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Prevalence and Detection of Malaria at a Tertiary Care Hospital in Southern Rajasthan, India

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A B S T R A C T

Malaria presents a diagnostic challenge to laboratories in most countries. The majority of malaria cases are found in countries where cost-effectiveness is an important factor and ease of diagnostic test performance and training of personnel are also major considerations. Most new technology for malaria diagnosis incorporates immunochromatographic capture procedures, with conjugated monoclonal antibodies providing the indicator of infection. Preferred targeted antigens are those which are abundant in all asexual and sexual stages of the parasite; currently interest is focused on the detection of histidine-rich protein 2 (HRP-2) from *Plasmodium falciparum* and parasite specific lactate dehydrogenase (pLDH). The total 1440 blood samples of suspected malaria cases were tested by rapid card method during the period of April 2015 to September 2015. Out of 1440 suspected cases, 100(14.4%) cases were positive for malaria. The male to female ratio was 2:1. Positive for *Plasmodium vivax* were (69%) and *Plasmodium falciparum* (31%).The infection is more common in the age group of >18 years. Rapid diagnostic tests offer the possibility of more rapid, non-microscopic method for malaria diagnosis. These tests are easy to perform and require little training to interpret the results as comparing with routine microscopic (gold standard) examination, which required time and need a technical expertise.

Introduction

Malaria is transmitted to humans by mosquitoes of the genus Anopheles. Malaria is known to be caused by four plasmodia species, namely *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*, with *P. falciparum* being the most lethal. There are increasing reports of a fifth human-infecting species, *Plasmodium knowlesi,* which has been described in south-east Asian countries

(Amexo *et al*., 2004; Beadle *et al*., 1994; Bell *et al*., 2005; Chin *et al*., 1968). In addition, of the five species that infect humans, *P. vivax* and *P. falciparum* cause 95% of infections. *P.vivax* may be responsible for 80% of the infections, because this species has the widest distribution in the tropics, subtropics and temperate zones. *P. falciparum* is generally confined to the tropics, *P. malariae* is sporadically distributed and *P. ovale* is

confined mainly to central West Africa and some South Pacific islands. The fifth human malaria, *P. knowlesi,* a malaria parasite of long-tailed macaque monkeys, has been confirmed in human cases from Malaysian Borneo, Thailand, Myanmar and the Philippines (Dutta *et al*., 1999). Diagnosis is complicated by *P. knowlesi* and *P. malariae*having similar morphology, and it is difficult to differentiate *P. falciparum, P. malariae* and *P. knowlesi* by microscopy (Jivabhai *et al*., 2014; Karlekar *et al*., 2012). For efficient treatment and management of malaria, rapid and accurate diagnostic testing is needed. Microscopy has been in use for over 100 years and is inexpensive, rapid and relatively sensitive when used appropriately. Microscopy is regarded as the 'gold standard' for malaria diagnosis (Kilian *et al*., 2000). However, the lack of skilled technologists in medical facilities in affected areas often leads to poor interpretation of data. Furthermore, microscopy is time consuming and labour intensive, cannot detect sequestered P. falciparum parasites and is less reliable at low-density parasitaemia (<50 parasites/ml blood) (Lee *et al*., 2009; Lee *et al*., 2011b; Leke *et al*., 1999).

Most of the point prevalence studies in India have been carried out for outbreak/epidemic investigations. There is very limited information on age and sex specific seasonal prevalence of malaria in different paradigms in the country. The burden is generally higher in men than women in all age groups. Children in the states of Assam (Ong *et al*., 2009), Arunachal Pradesh (Palmer *et al*., 1998), and Rajasthan (Patriia) had a higher incidence of malaria than adults. Malaria is considered to be immediately life threating and a patient with the diagnosis of *P. falciparum* or *P. knowlesi* malaria should be considered a medical emergency because the disease can be rapidly fatal.

Clinical misdiagnosis of malaria has been reported both in the public and private health sectors. Both under and over diagnosis of malaria has been observed in South East Asian countries (Amexo *et al.,* 2004). Hence it is necessary to use appropriate diagnostic tools to prevent unethical use of antimalarials in the malaria negative patients (WHO, 2006). The development and commercial availability of Rapid diagnostic tests either for individual species (for P. falciparum) or in combination (P. falciparum + P. vivax) or for all the species has revolutionized malaria diagnosis recently (Palmer *et al.,* 1998; Valecha *et al.,* 2003). The standard test particularly HRP-2 based for P. falciparum has acceptably high sensitivity and specificity (Beadle *et al.,* 1994). With the reducing cost of these tests, RDTs are now being increasingly deployed in the control programs in India. The major advantage is that RDT can be used for on-the-spot diagnosis and treatment of malaria and in many situations could be lifesaving as well as a useful tool for transmission control at the community level.

Although RDT's for malaria diagnosis have been introduced in the National Policy, there use should be further encouraged as a part of the EDPT policy being followed in India. Besides routine diagnosis during both active and passive case detection, they can also play an important role in the special situations e.g. in spot detection and treatment of cases in migrant population/refugees, in large projects where labor congregates and during outbreaks of malaria.Our aim of the study is to detect malarial parasitic infections among patients attending our tertiary care hospital and to know the prevalence.

Materials and Methods

This study was carried out at the department of Microbiology, central clinical laboratory,

Pacific institute of Medical Sciences, Udaipur, Rajasthan, over a period of April 2015 to September 2015.

Study type: Retrospective type of study.

Sample collection

The patient's name, age, sex, details of history and clinical examination findings were recorded. After obtaining informed consent, 2 ml of blood collected in EDTA (anticoagulant) containing vial from antecubital vein of all patients by taking sterile precautions.

Rapid diagnostic test (RDT)

Malaria antigens suitable for rapid diagnostic tests are HRP-2 and pLDH. The detection system for *P.falciparum* malaria is based on the detection of *P.falciparum* specific histidine rich protein-2 (Pf.HRP-2), which is a water soluble protein that is released from parasitized erythrocytes of infected individuals. The detection system for *P.vivax* malaria is based on presence of *P.vivax* specific pLDH.

Malaria Antigen utilizes the principle of immunochromatography. As the test sample flows through the membrane assembly of the device after addition of the clearing buffer, the colored colloidal gold conjugates of monoclonal anti-Pf. HRP-2 (IgG) antibody and monoclonal anti-*P.vivax* specific pLDH antibody complexes the HRP-2/pLDH in the lysed sample. This complex moves further on the membrane to the test region where it is immobilized by the anti-Pan specific pLDH (monoclonal) antibody and/or the monoclonal anti-Pf.HRP-2(IgM) antibody coated on the membrane leading to formation of pink-purple colored band/s which confirms a positive result. A band will appear under Pf at the test region in falciparum positive samples, while a band will appear under Pv in *vivax* malaria positive samples. Appearance of band under Pf as well as Pv in the test region suggests a mixed infection.

Results and Discussion

Total 1440 suspected cases were studied during the period of six months, out of which 100 cases were positive for malaria. The prevalence rate was 14.4%. Prevalence of malaria was more in males (63%) as compared to females (37%)[Graph 1].

Among the 100 cases of malaria positive cases, *Plasmodium vivax* was predominant (69%) and *Plasmodium falciparum* was (31%). In this study malaria infection was more common in the age group of >18 years (71%), as compared to ≤18 years (29%) [Table 1].

The present study detected all the positive malaria cases by rapid diagnostic method (Immuno-chromatography method). Immuno-chromatographic technique offers the possibility of more rapid, non-microscopic methods for malaria diagnosis, thereby saving on training and time. These tests are easy to perform and require little training to interpret the results.

However, microscopy is regarded as the 'gold standard' for malaria diagnosis (WHO, 1999). However, the lack of skilled technologists in medical facilities in affected areas often leads to poor interpretation of data. Furthermore, microscopy is time consuming and labour intensive, cannot detect sequestered P. falciparum parasites (Leke *et al.,*., 1999) and is less reliable at low-density parasitaemia [<50 parasites (ml blood) (Kilian *et al.,* 2000; Bell *et al.,* 2005)]. None the less, a good microscopist can differentiate species with microscopy.

The prevalence of malaria infection in our study was found to be (14.4%). Male to

female ratio was 2:1. Species distribution was *P.vivax* (69%) and *P.falciparum* (31%). Maximum prevalence was found to be in the month of July and Agust. Malaria infection detected in all age groups with maximum prevalence in age group >18 years.

There is a wide variation of reports of prevalence of malarial infection in India and other countries. This can be due to differences in geographical and climatic condition which affect mosquito breeding, socio-economic conditions of patients, knowledge about healthcare and public health practices.

Prevalence of malarial infection in our study was 14.4% which is similar to Singh *et al.,* of Navi Mumbai(16.58%),Pandey *et al.,* of Bilaspur (24.74%). However Hadiya*et al.,* from Gujarat and Karlekar *et al.,* from Gadchiroli (Maharashtra) reported very less prevalence of 2.10% and 4.28% respectively. This difference could be due to geographical and climate conditions.
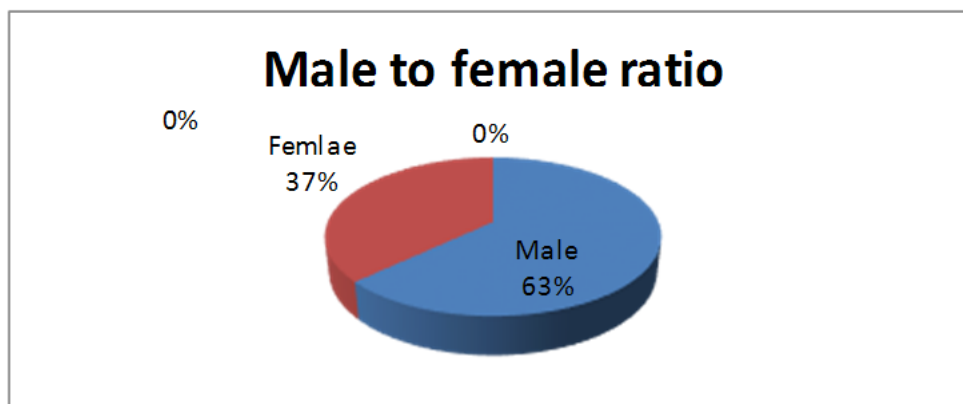
However, our study period is only 6 months (April to September), maximum numbers of cases were found in the months of July and August. Similar findings are reported by Singh *et al.,* Sachin *et al.,* Hadiya *et al.,* The high prevalence of malaria in this period could be due to collection of water in rainy season and mosquito breeding.

Regarding prevalence of species, *Plasmodium vivax* was 69%, *Plasmodium falciparum* 31%. Our findings are similar to Hadiya*et al.,* who reported *P. vivax* 61.41% *P. falciparum* 38.56%, but different from Karlekar *et al.,* who reported *Plasmodium vivax* 33.8% and *Plasmodium falciparum* 66.6%. The difference in prevalence of *Plasmodium vivax* and *Plasmodium falciparum* in different areas can be due to presence of endemicity of particular type and higher relapses in *vivax* type.

Table.1 Detection of Malaria antigens by rapid card method and distribution among male and females

Species of Malaria parasites	Children (≤18 years)		Adults (>18 years)		Total
	Male	Female	Male	Female	
P. falciparum	07	05	12	07	31
P. vivax	10	07	34	18	69
Total	17	12	46	25	100

Graph.1 Gender wise distribution of Malaria infection

Male to female ratio in our study was 2:1, which is similar to Karlekar *et al.,* and Singh *et al.,* reports. The difference in M:F ratio could be due to various reasons like body odour, which may attract mosquitoes, movement of males in wider areas, more chances of mosquito bites and some unknown inherent susceptibility. In our study the maximum number of cases of malaria occurred in the age group >18 years (63%). Our finding correlates with S.R. Karlekar *et al.,* who reported mean age group of 24.8 years and Singh *et al.,* reported 21-30 years of age. The reason of higher prevalence in this age group could be due to movement in wider areas possibly endemic, more chances of exposure to mosquito bites.

In conclusion, parasitological confirmation of suspected malaria using microscopy, the gold standard, is cumbersome and requires trained personnel, microscopes and a source of electricity. PfHRP2- and pLDH-based RDTs are the most commonly used. These tests are easy to perform and require little training to interpret the results. Therefore, malaria treatment based on RDTs, are quick in endemic and remote areas, where limited sources.

The present study reveals a prevalence rate of malarial infection (14.4%) in tertiary care hospital, Udaipur (India). Malarial infections were more in males than females and infection occurred in age group >18 years. This finding could be due to more chances of exposure of mosquito bites in endemic areas.

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