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ORIGINAL ARTICLE

RAPID DIAGNOSIS OF MALARIA USING PLASMODIUM LACTATE DEHYDROGENASE

Manjula M. R[1], Soumya N[2], Majigoudar S. S[3]

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ABSTRACT: BACKGROUND AND OBJECTIVES: A hospital based study was conducted to evaluate the efficacy of PLDH assay (immune chromatographic test) for the diagnosis of malaria, in comparison with the gold standard 'Microscopic examination' in the Department of Paediatrics, Chigateri Hospital and Bapuji child health and research Centre attached to J.J.M. Medical College, Davangere. **METHODS:** Blood samples from 160 children with clinical suspicion of malaria were tested by PBS study, and PLDH test. The PLDH assay (immune chromatographic test) is a rapid malaria diagnostic test which utilizes a dipstick coated with monoclonal antibodies against the intracellular metabolic enzyme PLDH (Plasmodium lactate dehydrogenase). Differentiation of malaria parasite is based on antigenic differences between PLDH isoforms. Results from the PLDH test were compared to those obtained by PBS. **STATISTICAL ANALYSIS:** All the statistical operations were done through SPSS for Windows, Version16. **RESULTS:** Out of 160 suspected malaria cases, 32 (20%) cases were confirmed positive by PBS (23 Pl. falciparum and 9 Pl. vivax), while 37 (23.1%) cases were detected positive by PLDH test (26 Pl. falciparum and 11 Pl. vivax infections). PLDH test showed sensitivities of 91.3% and 100% and specificities of 96.3% and 98.7%, respectively, and PPV's of 80.8% and 81.8% and NPV's of 98.5% and 100%, respectively, when compared to PBS study for detection of Pl. falciparum and Pl. vivax malaria. **CONCLUSION:** The PLDH test showed an excellent correlation with the PBS study in the identification of Pl. falciparum and Pl. vivax malaria. Thus, we can conclude that the integration of the PLDH test into the Indian health care infrastructure would provide an important and easy tool for the timely diagnosis of malaria.
KEYWORDS: Plasmodium, Malaria, PBS, PLDH.

INTRODUCTION: Malaria is the most common serious parasitic disease of human beings, killing one person every 12 seconds.[1] Estimates from WHO for 2008 suggested that 243 million cases of malaria (around 90% caused by plasmodium falciparum) resulted in 863 000 deaths, of which more than 80% occurred in children younger than 5 years of age in sub-Saharan Africa.[2] The magnitude of the problem is further compounded by plasmodium falciparum (Pl. falciparum) resistance to standard anti-malarial drugs adding to increased morbidity and mortality.[3] WHO recommends that malaria case management be based on parasite-based diagnosis in all cases.[4]

Microscopic examination of blood smears remains the gold standard for the diagnosis of malaria, but it is time-consuming and requires skilled operators.[5] The use of antigen detecting rapid diagnostic tests (RDTs) forms a vital part of this strategy, forming the backbone of expansion of access to malaria diagnosis as they provide parasite-based diagnosis in areas where good quality microscopy cannot be maintained.[4] This study intends to compare the ability of newly developed rapid diagnostic test, an immune chromatographic antigen detection assay for the diagnosis of malaria using plasmodium lactate dehydrogenase (PLDH), against the gold standard microscopy.

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METHODOLOGY: The 160 cases in the age group of 1-16years attending OPD / IPD with clinical features (fever, chills and rigors, anemia, Hepatosplenomegaly) consistent with malaria formed the study sample, which was conducted in the Department of Paediatrics, Chigateri Hospital and Bapuji Child Health And Research Centre attached to J.J.M. Medical College, Davangere between November 2010 to October 2012 after taking clearance from the Ethical committee. Children who were already being treated or partially treated for Malaria in the previous 4 weeks and Children with lung, liver or renal disorders were excluded from the study. Details of the history and clinical examination of suspected Malaria cases were recorded on a pre-structured proforma and an informed consent was taken from all the cases before drawing blood.

SAMPLING PROCEDURE: 2 ml of venous blood was drawn with aseptic precautions and collected into a sterile EDTA test tube. The following investigations were done on the day of admission: hemoglobin, total and differential leucocyte count, platelet count, peripheral smear (both thick and thin smear for malarial parasite). The stained smears were examined under the oil immersion field using a light microscope. Subsequently, the blood sample was subjected to antigen detection by using the Malarigen kit (PLDH assay) according to the manufacturer's instructions.

ETHICAL CLEARANCE: This study was approved by ethical committee of JJMMC, Davangere.

PATIENT CONSENT: Written informed consent obtained from all participants' parents.

STATISTICAL ANALYSIS: All the statistical operations were done through SPSS for Windows, Version16, (Statistical package for social sciences).

RESULTS: Results of the 2 tests employed in the diagnosis of malaria with respect to the species is shown in table 1. Of the 160 clinically suspected cases of malaria, the number of cases positive for malaria by PBS, and PLDH test were 32 (20%) and 37 (23.1%) respectively. Of the 32 smear confirmed cases, 23 were Pl. falciparum positive and 9 were Pl. vivax infection. Of the 37 cases tested positive for malaria by PLDH test, 26 were Pl. falciparum and 11 were Pl. vivax infection cases. Highest number of positive cases (38.5%) was in the age group of 9-11years followed by 12-16years, 12 cases (30.76%). Fever was the most common symptom (100%) followed by, chills and rigor (51.2%), sweating (15.6%), headache (38.5%) and pain abdomen (35.9%). On examination, 64.1% of the malaria cases had pallor, 89.7% had spleenomegaly and 74.3% had hepatomegaly.

There were no cases with features suggestive of complicated malaria. 41% of malaria cases had their Hb% in the 8-10 gm% range and with 28.2% cases in the 6-8 gm% range. 79.4% of malaria cases had platelet count < 1.5 lakh / cm. Comparison of PLDH test with Peripheral blood smears is shown in table 2 which shows that PLDH test and PBS showed 26 and 23 positive cases respectively for Pl. falciparum. Out of which 21 cases of Pl. falciparum were positive by both methods. 5 cases positive by PLDH test were negative by PBS. 2 cases positive by PBS test were negative by PLDH test. Chi-square analysis revealed a highly significant difference between the frequencies of these two tests. (X^2=111.2, P< 0.001HS) Considering microscopy as gold standard, the sensitivity, specificity, Positive predictive value (PPV) and Negative predictive value (NPV) and diagnostic efficiency of PLDH test were calculated. They were 93.8%, 94.5%, 81.1%, 98.4% and 94.4% respectively.

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DISCUSSION: In the present study 32 cases were detected by peripheral blood smear examination. But 37 cases were detected by PLDH test. 2 cases of Pl. falciparum detected by PBS were not detected by PLDH test. This may be explained by the fact that increased awareness of malaria among the general public has led to rampant misuse of antimalarial drugs in inadequate doses empirically for any fever. Since PLDH test detects PLDH which is produced only by viable parasites, the blood samples judged negative by PLDH test may have been dead parasites and not yet cleared from the host. This can also be due to insufficient enzyme production which occurs during early malarial infection or the patient blood samples contained parasites at concentration below the PLDH test kit detection level.[6] 7 cases detected by PLDH test kit, were not detected by PBS. Out of 7 cases, 5 cases were Pl.falciparum.so this may be explained by the fact that Pl. falciparum can sometimes sequester and may not be present in circulating blood.[6]

2 cases of Pl. vivax malaria missed by PBS were positive by PLDH test (Table 2). This could be due to the loss of parasites during processing of smear. A low level of parasitemia below that of detection could have also contributed to smear negativity, or it could be false positive cases due to the presence of cross reacting autoantibodies.[7] The present study had a positivity percentage of 20% for PBS study and 23.1% for PLDH test. This is comparable with studies done by Palmer CJ et al[8] and Jelinik T et al[9] which also had a minor difference in percentage of positivity between PBS study and PLDH test. In the present study, the PLDH test showed sensitivities of 91.3% and 100% and specificities of 96.3% and 98.7%, respectively, when compared with the traditional peripheral blood smear for the detection of Pl. falciparum and Pl. vivax malaria. These results are comparable with Palmer CJ et al[10] and Chayani. N et al.[11] In the present study the PLDH test showed a PPV's of 80.8% and 81.8% and NPV's of 98.5% and 100%, respectively, when compared to PBS study for detection of Pl. falciparum and Pl. vivax malaria. These results are comparable with studies done by Palmer CJ et al,[10] Jelinik T et al,[9] and Jamshaid Iqbal et al,[12] Chayani. N et al and Jamshaid Iqbal.et al.[13]

CONCLUSIONS: Microscopic analysis of stained blood smear has been the gold standard diagnostic technique for identifying malaria for more than a century. The major advantages of it are, it is least expensive, species differentiation is clear and quantitation of parasitemia is possible. But, it is time consuming, labor intensive and requires the service of a skilled technician. In the present study, designed to determine the efficacy of PLDH assay in the rapid diagnosis of malaria, the test showed sensitivities of 91.3% and 100% and specificities of 96.3% and 98.7% for detection of Pl. falciparum and Pl. vivax, respectively in comparison with the PBS.

The PLDH test meets many of the criteria for an ideal diagnostic test: it is simple, rapid, sensitive, specific, and easy to perform and does not require electricity, equipment or a trained technician. The major limitations are the high cost and its inability to indicate the severity of infection. Thus, quality RDT is a valuable complement to microscopy because it helps expand the coverage of parasite based diagnosis to the periphery and minimizes exclusively clinical diagnosis. The cost of improved malaria diagnosis will inevitably increase, whether by investment in microscopy or RDT's or both. However such investments offer a more promising strategy to deal with increasing costs of therapy driven by drug resistance.

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Test	Pl.f alciparum	Pl.vivax	X^2	P value
PBS	23	9	124.1	<0.001 HS
PLDH assay	26	11	149.3	<0.001 HS

Table 1: Results of the 2 tests employed in the diagnosis of malaria with respect to the species

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Plasmodium Species	PLDH Result	PBS result		
		Positive	Negative	Total
Pl. Falciparum	Positive	21	5	26
	Negative	2	132	134
	Total	**23**	**137**	**160**
	X^2=111.2; P <.001 HS			
Pl. Vivax	Positive	9	2	11
	Negative	0	149	149
	Total	**9**	**151**	**160**
	X^2=129.2; P<.001 HS			

Table 2: Comparison of PLDH test with Peripheral blood smears

AUTHORS:

1. Manjula M. R.
2. Soumya N.
3. Majigoudar S. S.

PARTICULARS OF CONTRIBUTORS:

1. Assistant Professor, Department of Paediatrics, Adichunchanagiri Institute of Medical Sciences, B. G. Nagara.
2. Assistant Professor, Department of Paediatrics, JJMMC, Davangere.
3. Professor, Department of Paediatrics, JJMMC, Davangere.

NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Manjula M. R,
51, Kalpatharu Bhavana,
Adichunchanagiri Institute of Medical Sciences,
B. G. Nagara, Nagamangala Taluk,
Mandya-571448, Karnataka, India.
Email: manjula.mmc@gmail.com

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