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Prevalence of Plasmodium Falciparum and Plasmodium Vivax Associated Infections in Malaria Suspected Patients Attending Community Health Centre, Manipur (Jiribam) N.E India

KEYWORDS: Malaria, N.E India, community health centre, Plasmodium

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ABSTRACT *Background and objective: A study was conducted to determine the prevalence of Plasmodium infections in malaria suspected patients attending community health centre, Manipur (Jiribam) N.E India.*

Methods: A total of 127 blood samples were examined for human malaria parasites by using microscopy and rapid malaria test strip.

Results: Laboratory diagnosis revealed that out of 127 samples, 67 were positive for Plasmodium falciparum/ P. vivax/ showing an overall prevalence of 52.75%. Of the 67 positive samples, 13 (10.2%) were P. vivax positive, 33 (25.9%) were P. falciparum positive and 21 (16.5%) were positive for both P. falciparum and P. vivax exhibiting P. falciparum mono-infection significantly higher in this region of Manipur followed by P. falciparum + P. vivax (mixed) infection and P. vivax mono-infection (p= 0.11).

Conclusions: With the findings of high prevalence of P. falciparum and P. vivax, during the study period, there is an urgent need of molecular epidemiological studies on antimalarial drug resistance pattern, pathophysiology of severe and complicated malaria with special reference to P. falciparum and P. vivax strain variations along with the socio-demographic profile of the malaria infected patients of the areas near future. It is also further recommended that surveillance be strengthened to contain further extension of malaria in north-eastern India.

Introduction

Malaria, sometimes called the "King of Diseases" is the most important infectious disease in tropical and subtropical regions and continues to be a major global health problem with over 40% of the world's population exposed to varying degrees of malaria risk in some 100 countries (WHO 2010, Tikar *et al.*, 2011). It has been estimated that over 500 million people suffer from malaria infections annually, resulting in about 1-2 million deaths, of whom 90% are children globally (Dev *et al.*, 2006). India having the largest population in the world at risk of malaria, with 85% living in malarious zones (Sharma, 1999) contributes to more than 78% of total malaria cases of Southeast Asia and *P. Vivax* alone accounts for more than 50% of annual malaria cases (Kumar *et al.*, 2007). In most endemic areas, *P. vivax* cohabits with *P. falciparum* and mixed infections with these two common species, while *P. vivax* being more difficult to control and eliminate than *P. falciparum* because of its tendency to relapse after resolution of the primary infection. In north-east India, it has been observed that disease distribution is geographically restricted but remains entrenched in population groups living in poverty particularly in foothill villages/inter border areas (Dev *et al.*, 2006). Among the seven sister states of north-east India, much of the research investigations related to malaria epidemiology and controls were reported from Assam (Dev, 2009). The introduction of PCR genotyping in malaria research has paved the way for major improvements in the understanding of parasite biology. The discovery of quinine is considered the most serendipitous medical discovery of the 17th century and malaria treatment with quinine marked the first successful use of a chemical compound to treat an infectious disease (Ghosh and Dash, 2007). So far the prevalence of malaria in northeast India is concerned no attempt has been initiated except the report from Garo hills of Meghalaya (Dev *et al.*, 2010). Keeping in mind the paucity of recent literature and being a malaria endemic area as frequently reported by local newspapers, the main objective of the study was to estimate the prevalence of *P. vivax* and *P. Falciparum* associated infection in malaria suspected patients attending the community health centre, Jiribam, Manipur, along with their respective rate of infection among different age groups and sex.

Materials and methods

A total of 127 blood samples collected during December/2012 to May/2013 from malaria suspected patients (58 males and 69 females) attending the Community Health Centre of Jiribam, Manipur, N.E India, were initially screened for the presence of *Plasmodium* by light microscopy. A 2ml of whole blood was collected under aseptic conditions by using disposable syringes and alcohol swabs. The collected blood was immediately transferred to the specimen vial containing anticoagulants (EDTA). Further, finger-prick blood samples were also collected and both thick and thin blood smears were prepared for microscopy examination.

Rapid diagnosis test (RDT)

Rapid diagnosis was done using one-step malaria *P.f/P.v* test kit (BioLine, Biostandard diagnostic Pvt. Ltd., Gurgaon, Haryana, India) as per the manufacturer's instructions. Serum was initially separated from blood sample through centrifugation at 10,000 rpm for 10 minutes at 25^{0}C (Refrigerated centrifuge, REMI). About, 10µl of serum was then added to the sample well of the test device along with 3-4 drops of assay diluents.

Statistical analysis:

The collected data was computerized using excel program and analyzed by SPSS version 8.0 (SPSS, Inc., Chicago, IL, USA). Prevalence of *Plasmodium* in different groups was compared using chi-square tests while Odds ratio was calculated in statistical software MedCalc (Acacialaan, Belgium).

Results

In this study out of 127 samples 67 sample were found to be positive for *P. falciparum/ P. vivax/* showing an overall prevalence of 52.75%. Of the 67 positive samples, 13 (10.2%) were *P. vivax* positive, 33 (25.9%) were *P. falciparum* positive and 21 (16.5%) were positive for both *P. falciparum* and *P. vivax*, showing *P. falciparum* mono-infection is significantly higher in this region of Manipur followed by *P. falciparum + P. vivax* (mixed) infection and *P. vivax* mono-infection (p= 0.11) (Table 1).

Volume : 4 | Issue : 5 | May 2014 | ISSN - 2249-555X

Type of Parasitic infection	No. infected N=127	Proportion	OR (95% CI)	P value
Plasmodium falciparum + *Plasmodium vivax* (mixed-infection)	21	16.5	1.73 (0.82, 3.64)	0.11
Plasmodium falciparum (mono-infection)	33	25.9	3.07 (1.53,6.18)	
Plasmodium vivax (mono-infection)	13	10.2	1*	

Table1: Summary of mono and mixed infection of P. falciparum and P.vivax in malaria suspected patients attending the Community Health Centre of Jiribam, Manipur.

The relationship between sex and *Plasmodium spp.* mono and mixed infection in human was calculated (Table 2). The study population comprised of 58 males and 69 females. According to these results, though there was no significant difference between males and females regarding the overall infection of *Plasmodium* infection and *P. vivax* infection, however *P. falciparum* infection was significantly (p= 0.013) more prevalent (34.78%) in female hosts as compared (15.51%) in male hosts (OR= 2.90; 95% CI= 1.22, 6.90) while mixed infection of *P. falciparum* and *P. vivax* was significantly (p=0.034) more prevalent (24.13%) in male hosts as compared (10.14%) in female hosts (OR= 2.81; 95% CI= 1.07, 7.55).

Table2: Relationship between sex and prevalence of *P. falciparum*, *P. vivax* and mixed infection in malaria suspected patients attending the Community Health Centre of Jiribam, Manipur.

Type of Parasitic infection	Male n=58 (%)	Female n=69 (%)	OR (95% CI)	P value
Plasmodium falciparum + Plasmodium vivax (multi-infection) (I)	14	7*	2.81 (1.07, 7.55)	0.034
Plasmodium falciparum (mono-infection) (II)	9*	24	2.90 (1.22, 6.90)	0.013
Plasmodium vivax (mono-infection) (III)	5 (8.6)*	8 (11.5)	0.71 (0.22, 2.33)	0.582
Over all infection (I+II+III)	25 (43.1)*	37 (53.62)	0.65 (0.32,1.32)	0.237

The relationship between age and *Plasmodium spp.* infection (*P. falciparum* and *P. vivax*) in human was also determined (Table 3). It was observed that the overall infection (*Pv/ Pf/ Pv + Pf*) had significantly (p= 0.023) highest prevalence (70.83%) in age groups of 31-40 years (OR= 4.25; 95% CI= 0.93, 19.26), followed by 68.96% in age groups of 21 – 30 years (OR= 3.88; 95% CI= 0.90, 16.72); 52.38% in age groups of 11 - 20 years (OR= 1.92; 95% CI= 0.43, 8.60); 43.75% in the age groups 41 – 50 years (OR= 1.36; 95% CI= 0.28, 6.58) and 30.76% in the age groups of 50 years and above (OR= 0.77; 95% CI=0.17, 3.43).

Table3: Relationship between age group and overall *Plasmodium* (*P.f* and *P.v*) infection in malaria suspected patients attending the Community Health Centre of Jiribam, Manipur.

Age group	Number Examined	Pf (I)	Pv (II)	Pf + Pv (III)	No. with infection	OR (95% CI)	P value
≤10	11	2	-	2	4	1*	0.023
11-20	21	4	4	3	11	1.92 (0.43, 8.60)	
21-30	29	10	3	7	20	3.88 (0.90, 16.72)	
31-40	24	10	2	5	17	4.25 (0.93, 19.26)	
41-50	16	3	2	2	7	1.36 (0.28, 6.58)	
≥51	26	4	2	2	8	0.77(0.17, 3.43)	

The relationship between season and *Plasmodium spp.* infection (*P. falciparum* and *P. vivax*) in humans was also determined (Table 4). Malaria cases were recorded in all months; however, with the commencement of the rainy season in April, there was sudden rise in cases. The results showed that the rate of infection was significantly (p= 0.023) more in April-May as compared to December (OR= 3.70; 95% CI= 1.18, 18.69).

Table 4: Relationship between season and overall *Plasmodium* (*Pf* and *Pv*) infection in malaria suspected patients attending the Community Health Centre of Jiribam, Manipur.

Months	No Tested	Pf (I)	Pv (II)	Pf + Pv (III)	No With infection	OR (95% CI)	P value
December	13	2(15.4)	1(7.7)	1(7.7)	4 (30.8)	1*	0.023
January	15	3(20)	1(6.6)	1(6.6)	5(33.3)	1.12 (0.22, 5.53)	
February	19	2(10.5)	3(15.8)	2(10.5)	7(36.8)	1.31 (0.29, 5.89)	
March	17	2(11.8)	3(17.6)	3(17.6)	8(47.1)	1.50 (0.34, 6.58)	
April	29	11(37.9)	3(10.3)	6(20.6)	20(68.9)	3.61 (1.21, 20.61)	
May	34	13(38.2)	2(5.9)	8(23.5)	23(67.6)	3.70 (1.18, 18.69)	

Discussion

The present study conducted from the patients attending the Community Health Centre of Jiribam, Manipur, was examined for presence of *Plasmodium* by light microscopy and rapid malaria test strip. The study population comprised of 58 males and 69 females. *Plasmodium* species were detected in 67 (52.8%) of the 127 blood specimens tested. *P. falciparum* mono-infection was detected in 33 (25.9%) specimens and *P. vivax* mono-infection was detected in 13(10.2%) specimens. In 21 (16.5%) specimens both *P. falciparum* and *P. vivax,* mixed infections were detected and negative results were observed in 60 samples (47.2%). *P. falciparum*, the most commonly encountered species and highly responsible for causing malaria in North-Eastern India as reported by Dev *et al.*, 2010, reveals that in West Garo hills district which is highly co-endemic for *P. falciparum* and *P. vivax* type of infections and *P. falciparum* being the most predominant type (> 82%) amongst the population showed similar results to this present investigation where the predominant organism was found to be *P. falciparum* followed by mixed infections by *P. vivax* amongst the 67 positive samples analysed and 33 samples that were found to be predominant of *P. falciparum* type. The present study also reports that *P. falciparum* infections were higher among the females than the males; whereas in males, mixed infections were significantly higher than females though there was no significant difference between males and females regarding the overall infections of plasmodium and *P. vivax* (Kumar et al., 2007, Rijken *et al*., 2012).

Furthermore, the age groups of 11-20; 21-30 and 31-40 years were predominant in terms of incidence and were also similar to those of many other studies conducted in the India or in different countries (Dutta et al., 1999). A comparatively higher positivity was observed towards the age groups of 21-30 (OR 3.88), followed by 31-40 (OR 4.25) and 11-20 (OR 1.92) respectively. Patients infected with *P. falciparum* and mixed infections differed significantly and the patients infected with *P. vivax* did not differ significantly with respect to age (Dutta et al., 1999).

Though malarial cases were recorded in all months from December 2012 to May 2013, however, with the commencement of the rainy season in March-April 2013, a sudden rise in the infections was observed (Dev *et al*., 2010, Barber *et*

Volume : 4 | Issue : 5 | May 2014 | ISSN - 2249-555X

al., 2013) due to poor coordinated vector control interventions, illiteracy, difficult terrain and access to healthcare services (Dash et al., 2008). Since the World Health Organization (WHO, 2010) has recognized the urgent need for new, simple, quick, accurate, and cost-effective diagnostic tests for determining the presence of malaria parasites, to overcome the deficiencies of light microscopy, numerous new malaria-diagnostic techniques have been developed. This, in turn, has led to an increase in the use of RDTs for malaria, which are fast and easy to perform, and do not require electricity or specific equipment (Bell et al., 2006, Tangpukdee *et al.*, 2009).

It has also been observed that when the parasitic levels are very low the information obtained by microscopy is restricted, and in some cases biased, by the inability to devote the necessary amount of time to the examination of blood smears. A missed diagnosis of *P.vivax* concurrent with *P. falciparum* is more problematic since these species could cause relapses, thereby compounding morbidity. Because of negative microscopical diagnosis untreated patients may be carriers of the malaria parasites in these particular areas (Zakeri*et al.*, 2002). The similarities or differences in the study of prevalence of *Plasmodium* may result from different geographical locations, environmental and climatic conditions of the study area, practices such as healthcare and education programmes, socioeconomic standards and hygienic conditions. Recent developments in molecular biological technologies have permitted extensive characterization of the malaria parasite and are generating new strategies for malaria diagnosis. PCR-based techniques are a recent development in the molecular diagnosis of malaria, and have proven to be one of the most specific and sensitive diagnostic methods, particularly for malaria cases with low parasitemia or mixed infection (Morassin *et al.*, 2002).

Conclusions

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. With the findings of high prevalence of *P. falciparum* and *P. vivax,* within the study area, there is an urgent need of molecular epidemiological study of antimalarial drug resistance pattern, pathophysiology of severe and complicated malaria with special reference to *P. falciparum* and *P. vivax* strain variations along with the socio-demographic profile of the malaria infected patients of the areas in near future. Also, emphasis needs to be given to other ecofriendly methods of vector control, such as biocontrol with larvivorous fish and biolarvicides (Ghosh and Dash 2007). Insecticides are currently most practical in controlling mosquito vector, and therefore cannot be overlooked and effective resistance management mainly depends upon early detection of the status of resistance, therefore monitoring of insecticide resistance at regular intervals is necessary so that an effective management strategy can be designed.

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REFERENCE

1. Barber BE, William T, Grigg M, Yeo TW, Anstey NM: Limitations of microscopy to differentiate Plasmodium species in a region co-endemic for Plasmodium falciparum, Plasmodium vivax and Plasmodium knowlesi. Malaria Journal 2013, 12:8 | 2. Bell D, Wongsrichanalai C, Barnwell JW: Ensuring quality and access for malaria diagnosis: how can it be achieved? Nat Rev Microbiol 2006; 4: S7-S20. | 3. Dev V: Integrated disease vector control of malaria: a success story based in Assam, northeastern India. ICMR Bulletin 2009, 39:21-28. | 4. Dev, V. and Sharma VP: Persistent transmission of malaria in Sonapur PHC, Kamrup district, Assam. J Parasitic Dis 1995, 19: 65–68. Dev Vas, Barlind M Sangma, Aditya P Dash: Persistent transmission of malaria in Garo hills of Meghalaya bordering Bangladesh, north-east India, Malaria Journal 2010, 9:263. | 5. Dev V, Dash AP, Khound K: High-risk areas of malaria and prioritizing interventions in Assam. Curr Sci 2006, 90:32-36. | 6. Dutta P, Khan AM, Mahanta J: Problem of malaria in relation to Socio-cultural diversity in some ethnic communities of Assam and Arunachal Pradesh. J Parasitic Dis 1999, 23: 101–104. | 7. Dash AP, Valecha N, Anvikar AR, Kumar A: Malaria in India: Challenges and opportunities. J Biosci 2008, 33:583-592 | 8. Dash AP, Adak T, Raghavendra K, Singh OP: The biology and control of malaria vectors in India. Current Science. 2007; 92:1571–1578. | 9. Ghosh SK, Dash AP: Larvivorous fish against malaria vectors: a new outlook. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2007; 101:1063–1064 | 10. Kumar A, Valecha N, Jain T, Dash AP: Burden of malaria in India: retrospective and prospective view. Am J Trop Med Hyg 2007, 77:69-78. | 11. Morassin B, Fabre R, Berry A, Magnaval JF: One year's experience with the polymerase chain reaction as a routine method for the diagnosis of imported malaria. Am J Trop Med Hyg 2002; 66: 503-508. | 12. Rijken MJ, McGready R, Boel ME, Poespoprodjo R, Singh N, Syafruddin D, Rogerson S, Nosten F: "Malaria in pregnancy in the Asia-Pacific region". Lancet Infectious Diseases 2012, 12 (1): 75–88. | 13. Sharma VP: Current scenario of malaria in India. Parassitologia 1999; 41:349–353. | 14. Tangpukdee N, Duangdee, Wilairatana P and Krudsood S. Malaria Diagnosis: A Brief Review. Korean J Parasitol.Vol. 47 2009, No. 2: 93-102. | 15. Tikar SN, Mendki MJ, Sharma AK, Sukumaran D, Vijay Veer, Shri Prakash, Parashar, BD: Resistance Status of the Malaria Vector Mosquitoes, Anopheles stephensi andAnopheles subpictus Towards Adulticides and Larvicides in Arid and Semi-Arid Areas of India. J Insect Sci. 2011, 11: 85. | 16. World Health Organization. WHO information consultation on recent advances in diagnostic techniques and vaccines for malaria: a rapid dipstick antigen capture assay for the diagnosis of falciparum malaria. Bull World Health Organ 1996, 74: 47-54. | 17. Zakeri S, Najafabadi ST, Zare A, Djadid ND: Detection of malaria parasites by nested PCR in south-eastern, Iran: Evidence of highly mixed infections in Chahbahar district. Malaria Journal 2002, 1:2. |