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ANALYSIS OF MEROZOITE SURFACE PROTEIN-3B IN *PLASMODIUM VIVAX* ISOLATES COLLECTED FROM SOUTHWESTERN COASTAL REGION OF INDIA

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ABSTRACT

Background: Malaria is one of the major infectious diseases and is a global health threat. Malarial parasites exhibit high genetic diversity, Which is one of the reasons hampering the vaccine development. Analysis of genetic variations in known vaccine candidates is crucial for developing an effective vaccine. The current study was aimed at analysing one of the members of the merozoite surface family of proteins, MSP- 3β, of *Plasmodium vivax* (*Pv*) in parasite samples collected from patients from southwestern coastal region of India, where prevalence of *P. vivax* predominates compared to *P. falciparum.* **Method:** A total of 25 field isolates were analysed in the study. Allelic diversity at the *Plasmodium vivax* merozoite surface protein-3β locus was investigated using a combined polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) procedure. **Results:** PCR products of *Pv*MSP-3β revealed three allelic variants. Digestion with Pst 1 restriction enzyme yielded suballelic variants. A total of five different alleles were observed in the current study. **Conclusion:** Even though the samples were limited, *Pv*MSP-3β was found to be significantly variant in the isolates from this area. Further studies using substantially more samples would provide definitive information about the extent of polymorphisms in *P. vivax* isolates in this area.

KEYWORD: Malaria, merozoite surface protein 3 beta, Plasmodium vivax, Mangalore.

INTRODUCTION

Malaria is one of the major global health problems, particularly in developing countries. About 3.2 billion people are at risk contracting the disease and ~1.2 billion are at high risk.[1] Malaria control strategies are confounded by several factors including drug resistance, insecticide resistance, scarcity of rapid diagnosis and treatment, and lack of vector control measures. Moreover, an effective vaccine is still not available. Thus, the burden of malaria still remains high. Research on potential vaccine candidates is underway and the European health agency has recently sponsored the use of malaria vaccine RTS/S.[2]

Malaria vaccine development is hampered by antigenic diversity and immune evasion ability of malaria parasites. Understanding the extent of polymorphisms is crucial for developing an effective vaccines. Proteins expressed on the surface of merozoites, including apical membrane antigen-1 (AMA1), merozoite surface protein-1 (MSP1) and Duffy binding protein (DBP) have been suggested as potential vaccine candidates. Since antigenic diversity is a major challenge in vaccine development, the assessment of parasite genetic diversity would provide critical information needed for advancing malaria vaccine.

Compared to *P. falciparum*, *P. vivax* remains widely distributed in many regions of the globe and half of the world's population is at risk of infecting with this parasite species.[13] The overall disease burden, economic loss and mortality from *P. vivax* infection have been underestimated.[12] Compared to *P. falciparum,* relatively less is known about the genetic diversity of *P. vivax.*[10,11] MSP3 family of genes (*pvmsp*-3α, *pvmsp*-3β, and *pvmsp*-3γ) of *P. vivax* have been characterized. *Pv*MSP-3β gene has central alanine rich domain and it is predicted to form coil-like tertiary structure.[7] Large deletion/insertion mutations are present in this region, making this protein highly polymorphic.[4]

Mangaluru, a southwestern coastal region of India is endemic to malaria and *P. vivax* is the major parasite species in this region. The current study was aimed at analysing the genetic diversity of MSP-3β of *P. vivax* (PvMSP-3) isolates collected from this area.

METHODS

Sample collection and ethics

Sample were collected during rainy season from June 2014 to July 2014. A total of 25 isolates from people infected with *P. vivax* who seeked treatment at Wenlock District Hospital, Mangaluru. After obtaining informed consent from participants, finger prick blood samples were collected on Whatman No 3MM filter paper. Air dried filter samples were placed in plastic bags and stored at *-80°C* until used. The study design was in accordance with the ethical guidelines of Indian Council of Medical Research (ICMR) and the National Institutes of Health, USA. The study protocols were approved by the Institutional Review Board of Kuvempu University, Shivamogga, Karnataka, India, and Pennsylvania State University College of Medicine, Hershey, PA, USA.

DNA extraction

Blood spots on each filter paper sample was incubated with 0.5% saponin in phosphate buffered saline at 4°C overnight as described earlier.[3] The samples were then washed with 1X PBS for 30 minutes, transferred to new tubes containing 5% Chelex 100 and vortexed for 30 seconds followed by heating at 99°C for 15 minutes and centrifuged at 14 K rpm in a microcentrifuge for 2 minutes. Supernatants were collected and stored at -20°C until used.

Amplification of *Pv*MSP-3β

*Pv*MSP-3β gene was amplified by nested PCR as described earlier.[5] The PCR conditions used were: initial denaturation at 95°C/30 sec, denaturation at 95°C/30 sec, annealing at primer dependent temperature /30 sec and extension at 68°C/60sec for 30 cycle and final extension at 68°C/5 minute. Two per cent agarose gel was stained with ethidium bromide in order to visualise the PCR separated product, under UV illumination.

RFLP analysis of *Pv*MSP-3β PCR products

Pst 1 restriction enzyme was used for RFLP analysis. The total volume of the reaction mixture was kept as 20 µl composed of 1.2 µl of *Pst* 1 buffer, 8.8 µl of PCR water, 1.0 µl of enzyme and 10 µl of product of *msp-3β* gene.

RESULTS

Of the total of 25 isolates analysed in the study, the gene was amplified in 20 isolates. Among these 20 isolates, six were mixed and remaining 14 revealed different sizes labeled as Type A and Type B. Digestion with Pst 1 enzyme yielded different fragment sizes within each group. Bands between 1.7 kb and 2 kb in the PCR products were grouped into Type A and those between 1.4 and 1.5kb were grouped as Type B, which is considered as reference strain (Belem) without any insertions. In Type A, both 1700 kb and 2000kb genotype was observed in 5 isolates. Type B was observed in 7 isolates. Five different alleles were observed in both types as shown in the table.

Table 1: *Pv*MSP-3β genotypes with different alleles.

No	Genotype	Pst restriction fragments	Number of samples showing the allele
1	A-2000	A1: 800+350+150 A2: 800+ 600+300	3 2
2	A-1700	A3: 850+800	2
3	B-1400	B1: 1400+ 800+600 B2: 800	6 1

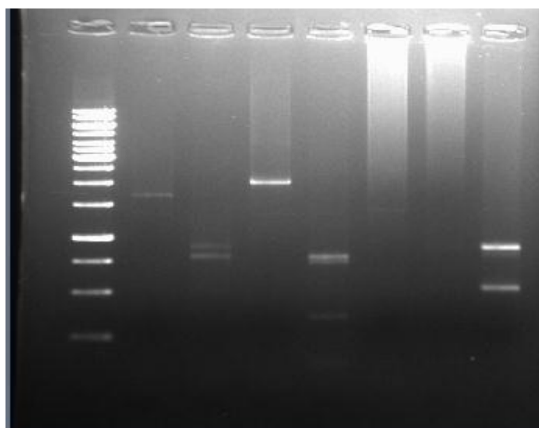

Figure 1: PCR products of *Pv*MSP-3β gene and restriction fragment length polymorphism patterns after Pst 1 digestion. Lane 1: 1kb adder; Lane 2: Type A; Lane 3: Allele A3; Lane 4: Type A; Lane 5: Allele A1; Lane 6: Type B; Lane 7: Allele B2; Lane 8: Allele B1.

DISCUSSION

Studies on genetic diversity and population structure of *P. vivax* parasites provides important information for evaluation of new drugs and vaccines.[9] Several merozoites surface antigens of malaria parasites have been identified as potential vaccine candidates.[14] The aim of this study was to analyse the polymorphisms in MSP-3β gene in *P. vivax* isolates from Managalore area, an highly malaria endemic place where *P. vivax* infection predominates over *P. falciparum*. We identified five different alleles of *Pv*MSP-3β gene. Based on size differences in PCR products, these alleles were grouped into two *Pv*MSP-3β types, Type A and Type B. Type C

was not observed in any of the isolates similar to the previous study from India.[8] Thus, these results are in contrast to the three major size types of *Pv*MSP-3β alleles observed in the *P. vivax* strains from other parts of India and southeast Asia.[6] Within each allele variants, different sub allelic variants were observed. Since *Pv*MSP-3β is considered as a potential vaccine candidate, further analysis on polymorphisms is desirable.

CONCLUSION

Although the study was conducted with a limited number of samples, the observed five different alleles of *Pv*MSP-3β points to extensive sequence diversity. Further detailed studies using a large number of samples is needed to gain information about the suitability of *Pv*MSP-3β as a malaria vaccine candidate.

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