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Original Article

Microbiology Section

Prevalence of *Plasmodium falciparum* and *Plasmodium vivax* Infections among Malaria Suspected Patients Attending a District Hospital, Assam

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ABSTRACT

Introduction: Malaria is a major public health problem in Assam and other North-Eastern states of India. Lakhimpur district is also one of the important malaria endemic regions of Assam, where several malaria outbreaks occurred in the recent past. The district hospital received the highest numbers of Malaria suspected patients from every corners of the district, which includes various age groups, caste and communities and belongs to different socio-economic status. Thus, it may help to estimate the prevailing burden of malaria in this geographical area for framing policy and action for malaria control.

Aim: To understand the malaria epidemiology and related risk among the suspected patients.

Materials and Methods: We conducted a cross-sectional hospital based study on total 1362 malaria suspected patients attending North Lakhimpur Civil Hospital which was carried out from May, 2013 to June, 2015. The *Plasmodium* spp. detection was carried out by microscopic examination followed by immuno chromatography based rapid test kits. The relationship between age, sex and *Plasmodium* spp. infection were also examined.

Results: Total 1362 blood samples tested, out of which 53 diagnosed as malaria positive. The overall Slide Positivity Rate (SPR) was 3.89. Total 45 (84.9%) patients infected with *P. falciparum*, 6 (11.3%) with *P. vivax* and 2 (3.8%) with both *P. falciparum* and *P. vivax* parasites. *P. falciparum* was the most prevalent infection. Monsoon season (May, June and July) was the peak period of the year for malaria transmission in Lakhimpur. Highest Slide Positivity Rate (6.9) was observed in the age group of 5-15 years. Highest *P. falciparum* infection occurred in the age group 5-15 years and highest *P. vivax* infection found in age group of 16-30 years. The two age groups 5-15 and 16-30 years were found as vulnerable groups for *Plasmodium* spp. infection. No significant relationship between sex and *Plasmodium* spp. infection was observed. The Bengali (9.1%) and Tea Garden community (7.7%) of the district demonstrated highest seropositivity rate.

Conclusion: A significant burden of malaria still prevailing in this borderline district of the state. Early prompt diagnosis with enhanced malaria surveillance, community education and awareness regarding adult vector control, minimization of local breeding sites are highly recommended.

Keywords: Epidemiology, Microscopy, Rapid diagnostic test, Risk factor

INTRODUCTION

Malaria globally affects about 214 million people annually and accounts for nearly 4, 38,000 deaths [1]. Malaria is endemic in 104 tropical and subtropical countries of the world and still remains as one of the leading infectious diseases [2]. In India also, malaria is a major public health problem. About 95% population in the country resides in malaria endemic areas [3]. In North-eastern parts of India, malaria is endemic in most of the states including Assam [4,5] and it continues to obstruct the equitable socioeconomic development of the region [6]. *P. falciparum* remains the predominant malaria parasite species in Assam causing 58%-68% of the malaria cases [4,5]. In North-eastern states including Assam, the malaria transmission is perennial and persistent [7]. Due to conducive environment to the proliferation and survival of vectors throughout the year, focal outbreaks are common in this part of India at any period of time in the year [6,8,9]. In the year 2006, severe malaria epidemics occurred in Assam with total 1707 death [3]. In that year, in Lakhimpur district of Assam, nearly 200 malaria death due to *P. falciparum* infection was reported [3,10]. The present study was undertaken to determine the current prevalence rate of *P. falciparum*/*P. vivax* associated infections in this malaria endemic region of Lakhimpur district of Assam.

MATERIALS AND METHODS

Study Design and Sample Collection

This was a hospital based cross-sectional study of malaria

suspected patients attending North Lakhimpur Civil Hospital and tested at District Public Health Laboratory, Lakhimpur, which is a malaria endemic area in Assam, India. The study was carried out from May, 2013 to June, 2015. Total 1362 malaria suspected patients were screened, who carries the necessary prescription/laboratory investigation slip issued by the clinicians for malaria tests. Out of the patients, 713 were males, 649 were females, 71 were <5 years age, 290 were in 5-15 years, 479 were in 16-30 years, 461 were in 31-60 years age group and 61 patients were >60 years of age. Around 2 ml of whole blood from the patients was collected using disposable syringe and immediately transferred to EDTA vial. Before collecting blood samples and necessary demographic information, the verbal consent was obtained from both the patient and guardian to perform the tests. The patients coming without the required prescription/ laboratory investigation slip and those who did not wish to give their blood samples were exempted from the study. As this study was based on the analysis of routine laboratory investigation works, so the ethical approval was not necessary.

Laboratory Methods

Malaria Microscopy

Total one thousand three hundred sixty two (1362) blood smears collected from the suspected subjects were screened for malaria parasite infection in peripheral blood smear using microscopic technique. Both thick and thin films were prepared and allowed to dry. Smears were stained with the JSB staining solution [11]. Briefly, the smear were dipped in coupling jar with JSB-2 for 2-3 times and washed in tap water for 2-3 times. Then in coupling jar with JSB-1, the smears were dipped in for 40-60 seconds, washed with tap water, allowed to dry and observed in microscope (Magnus, MLX, India) under 10X eye pieces and an oil immersion objective of 100X magnification using standard method and were examined for at least 100 microscopic fields for malaria parasite identification [12].

Serology

Rapid one step Malaria Histidine-Rich Protein-II (HRP-II for *P. falciparum*) and *Plasmodium* Lactate Dehydrogenase (pLDH for *P. vivax*) antigen rapid test from SD BIOLINE Anti-Malaria *P. falciparum*/*P. vivax* kit (SD Bio Standard Diagnostics Pvt. Ltd., India) were used according to manufacturer's instructions for detection of malaria parasites. The kit allowed to come to room temperature first. Then 5 µl of whole blood added to the round circle of the card, followed by 4 drops of assay diluents added to the diluents area of the card and waited for 15-30 minutes. Then the results were observed and recorded [13,14].

STATISTICAL ANALYSIS

The percent of positive cases among the blood smears examined was the slide positivity rate (SPR). The slide *P. falciparum* rate (SfR)/ slide *P. vivax* rate (SvR) and slide mixed rate (SmR) was the proportion of blood smears found positive for *P. falciparum*/*P. vivax*/ mixed. Using the statistical software SPSS version 17.0 (SPSS, Chicago, IL, USA), the statistical analysis was carried out. In the form of percentage and numbers, the qualitative data were presented. Using Pearson's chi-square test, the *Plasmodium* spp. prevalence in different sub groups was compared. With 95% confidence interval (CI), an odds ratio (OR) was computed by the univariate logistic regression analysis for statistically significant factors if any. Different risk factor evaluation for malaria infection has been carried out by this method. Taking the <5 years age group as reference group, the relationship of age group and *Plasmodium* spp. infection was studied.

RESULTS

Among the patients tested, total 53 cases were detected as malaria positive and the overall Slide Positivity Rate (SPR) was 3.89. Out of these 53 malaria positive cases, 45 (84.9%) patients were infected with *P. falciparum*, 6 (11.3%) patients were infected with *P. vivax* and 2 (3.8%) patients were infected with both *P. falciparum* and *P. vivax* parasites. The respective Slide Infection Rates were 3.30 SfR, 0.44 SvR and 0.15 SmR [Table/Fig-1]. The *P. falciparum* mono-infection found to be highest among the malaria positive cases, followed by *P. vivax* mono-infection and both *P. falciparum* and *P. vivax* mixed infection.

Among the three consecutive study years, the highest SPR was observed in the year 2015-16 (6.02), followed by 2014-15 (4.74) and 2013-14 (1.24) [Table/Fig-1]. The highest SfR (5.30) was also observed in the year 2015-16, followed by 2014-15 (SfR 4.53). However, the highest percentage of *P. falciparum* cases among the malaria positive was found in the year 2014-15 (95.5%), followed by the year 2015-16 (88.0%). The year 2013-14 revealed more SvR (0.62) and *P. vivax* cases in percentage (50%) than in the year 2014-

Study Year	BSE/n (SPR)*	Pf (%)/Pv (%)/mixed(%)†	SfR/SvR/SmR‡
2013-14 (May-13 to April-14)	483/6 (1.24)	2(33.3)/3(50.0)/1(16.7)	0.41/0.62/0.21
2014-2015 (May-14 to April-15)	464/22 (4.74)	21(95.5)/1(4.5)/0(0)	4.53/0.22/0
2015-2016 (May-15 to June-15)	415/25 (6.02)	22(88.0)/2(8.0)/1(4.0)	5.30/0.48/0.24
Total (May-13 to June-15)	1362/53 (3.89)	45(84.9)/6(11.3)/2(3.8)	3.30/0.44/0.15

[Table/Fig-1]: Year-wise malaria incidence among the suspected patients attending the district Hospital.
*BSE= Blood Smear Examined; n= Malaria positive case; SPR= Slide Positivity Rate
†*Pf= P. falciparum; Pv= P. vivax; mixed= P. falciparum* and *P. vivax* mixed infection
‡SfR= Slide *falciparum* Rate; SvR= Slide *vivax* Rate; SmR= Slide mixed infection Rate

15 and 2015-16. Two cases with *P. vivax* and *P. falciparum* mixed infection diagnosed in the year 2013-14 and 2015-16 each. Out of the total 53 malaria cases, *P. falciparum* demonstrated itself as the most prevalent infection with the no. of 45 in Lakhimpur district [Table/Fig-1].

The month-wise distribution study of *P. falciparum* and *P. vivax* cases across the three study years revealed that the month of June was the peak time for malaria parasite infection in Lakhimpur with total 35 malaria positive cases, out of which 30 were *P. falciparum* and 5 were *P. vivax* cases [Table/Fig-2]. In the month of May, the 2nd highest malaria positive cases (11) were recorded, out of which 10 cases were *P. falciparum* and 1 was *P. vivax* case. In the month of July, total 3 nos. of *P. falciparum* cases were recorded in the year 2014. So, the months of May, June and July were found to be the peak period of the year for malaria transmission in Lakhimpur.

The distribution and relationship study between the age group, sex and *Plasmodium* spp. infection revealed the highest Slide Positivity Rate (SPR) 6.9 in the age group of 5-15 years, followed by SPR 4.2 in the age group of <5 years, SPR 3.8 in 16-30 years, SPR 3.3 in >60 years and SPR 2.2 in 31-60 years age group [Table/Fig-3]. The highest numbers of *P. falciparum* infection (18) was found in the age group of 5-15 years, whereas, the highest numbers of *P. vivax* infection (4) was observed in the age group of 16-30 years. The age groups 5-15 and 16-30 years each also demonstrates one (1) *P. falciparum* and *P. vivax* mixed infection.

Taking the <5 years age group as reference group, the relationship of age group and *Plasmodium* spp. infection was also studied, where it was observed that the vulnerability of *Plasmodium* spp. infection was two fold increased in the age group of 16-30 years (OR=2.19; 95% CI 0.50-9.61) as compared to the reference age group, although it was not statistically significant. However, no other significant relationship in terms of *Plasmodium* spp. infection was observed in other age groups [Table/Fig-3].

The distribution and relationship of sex and *Plasmodium* spp. was also checked in the study, which revealed that the males have slightly more SPR (4.1), Pf (23) and Pv (4) numbers than the females (SPR 3.7, Pf 22, Pv 2). The two (2) numbers of the *P. falciparum* and *P. vivax* mixed infections also found in the male group only. Hence, the males were found to be slightly more vulnerable to the malaria infection than the females. However, no statistically significant relationship were observed between the sex and *Plasmodium* spp. infection (OR=1.10; 95% CI 0.64-1.92) in our study in Lakhimpur district of Assam [Table/Fig-3].

The distribution and relationship between the type of

Age Group (years)	BSE/n (SPR)*	Pf/Pv/ mixed†	OR (95% CI)	p-value
<5	71/3 (4.2)	3/0/0	1.00 (Ref)	
5-15	290/20 (6.9)	18/1/1	1.30 (0.21-8.01)	0.77
16-30	479/18 (3.8)	13/4/1	2.19 (0.50-9.61)	0.30
31-60	461/10 (2.2)	9/1/0	1.15 (0.26-5.09)	0.85
>60	61/2 (3.3)	2/0/0	0.65 (0.14-3.06)	0.59
Sex				
Female	649/24 (3.7)	22/2/0	1.00 (Ref)	
Male	713/29 (4.1)	23/4/2	1.10 (0.64-1.92)	0.73

[Table/Fig-3]: Relationship between age group, sex and malaria spp. infection among the suspected patients.
*BSE= Blood Smear Examined; n= Malaria positive case; SPR= Slide Positivity Rate
†*Pf= P. falciparum; Pv= P. vivax; mixed= P. falciparum* and *P. vivax* mixed infection

Months	*Pf* (*mono-infection*)				*Pv* (*mono-infection*)				Total (2013-15)
	2013	2014	2015	Total	2013	2014	2015	Total	Total *Pf* + *Pv*
May	0	0	10	10	0	0	1	1	11
June	1	17	12	30	3	0	2	5	35
July	0	3	-	3	0	0	-	0	3
Aug	0	0	-	0	0	0	-	0	0
Sept	0	0	-	0	0	0	-	0	0
Oct	0	0	-	0	0	0	-	0	0
Nov	0	0	-	0	0	0	-	0	0
Dec	1	0	-	1	-	0	-	0	1
Jan	-	0	0	0	-	0	0	0	0
Feb	-	0	0	0	-	0	0	0	0
Mar	-	0	1	1	-	0	0	0	1
April	-	0	0	0	-	0	0	0	0
Total	2	20	23	45	3	0	3	6	51

[Table/Fig-2]: Monthly and yearly distribution of malaria parasite infection among the patients.

Plasmodium spp. and sex was also studied. However, no significant relationship was observed in terms of types of malaria parasite infection (*P. falciparum*, *P. vivax* and both mixed) and sex in our study [Table/Fig-4].

The community-wise distribution of positive and negative cases across the study subjects revealed that the highest seropositivity in percentage was observed in the Bengali community (9.1% of total BSE among the community), followed by Tea-Garden community (7.7%), Nepali (6.5%). No malaria positive case was observed in the Missing and Muslim community in our current study [Table/Fig-5].

Type of Infection	Male (%) n= 713	Female (%) n= 649	OR (95% CI)	p-value
Pf	23 (3.2)	22 (3.4)	0.55 (0.10-2.99)	0.49
Pv	4 (0.6)	2 (0.3)	1.05 (0.58-1.90)	0.88
Mixed	2 (0.3)	0 (0)	0.0	0
Overall infection	29 (4.1)	24 (3.7)	1.01 (0.52-1.57)	0.73

[Table/Fig-4]: Relationship between sex and type of malaria parasite infection among the malaria positive cases.
n= Total no. of suspected Male/ Female patients

Sl. No.	Community	Total BSE*	Malaria (+)ve (%)	Malaria (-) ve (%)
1	Ahom	288	4 (1.4)	284 (98.6)
2	Brahmin, Kalita	67	2 (3.0)	65 (97.0)
3	Tea Garden	455	35 (7.7)	420 (92.3)
4	Missing	123	0 (0)	123 (100.0)
5	Kachari	58	2 (3.4)	56 (96.6)
6	Koch-Rajbongshi, SC	67	2 (3.0)	65 (97.0)
7	Bengali	33	3 (9.1)	30 (90.9)
8	Nepali	46	3 (6.5)	43 (93.5)
9	Muslim	182	0 (0)	182 (100.0)
10	Others (Panjabi, Bihari, Harijan, Desuali)	43	2 (4.7)	41 (95.3)
11	Total	1362	53	1309

[Table/Fig-5]: Community wise distribution of malaria among the suspected patients.
*BSE= Blood Smear Examined

DISCUSSION

In this study, *P. falciparum* was found as the most prevalent malarial infection among the cases, followed by *P. vivax* mono-infection and both *P. falciparum* and *P. vivax* mixed infection. Many earlier studies also reported that in Indian sub-continent, the majority of malaria infections are contributed by *P. falciparum* and *P. vivax* [3,9,15].

Monsoon season, as expected (May, June and July) is found as the peak period of the year for malaria transmission in Lakhimpur. However, this month wise distribution of cases will be more conclusive, when more month-wise data of many years can be included and analyzed. In north east region of India, the malaria transmission is persistent. High sero-prevalence of malaria during the rainy season from April to September has been reported earlier in many studies in north east region of India including Assam [6,16,17] and in some other countries [18-20].

In our study, highest Slide Positivity Rate (6.9) was observed in the age group of 5-15 years. Highest *P. falciparum* infection was found in the age group 5-15 years and highest *P. vivax* infection in 16-30 years group. Hence, these two age groups found as vulnerable groups for *Plasmodium* spp. infection, as most of the persons of these age groups generally engaged in outdoor activities for working and playing. Earlier, Rabha et al., also reported more *P. falciparum* infection in the 5-15 years of age group. Anirudh et al., and Alemu et al., also reported a significantly high prevalence of *Plasmodium* spp. (*P. falciparum* and *P. vivax*) in patients of the age groups 21-30 years and 31-40 years [18,21,22].

Our study revealed that males were comparatively more vulnerable to the malaria infection than the females, which may be due to their most common outdoor activity, where the mosquito biting is more possible. Many earlier studies reported differently about gender reservation for malaria prevalence, as few studies [5,17] reported as females have higher seropositivity rates than males, whereas few others reported that males and females were equally infected by malaria parasites [9,16,21,23].

Malaria distribution in North-eastern states of India including Assam is mainly confined to the population living in poverty [21,24]. The same situation is also reflected among different communities in this current study in Lakhimpur, where most of the people including the Bengali and Tea Garden communities have low socio-economic status.

LIMITATIONS

As this cross-sectional study was based on some routine hospital investigations, so more clinical and behavioral information from the patients cannot be obtained, due to which more risk factor analysis for malaria was not possible. Moreover, it could have better if we can recruit some patients from the block level PHCs of the districts for more homogeneous representations and better understanding of the disease epidemiology.

CONCLUSION

Our study revealed that the district still have the potential to suffer Malaria outbreak, if necessary interventions are not done for prevention and control of the disease. Early diagnosis and control activities well ahead of the pre-monsoon and monsoon season should target more to the communities with lower socio-economic status and the young age groups.

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