Int J Biol Med Res. 2012; 3(1): 1356-1358

Contents lists available at BioMedSciDirect Publications

International Journal of Biological & Medical Research

Journal homepage: www.biomedscidirect.com

Original Article

Seroprevalence of Malaria by using dipstick method (Malarigen kit) in the tertiary care hospital

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ARTICLE INFO

Keywords:
pLDH assay
Plasmodium
Malarigen kit
Seasonal variation

ABSTRACT

Background and objective: Microscopic examination of blood smears for malaria parasite is laborious , time consuming and requires skilled operators. Immunochromatographic dip stick assays provide a potential alternative . One such dipstick method (Malarigen kit) assay is based on detection of the *Plasmodium* intracellular metabolic enzyme lactate dehydrogenase(LDH). The differentiation of malarial parasites is based on the antigenic difference between the *Plasmodium* lactate dehydrogenase (pLDH) isoforms. The rapid and specific diagnostic tests to identify individuals infected with malaria is paramount importance in efforts to control public health impact. Therefore, the test is valuable in emergency for rapid diagnosis of malaria. A study was therefore undertaken to find out the seroprevalence of malaria by using dipstick method. Material **Methods:** This study was conducted in the Department of Microbiology, J.J.M. Medical College,Davangre. Patients of both sexes and of all age groups with clinically suspected from September 2006 to November 2011 malaria were studied. Venous blood was collected by venipuncture into EDTA tubes for antigen detection. Results: Totally 4607 patients were screened, who were clinically suspected malaria, 2407 were males and 2200 were females with all age groups. A total of 492 cases were positive for malaria with incidence 10.67%.The *P.falciparum* were 272(55.3%) and *P.vivax* were 220(44.7%). Among 492 positive cases, males were 272 (55.3%), females were 220(44.7 %). In our study, the cases were more during rainy season 280(57%) between July to October. Conclusion: The rapid dipstick method (Malarigen kit) is a simple, sensitive, rapid test (10-15 minutes), ease of performance, interpretation, can be useful tool for the detection of *Plasmodium falciparum* and Plasmodium vivax where the laboratory support is limited in the rural areas.

1. Introduction

Malaria is a major public health problem in India. The National Vector Borne Diseases Control Program (NVBDCP) reported about 1.67 million cases of malaria (including 0.75 million *P. falciparum* cases) and 1487 deaths in the country in 2006.[1] Malaria kills about one million children, under five years of age, each year worldwide.[1] One of most pronounced problems in controlling the morbidity and mortality caused by malaria is limited access to effective diagnosis and treatment in areas where malaria is endemic[2].In India, healthcare is provided by para-health care workers at the domiciliary or village level[3] .In such situations, it usually takes one week for collection of smear ,transporting it to the primary health centre[4]. (PHC) and obtaining laboratory confirmation of the diagnosis. All cases with fever are presumptively treated as being due malaria and given chloroquine. This may result in overtreatment and has a bearing on the malarial parasite developing resistance to chloroquine. The WHO now emphasizes full treatment of patients after identification of malarial species[4].Identification of the malarial parasite requires microscopic observation of the parasite on a stained blood smear, which is laborious and depends on quality of the stained preparation as well as of the microscopes, besides requiring considerable skill for its interpretation[5]. Decentralization of slide examination from regional or district level laboratories to PHCs has resulted in deterioration of the quality of slide

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examination, mainly due to technical problems such as poor maintenance of microscopes, improper staining and inadequate supervision[6]. All these limitations necessitate consideration of alternate strategies for the diagnosis of malaria, which are reliable, rapid and technically less demanding.

The development of rapid diagnostic tests (RDTs) based on immunochromatographic technique (ICT) is a hall mark that has revolutionized the diagnostic facility for malaria[7]. The ICT is a RDT that was initially devised to diagnose *P.falciparum* infection based on the parasite specific histamine rich protein II antigen which was subsequently modified to identify simultaneously the malarial infection at species level also [8,9]., *P.falciparum* infections have increasingly been reported also from some part of India. Therefore, this study was carried out to recognize the present scenario of malaria by using dipstick method (Malarigen kit) in the tertiary care hospital.

2. Material and Methods

This study was conducted at Department of Micriobiology ,J.J.M,Medical College, Davangere, Karnataka, India from September 2006 to November2011. The suspected cases of malaria, attending the OPD and in patients of Chigateri General Hospital and Bapuji Hospital were include in this study. The sample were collected from febrile cases, clinically suspected of malaria, before starting any treatment . A total of 4607 samples were tested by antigen detection kit. Ethical clearance was taken from the institutional ethical committee.

Sample collection: With all aseptic precaution, 1ml of venous blood was collected and transferred into a sterile bottle containing EDTA. The blood sample was subjected to antigen detection by using the Malarigen kit (pLDH assay) according to the manufacture's instructions (ASPEN LABORATORIES).

3. Results

A total of 4607 blood samples were tested, among them 2407 were males and 2200 were females with all age groups, by using rapid dipstick method for detection of malaria. A total of 492 cases were positive for malaria with incidence 10.67%. Of these, *P.falciparum* were 272(55.3%) and *P.vivax* were 220 (44.7%). The distribution of malaria cases, screened are among the total cases shown in Table-1.

Table 1: Result of antigen detection test (Malarigen kit)

	Number	Percentage	Number	Percentage
Positive cases	492	10.67		
P.f			272	55.3
P.v			220	44.7
Negative cases	4115	89.33		

P.f – Plasmodium falciparum *P.v – Plasmodium vivax*

The highest malaria cases were seen in children between the 0-14 years with 456 positive cases. A total of 492 positive cases, males were 272 and females were 220 shown in Table -2.

Table2: Species richness of mosquito fauna recorded during study period in the study area.

	Male			Female		
Age	P.f	P.v	Total	P.f	P.v	Total
0-1year	81	4	12	4		4
1-14years	52	92	244	88	108	196
15-30 years		16	16	8		8
31-50years				48		48
>50 years						
P.f	**160**	**112**	**272**	**112**	**108**	**220**

P.f – Plasmodium falciparum *P.v – Plasmodium vivax*

In our study, the malaria cases were more during the rainy season 280(57%) between July to October–Table-3.

Table 3: Seasonal distribution of malaria positive cases.

Month	Total cases	P.f	P.v
March-June	84	44	40
July-October	280	160	120
November-February	128	68	60
Total	492	272	220

P.f – Plasmodium falciparum *P.v – Plasmodium vivax*

4. Discussion

Several trials worldwide have reported that pLDH assay is an effective diagnostic test for malaria with a sensitivity of P. *falciparum* and *P.vivax* detection ranging from 94% to 95% and from 88 % to 96% respectively.[10,11]A hospital and a field based study from central India [12].also reported that pLDH assay was a useful test for diagnosis of malaria as well as for monitoring treatment. In recent years multiple studies have found that rapid dipsticks have excellent sensitivity and specificity when compared with conventional microscope.[13, 14] A P. *falciparum* infection may be easily missed that when the parasite is sequestered [10] in deep capillaries (spleen ,liver, bone marrow)and are present in insufficient numbers for detection in blood films.[15].as might happen in patients partially treated for malaria. pLDH assay may provide a more precise diagnosis of *P.falciparum* infection in such cases.

The incidence of malaria worldwide is estimated to be 300 - 500 million cases each year. In India there is a consistently declining trend in the annual incidence since 1997. About 1.5 - million cases have been reported annually in India, with the majority being P. vivax cases. In this study the incidence of malaria is 10.67%, with a predominance of *P. falciparum* 272(55.3%) followed by *P. vivax* 220(44.7%).This is low when compared to other studies. [16]

Malaria affects all age groups. In our study, the highest number of positive cases is in the age group of 0 - 14 years, which shows that malaria more commonly affects the pediatric age group. These findings are consistent with the previous studies. [17]

Among 492 positive cases, males were 272(55.3%) and females were 220(44.7%), this finding was similar to other studies [17].In our study, the cases were more during rainy season 280 (57%) July-October and was comparable to other studies.[17] This shows the association of malaria with rainfall. Rain in general provides opportunities for the breeding of mosquitoes and it also increases atmospheric humidity, which is necessary for the survival of mosquitoes.

It is interesting to note that a general trend in increase of malaria cases, particularly due to *P.falciparum* has been seen for the last few years especially during and after rainy season in and around Davangere. Epidemiological studies, to be undertaken to establish the locus of *P. falciparum* malaria in the area including the surveillance under the Global Malarial Stratergy [18].

The pLDH assay indicated mixed infections with P.falciparum and *P.vivax* as *P.falciparum*, which is acceptable given the configuration of the test [11],where *P.falciparum* could react with both monoclonal antibodies on strip. Though this format is advantageous, given the potentially fatal nature of *P.falciparum* infection, this will inevitably miss infections due to non *P.falciparum* species. The incorporation of *P. vivax* specific antibody in the dipstick which does not cross react with *P.falciparum* would obviate this problem.

There are compelling reasons to justify the implementation of a rapid malaria diagnostic test in the field. Rural clinics have difficulty to diagnose malaria on site due to a lack of microscopes and trained technicians to evaluate blood films. Moreover patient follow up is difficult due to economic constraints. Many people cannot afford transportation, so they walk several hours, some carrying small children to reach local clinic, once they have seen, they do not return. Diagnosis must therefore be immediate in order to provide proper treatment.

Malaria is a life threatening infection impacting the most developed countries of the world along of the world lacking basic healthcare infrastructure. Increasing burden of disease, emerging antimalarial drug resistance, are placing greater emphasis on rapid and accurate diagnosis of patients infected with malaria .Given the difficulty performing microscopy, especially in endemic areas, alternative diagnostic strategies are needed. A highly effective RDTs could overt over 1 lakh malaria related deaths and about 400 million unnecessary treatments [19]. In addition, it is likely that RDTs will be cost effective due to improved treatment and health outcomes for febrile disease not due to malaria along with cost saving associated with antimalaria drugs [20]. Although there is a limitation of detecting mixed infections, of all species of *Plasmodium* and infections at low concentrations of parasites, along with an inability to monitor response to therapy. This method can be of great use in a field survey as well, in case of a negative result, microscopy is recommended. Therefore RDTs do not eliminate the need to obtain thick and thin smears and maintaining expertise in microscopy is still a global priority until a new gold standard is developed. However, malaria RDTs are ushering in a new era of diagnosis to improve the global healthcare system.

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