RESEARCH ARTICLE
SEROPREVALENCE OF PLASMODIUM FALCIPARUM AND PLASMODIUM VIVAX INFECTIONS AMONG MALARIA SUSPECTED PATIENTS ATTENDING COMMUNITY HEALTH CENTER, JIRIBAM, MANIPUR

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ARTICLE INFO

Article History:
Received 16th, December, 2013
Received in revised form 26th, December, 2013
Accepted 15th, January, 2014
Published online 28th, January, 2014

Key words:
Malaria, P. falciparum, P. vivax, Serology, Female, Rainy season

ABSTRACT

Background: Malaria is major public health alarm in the socio-economic development of north-eastern states of India. There are limited data on the prevalence of P. falciparum and P. vivax associated infections from Manipur.

Materials & Methods: We conducted a cross-sectional study in the Community Health Center, Jiribam, the western gate of Manipur, evaluating prevalence rate of P. falciparum and P. vivax using microscopic examination of Giemsa-stained thick and thin blood films followed by immunochromatographic rapid test.

Results: Seropositivity for at least one Plasmodium spp. was found in 27.4% (95% CI = 22.0, 33.4) of the 234 suspected patients recruited. P. falciparum was detected in 13.2% (31/234) while P. vivax and mixed infections were detected in 9.4% (22/234) and 4.7% (11/234) individuals, respectively (p= 0.011). Overall infection was significantly higher in female (p= 0.016; OR = 2.03; 95% CI = 1.13, 3.63), in the age group of 21–30 years (OR = 4.25; 95% CI = 0.93, 19.26), 31–40 years (OR = 3.88; 95% CI = 0.90, 16.72) and in the month of May (p= 0.021; OR = 4.58; 95% CI = 1.76, 11.93), particularly with the onset of rainy season.

Conclusion: High seroprevalence rate suggests a well supported possibility of drug resistant P. falciparum and P. vivax strains, while their significant higher prevalence in female hosts emphasizes the need of routine screening of malaria during pregnancy.

INTRODUCTION

More than half of the world's population in approximately 100 countries is exposed to malaria [1]. The World Health Organization estimates that 300–500 million cases of malaria infections, with 700,000–2.7 million deaths globally occurring each year [2]. Malaria is major public health concern in the north-eastern states of India that continues to deter the equitable socio-economic development of the region. Among seven sister states of north-east India, much of the research investigations related to malaria epidemiology and control were reported from Assam [3]. However, though malaria endemic area in West Manipur, carried out between December, 2012 and May, 2013. Around two milliliter (ml) of whole blood was collected from 234 malaria suspected patients by using disposable syringe and immediately transferred to EDTA vial.

MATERIALS AND METHODS

Study site and sample collection

This was a cross sectional study of malaria suspected patients attending Community Health Center at Jiribam, a malaria endemic area in West Manipur, carried out between December, 2012 and May, 2013. Around two milliliter (ml) of whole blood was collected from 234 malaria suspected patients by using disposable syringe and immediately transferred to EDTA vial.

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All blood samples were collected with the consent of each patient and ethical clearance was obtained from the Institutional Ethical Committee (IEC), G. C. College, Silchar, prior to the study.

Microscopy
All the blood samples were subjected to microscopy within 1 to 2 h of collection. Giemsa-stained thick and thin blood films were used for the detection of parasites. Briefly, all the air dried sample’s smears were fixed in methanol and then stained in a 1:10 diluted Giemsa (pH 7.2) for 15–20 minutes. Tap water is used to wash off the stain and the smear was examined microscopically (Olympus CX-31, Japan) under 10X eyepieces and an oil-immersion objective of 100X magnification using standard method [10].

Serology
Qualitative immunochromatographic rapid test for detection of antibodies of all isotypes (IgG, IgM, IgA) specific to \textit{P. falciparum} and \textit{P. vivax} simultaneously in human serum was conducted using SD BIOLINE Anti-Malaria P.f/v (Standard Diagnostics, Inc., Kyonggi province, Korea) according to manufacturer’s instructions with some modifications. Briefly, suspected patient’s blood was centrifuged at 6000rpm for 10 mins at 4°C for serum separation. Approximately, 10µl of serum along with 4 drops of assay diluents was then loaded to the sample well of the test device and interpreted after 10-15 mins.

Statistical analysis
Statistical analysis was carried out using the statistical software SPSS version 16.0 (SPSS, Chicago, IL, USA). Range and percentage were used to describe different characteristics of study subjects as appropriate. The qualitative data were presented in the form of number and percentage. Prevalence of \textit{Plasmodium} spp. in different groups was compared using Pearson’s chi-square test. A cross sectional study carried out in the adjoining state, slide positivity rate of 42.8 was found among seven tea estates of Nagaon and Udalguri districts of Assam [12]. Similarly, an overall smear parasite rate (SPR) for the year ranged from 6.4% in 2002 to 18.8% in 2009 from Garo hills of Meghalaya bordering Bangladesh [3]. Though our study includes a small dataset, the high seroprevalence rate suggests a well-supported and continuous transmission of \textit{P. falciparum} and \textit{P. vivax} associated

Table 1 Summary of mono and mixed infections of \textit{P. falciparum} and \textit{P. vivax} in malaria suspected patients attending the Community Health Centre of Jiribam, Manipur

<table>
<thead>
<tr>
<th>Type of Parasitic infection</th>
<th>No infected</th>
<th>Proportion</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Plasmodium vivax} (mono-infection)</td>
<td>22</td>
<td>9.4</td>
<td>6.29, 14.07</td>
<td></td>
</tr>
<tr>
<td>\textit{Plasmodium falciparum} (mono-infection)</td>
<td>31</td>
<td>13.2</td>
<td>9.31, 18.43</td>
<td></td>
</tr>
<tr>
<td>\textit{Plasmodium falciparum} + \textit{Plasmodium vivax} (mixed-infection)</td>
<td>11</td>
<td>4.7</td>
<td>2.49, 8.48</td>
<td>0.006</td>
</tr>
</tbody>
</table>

An odds ratio (OR) and 95% confidence interval (CI) was computed by the univariate logistic regression analysis for statistically significant factor showing level of statistical significance p<0.05.

RESULTS AND DISCUSSION
In north-east India, disease distribution is geographically restricted but remains entrenched in population groups living in poverty particularly in foothill villages/inter-border areas [3]. This study provides for the first time \textit{Plasmodium} spp. (\textit{P. f} and \textit{P. v}) seroprevalence data for Jiribam, the western most boundary of the state Manipur, India. In this cross sectional study, 64 out of 234 blood samples tested were positive for \textit{P. falciparum/ P. vivax}, which corresponds to an overall seropositivity rate of 27.4% (Table 1).

Table 2 Relationship between sex and prevalence of mono and mixed infections in malaria suspected patients attending the Community Health Centre, Jiribam, Manipur

<table>
<thead>
<tr>
<th>Type of Parasitic infection</th>
<th>Male n=132 (%)</th>
<th>Female n=102 (%)</th>
<th>OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Plasmodium vivax} (mono-infection)</td>
<td>14 (10.6)</td>
<td>8 (7.8)</td>
<td>1.39 (0.56, 3.46)</td>
<td>0.470</td>
</tr>
<tr>
<td>\textit{Plasmodium falciparum} (mono-infection)</td>
<td>10 (7.5)</td>
<td>21 (20.6)</td>
<td>3.16 (1.42, 7.07)</td>
<td>0.003</td>
</tr>
<tr>
<td>\textit{Plasmodium falciparum} + \textit{Plasmodium vivax} (mixed-infection)</td>
<td>4 (3.0)</td>
<td>7 (6.7)</td>
<td>2.36 (0.67, 8.28)</td>
<td>0.288</td>
</tr>
<tr>
<td>Over all infection (\textit{P. f} + \textit{P. f} + Mixed)</td>
<td>28 (21.2)</td>
<td>36 (35.3)</td>
<td>2.03 (11.3, 6.63)</td>
<td>0.016</td>
</tr>
</tbody>
</table>

Of the 64 positive samples, 22 (9.4%) were positive for \textit{P. vivax}, 31 (13.2%) were \textit{P. falciparum} positive and 11 (4.7%) were positive for both \textit{P. falciparum} and \textit{P. vivax} , showing \textit{P. falciparum} mono-infection is significantly higher in this region of Manipur followed by \textit{P. falciparum} + \textit{P. vivax} (mixed) infection and \textit{P. vivax} mono-infection (p= 0.011). Overall seroprevalence rate observed in our study using strip test was 27.4% (95% CI = 22.04-33.39). Recently, in the Republic of Djibouti, \textit{P. falciparum} and \textit{P. vivax} seroprevalence rates of 31.5% and 17.5% were found respectively [11].

Table 3 Relationship between age group and overall \textit{Plasmodium} spp. (\textit{P. f} and \textit{P. v}) infection in malaria suspected patients attending the Community Health Centre, Jiribam, Manipur

<table>
<thead>
<tr>
<th>Age group</th>
<th>Number examined</th>
<th>\textit{P. f}</th>
<th>\textit{P. v}</th>
<th>\textit{P. f} + \textit{P. v}</th>
<th>No with infection (%)</th>
<th>OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 10</td>
<td>38</td>
<td>2</td>
<td>3</td>
<td>-</td>
<td>5 (13.2)</td>
<td>2.51 (0.72, 8.72)</td>
<td></td>
</tr>
<tr>
<td>11-20</td>
<td>29</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>8 (27.6)</td>
<td>4.60 (1.56, 13.55)</td>
<td>0.032</td>
</tr>
<tr>
<td>21-30</td>
<td>56</td>
<td>11</td>
<td>7</td>
<td>5</td>
<td>23 (41.1)</td>
<td>3.12 (1.03, 9.39)</td>
<td></td>
</tr>
<tr>
<td>31-40</td>
<td>53</td>
<td>9</td>
<td>5</td>
<td>3</td>
<td>17 (32.1)</td>
<td>1.98 (0.53, 7.34)</td>
<td></td>
</tr>
<tr>
<td>41-50</td>
<td>26</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>6 (23.1)</td>
<td>1.22 (0.32, 4.67)</td>
<td></td>
</tr>
<tr>
<td>≥ 51</td>
<td>32</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>5 (15.6)</td>
<td>1.22 (0.32, 4.67)</td>
<td></td>
</tr>
</tbody>
</table>

1* = reference category, CI= confidence interval
infection in this geographical region of India. The relationship between sex and Plasmodium spp. mono and mixed infection was calculated (Table 2). We have observed significant difference between male and female in terms of overall seropositivity which was more prevalent (35.3%) in female hosts as compared (21.2%) in male hosts (p= 0.016; OR= 2.03; 95% CI= 1.13, 3.63), while P. falciparum (mono-infection) is also significantly more prevalent (24.13%) in female hosts as compared female hosts (p= 0.003; OR= 3.16; 95% CI= 1.42, 7.07). However, there was no significant difference in terms of P. vivax and mixed infections between males and females. The results indicated that females have higher seropositivity rates than males. Overall high prevalence rate of Plasmodium spp. (P. f and P. v) in female hosts may be explained by increased outdoor activities of females in the study area particularly in pineapple gardens. High prevalence of malaria in female hosts in this geographical area emphasizes the need of routine screening of malaria during pregnancy as malaria in pregnancy has emerged as a major hidden public health problem globally [13, 14, 15].

Table 4 Relationship between season and overall Plasmodium spp. (P. f and P. v) infection in malaria suspected patients attending the Community Health Centre, Jiribam, Manipur

<table>
<thead>
<tr>
<th>Months</th>
<th>No examined</th>
<th>P. f</th>
<th>P. v</th>
<th>P. f + P. v</th>
<th>No with infection (%)</th>
<th>OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>December</td>
<td>61</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>8 (13.1)</td>
<td>1*</td>
<td></td>
</tr>
<tr>
<td>January</td>
<td>27</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>6 (22.2)</td>
<td>1.89 (0.59, 6.12)</td>
<td></td>
</tr>
<tr>
<td>February</td>
<td>31</td>
<td>5</td>
<td>3</td>
<td>-</td>
<td>8 (25.8)</td>
<td>2.30 (0.77, 6.89)</td>
<td></td>
</tr>
<tr>
<td>March</td>
<td>20</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>5 (25.0)</td>
<td>2.21 (0.63, 7.75)</td>
<td></td>
</tr>
<tr>
<td>April</td>
<td>51</td>
<td>8</td>
<td>8</td>
<td>3</td>
<td>19 (37.3)</td>
<td>3.93 (1.54, 10.02)</td>
<td>0.021</td>
</tr>
<tr>
<td>May</td>
<td>44</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>18 (40.9)</td>
<td>4.58 (1.76, 11.93)</td>
<td></td>
</tr>
</tbody>
</table>

* = reference category, CI= confidence interval

Table 3 showed age-dependency relationship of the prevalence rate of Plasmodium spp. infection in univariate analysis. Over all seropositivity (70.83%) was significantly highest in the age group 21–30 years (OR= 4.25; 95% CI= 0.93, 19.26), followed by 68.96% in age group 31–40 years (OR= 3.88; 95% CI= 0.90, 16.72), 52.38% in age group 11–20 years (OR= 1.92; 95% CI= 0.43, 8.60), 43.75% in the age group 41–50 years (OR= 1.36; 95% CI= 0.28, 6.58) and 30.76% in the age group above 50 years and above (OR= 0.77; 95% CI=0.17, 3.43) compared to those aged more than 10 year (13.2%). A significantly high prevalence of Plasmodium spp. (P. f and P. v) among the suspected patients in the age groups 21-30 years and 31-40 years in the study sites is in agreement with what was reported elsewhere [16, 17]. We observed a significant impact of the season on the prevalence of the parasite (Table 4). During this six month study we observed that with the commencement of the rainy season, there was sudden rise in seropositivity rate. The results showed that the overall prevalence rate of P. falciparum and P. vivax was significantly more in April (OR= 3.93; 95% CI= 1.54, 10.02) and May (OR= 4.58; 95% CI= 1.76, 11.93) as compared to December (p= 0.032). As expected, with the onset of rainy season a significant increase in the prevalence rate was observed supporting earlier observation made in Ethiopian population [16, 18]. A positive correlation between rainfall and the prevalence of P. vivax infection was observed in Northern Thailand [19]. However, a study conducted in Northern Senegal reported higher prevalence of P. falciparum in January compared to June [20].

CONCLUSION

In conclusion, this study reflects for the first time Plasmodium spp. seroprevalence data for Western part of Manipur, India. While, high seroprevalence rate suggests a well supported possibility of drug resistant P. falciparum and P. vivax strains. Moreover, high prevalence of malaria in female hosts in this geographical area emphasizes the need of routine screening of malaria during pregnancy as malaria in pregnancy has emerged as a major hidden public health problem globally.

Acknowledgement

Our modest acknowledgement goes to the Department of Science and Technology (DST), Govt. of India for providing infrastructural facilities (SR/SO/AS-36/2008).

References


