

COMPARISON BETWEEN MICROSCOPY AND RAPID DIAGNOSTIC TESTS IN DIAGNOSIS OF MALARIA AT A TERTIARY CARE MEDICAL INSTITUTION IN UTTARAKHAND (A 3-YEAR STUDY)

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Received: 21 August 2017, Revised and Accepted: 28 October 2017

ABSTRACT

Objectives: Malaria is one of the most prevalent parasitic diseases all over the world including India. Although the microscopic study of stained peripheral blood smear (PBS) is a gold standard of malaria diagnosis due to some subjective errors, rapid diagnostic tests (RDTs) can be a suitable alternative. This study was conducted to estimate the prevalence and demographic details of malaria cases along with a comparison between the two most common screening methods: PBS and RDTs.

Methods: Demographic profile, the prevalence of malaria in this region of Uttarakhand and evaluation of efficacy of RDT as a screening method was performed. Analysis of PBS microscopy for malaria parasites was performed and compared with immunochromatography based RDT over a duration of 3 years.

Results: Out of total 2982 clinically suspected patients of malaria, 132 were found to be positive by either of the two methods. Prevalence of malaria was 4.4% in our study. *Plasmodium vivax* was the predominant species isolated (95%). Males outnumbered females with a ratio of 2.1:1. The most common age group affected was 30–49 years. Sensitivity and specificity of RDT was found to be 91.8% and 93.8%, respectively. Positive predictive value and negative predictive value were found to be 97.8% and 98.9%, respectively.

Conclusion: We conclude that Uttarakhand is a low prevalence area for Malaria and the RDT based on malaria antigen (whole blood) method is as specific and sensitive as the traditional PBS microscopy. Thus, it can be used as an alternative to PBS microscopy.

Keywords: Rapid diagnostic test, Sensitivity, Specificity, Microscopy, Malaria.

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INTRODUCTION

Malaria has been a continuous socioeconomic burden among almost all of the developing countries including India. Historically, malaria in our country was predominantly caused by *Plasmodium vivax*, accounting for 53% [1] of the estimated cases and also by far we have the greatest estimated *P. vivax* burden of any country. *P. vivax* accounts for approximately a third of all malaria cases in India while a study reported that states such as West Bengal, Gujarat, Madhya Pradesh, Orissa, Andhra Pradesh, and North-East being highly endemic for *P. falciparum* and they contribute to around 97% of the total *P. falciparum* affected cases in the country [2].

Microscopy and rapid diagnostic tests (RDTs) represent the two most commonly used methods for detection of malaria with the largest impact on malaria control today [3]. Although light microscopy is considered as gold standard, it has its own characteristic strengths and limitations. It is prone to inherent errors due to sample handling, staining, and individual reader techniques [4-6]. According to Kilian *et al.* and Bell *et al.*, in case of low parasitemia (<50/ml) microscopy is less reliable [7,8]. In contrast, RDT kits are equally sensitive, specific, and stable under operational conditions and does not require extensive training or any equipment to perform or to interpret the results, and commercially available with all necessary reagents [9-11]. Moody and Leke *et al.* also reported that results of RDTs are rapidly available and less liable of being falsely negative due to parasite sequestration [12,13]. Antimalarial drug resistance and economic loss due to increased morbidity and mortality, there is an urgent requirement of improvement of parasite-based diagnostic methods with good quality and their availability to people living in endemic areas.

METHODS

This retrospective study was conducted in a tertiary care medical institution at Rishikesh, Uttarakhand. Blood samples were collected in Ethylenediaminetetraacetic acid vials from 2982 patients who were clinically suspected for malaria. Thick and thin smear microscopy and RDTs were done on these blood samples collected over 3 years. The patient's name, age, sex, details of fever, and other symptoms and clinical examination findings were recorded.

Thick and thin films were made within 10 min of collection and stained by Leishman's stain. RDT based on Lactate dehydrogenase/Histidine-rich Protein-2 antigens was carried out on aliquots of whole blood.

As per diagnostic modality requested by clinicians, samples were divided into three groups.

1. Group 1 represents the samples which were only sent for peripheral blood smear (PBS) microscopy.
2. Group 2 represents the samples which were only sent for RDT.
3. Group 3 represents the samples which were sent for both PBS microscopy and RDTs.

Statistical analysis

All data were analyzed using Statistical Package for the Social Sciences (SPSS) version IBM SPSS-23. Data are represented in the form of frequency and percentage.

RESULTS

Out of 2982 samples screened for malaria, 132 samples tested positive. Among 132 positive samples, 41 were detected in Group 1, 42 were

Table 1: Demographic details of the malaria patients (n=132)

Gender	Male (n=89) mean±SD	Female (n=43) mean±SD
Age (years)		
0-18	12.21±2.92	13.75±3.09
19-29	23.09±2.55	24.15±2.17
30-49	38.02±5.67	35.85±4.54
≥50	58.11±5.9	54.83±2.96
Geographic location	Haridwar, Pauri Garhwal, Bijnor	
Seasonal distribution	July-September	
Species isolation	<i>P. vivax</i> (95%;125/132) <i>P. falciparum</i> (5%;7/132)	

SD: Standard deviation, *P. falciparum*: *Plasmodium falciparum*, *P. vivax*: *Plasmodium vivax*

Table 2: Sensitivity, specificity, PPV and NPV of RDT

Statistical analysis	Total screened (n=2982)	Malaria positive (n=132)
Group 1	1521	41
Group 2	1045	42
Group 3	416	49
Sensitivity	91.8%	
Specificity	93.8%	
PPV	97.8%	
NPV	98.9%	

PPV: Positive predictive value, NPV: Negative predictive value, RDT: Rapid diagnostic test

detected in Group 2, and 49 were detected in Group 3. Prevalence of malaria was found to be 4.4%. Predominant species detected in our study was *P. vivax* (n=125/132, 95%), maximum prevalence was found to be in the month of July-September. Males (67.4%) outnumbered females (33.3%) in our study with ratio being 2.1:1 (Table 1). The most common age group affected was between 30 and 49 years of age with 12.1% in children in age group of 0-18 years.

Out of 49 malaria positive samples using both methods, four samples were found to be malaria positive by PBS microscopy only and 45 were found malaria positive by both of the methods and one by RDT alone. Sensitivity and specificity of RDT was found to be 91.8% and 93.8% by taking PBS microscopy as gold standard (Table 2).

DISCUSSION

Malaria is a major vector-borne disease in India. Extensive geographical areas, climatic diversity and variable malaria epidemiology in India is associated with high parasite genetic diversity and rapidly evolving drug resistance. Thus, epidemiological studies along with their screening methods and modern biological studies could help to find enduring solutions in future. Malaria is a major global public health concern. Annually, there occurs approximately 300 million of clinical cases and over 1 million of deaths worldwide due to malaria [14]. Prevalence rate of malaria at our tertiary care was found to be 4.4%. Jivabhai *et al.*, from Gujarat and Karlekar *et al.*, from Gadchiroli (Maharashtra) also reported a similar prevalence of 2.10% and 4.28%, respectively [15-18]. However, Kumar *et al.* from Udaipur, Rajasthan (14.4%), Singh *et al.* from Mumbai (16.58%), Sahu *et al.* from Orissa (16.5%) Pandey and Manwani from Bilaspur (24.74%), Baruah *et al.* (30.2%) from Nagaland, Das *et al.* from Assam (49.1%) [19-22], and Satyanarayana *et al.* from tribal belt of Andhra Pradesh (69.1%) reported a much higher prevalence. Some of these studies are hospital based, and others have conducted field survey's [23]. Hence, variations in prevalence might be due to different study settings, differing ecological conditions, along with socioeconomic conditions of patients and local public health practices which determine mosquito breeding and spread. Like these studies, most of the malaria cases were detected in the mosquito breeding rainy season of July-September.

In the current study, *P. vivax* was the predominant species identified in 95% cases followed by *P. falciparum* (5%). Our findings were similar to Kumar *et al.* from Rajasthan and Jivabhai *et al.* from Gujarat, who have reported *P. vivax* as the predominant species in 69% and 61.41% cases,

respectively [15-17]. However, *P. falciparum* as the predominant species have been reported from studies from Nagaland (76.5%), Assam (97.1%), and Orissa (89.1%) [19,21,22]. The difference in prevalence in different areas is due to the regional endemicity of a particular *Plasmodium* species.

Malaria is usually diagnosed using clinical criteria, with microscopy as the current gold standard even though it is not appropriate in many health-care settings. In recent years, RDTs have been considered as an ideal alternative method for diagnosing malaria [24,25]. Today's multi-million dollar investment in antimalarial drug and vaccine development should be accompanied by a parallel commitment to improve diagnostic tools and their availability to those living in endemic malaria areas. Wongsrichanalai *et al.* Suggested that RDT is a valuable complement to microscopy because it helps in reducing the over diagnosis based on clinical symptoms only and thus widens the coverage of parasite-based diagnosis to the periphery [6]. Similarly, Jessica *et al.* recommended the use of RDT in conjunction with microscopy [25,26]. These studies are in concordance with our study where sensitivity and specificity of RDTs was found to be 91.8% and 93.8%, respectively.

CONCLUSION

Malaria should always be considered as a medical emergency, and an early diagnosis should be made so that we could prevent its further spread in community. Although RDTs are best alternative to microscopy till date, their high cost and low specificity have been a matter of concern. However, in contrast, we found high sensitivity as well as high specificity of RDT as compared with microscopy in our study.

Thus, we conclude that RDTs can be used for confirming clinical diagnosis and for starting early and prompt treatment of malaria which would surely decrease the morbidity and mortality in the hospital as well as in field settings especially in a low prevalence area like Uttarakhand.

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