parasite was noted after careful screening of the smears and few cases were partially treated cases before visiting this hospital. Compared to Peripheral smear RDTs are more sensitive and specific for diagnosis of P Falciparum and mixed infections.

This is important because Falciparum causes severe disease and has high mortality requiring urgent intervention, whereas P. Vivax needs to be treated with primaquine to prevent relapses of malaria. The advantages of RDTs are that it is simple, easy to perform, no instruments or electricity required and interpretation is also easy. But the disadvantage is parasite density cannot be assessed and cannot be used to assess response to treatment as it can be positive for 7-14 days after treatment.[23] And with > 60 brands being marketed in India there is always confusion about which RDT kit to use. Pf /Pan specific RDTs cannot differentiate mixed infection (Pf with Pv) from P. Falciparum infections. But recently it is found P.Vivax also can lead to serious disease and no

longer can be considered as benign malaria.[24] Hence when Pv/Pan specific RDT kit is used, mixed infections are to be confirmed with peripheral smear examination. However, newer Pf/Pv specific RDT kits can differentiate mixed from P. falciparum infections. Peripheral smear though inexpensive of the two is laborious to perform, less sensitive, requires electricity, microscope and skilled technician to interpret. Results depend on quality of the smears.[25] But the advantages of peripheral smears are it is cheaper than RDT, parasite density can be assessed and it can also be used as quality control measure to check efficiency of RDTs.

Conclusion

Our evaluation shows that malaria antigen card test is a simple, reliable and rapid test for the diagnosis as well for speciation of the malarial parasite. The test can be a promising alternative to microscopy in urban and rural areas of our country.

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