

Diversity of Merozoite Surface Protein-3 β Gene of *Plasmodium vivax* Isolates from Iran

A Shahbazi¹, A Raeisi², *M Nateghpour³, H Mirhendi³, M Mohebbali³, M Asmar⁴

¹Dept. of Parasitology, Faculty of Medicine, Tabriz University of Medical Science, Tehran, Iran

²Center of Disease Management Ministry of Health, Tehran, Iran

³Dept. of Medical Parasitology and Mycology, School of Public Health, Medical Sciences/University of Tehran, Iran

⁴Dept. of Parasitology, Pastor Institute of Iran, Tehran, Iran

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Abstract

Background: *Plasmodium vivax* malaria accounts for approximately 88% of malaria cases in Iran. There is limited information on genetic diversity of *P. vivax* in the country and a need to develop and apply an effective vaccine against the disease is necessary. Among many potential candidates, *MSP-3 β* gene is promising target. This study was designed and carried out to determine the variation of this gene as genetic marker in population of malarious areas of Iran.

Methods: Blood sample of 85 *P. vivax* isolates from four southern and east-southern provinces of the country assessed for polymorphism of *PvMSP-3 β* gene by PCR/RFLP method.

Results: Based on the size of PCR product of the gene, 7 genetically different types of parasite has been distinguished. Two alleles were simultaneously visible in 19 % of the cases. Results from PCR/RFLP analysis of *PvMSP-3 β* gene showed at least 15 allelic groups. Multiple infections have been found in 2.4 % of the cases.

Conclusion: *PvMSP-3 β* gene was highly diverse in *P. vivax* isolates of malarious areas of Iran, and can be a suitable marker for population genetic studies of *P. vivax*. More investigations on *PvMSP-3 β* genes are needed to reveal genetic structure of *P. vivax* in Iran.

Keywords: *Plasmodium vivax*, *PvMSP-3beta*, *PCR/RFLP*, *Iran*

Introduction

Malaria is the most serious diseases to affect peoples in developing countries with tropical and subtropical climates (1). Human malaria caused by *Plasmodium vivax* rarely is similar to *P. falciparum*, but it causes a debilitating febrile illness in approximately 90 million people each year. Sever and widespread morbidity associated with endemic *P. vivax* malaria in Asia and the Americas produces a heavy social and economic cost (2). In Iran 88.3% of all malaria cases (18966) in 2005 caused by *P. vivax* and its transmission is almost located in southern and east-southern provinces of the country (Sistan and Bluchestan, Hormozgan, Kerman and Boushehr) (3). Although large amount of investment have been made in attempts to develop a vaccine

against malaria, none have been developed so far. The major problem in vaccine development is the antigenic diversity of vaccine candidates among parasites. Thus, the variation of immunodominant region is a serious obstacle. Therefore, the genetic variation study for the antigens of potential vaccine candidates e.g. *CSP* (circumsporozoite protein), *MSP* (merozoite surface protein), and *DBP* (Duffy binding protein) is very important and continues to grow steadily in both *P. falciparum* and *P. vivax* (1).

Some genes encoding for *p. vivax* MSPs are *PvMSP-1(4)*, *PvMSP-3 α* , *PvMSP-3 β* and *PvMSP-3 γ* (5, 6), *PvMSP-4* and *PvMSP-5* (7) and *PvMSP-9* (8). *PvMSP-3 α* , *PvMSP-3 β* and *PvMSP-3 γ* are members of a multy-gene family of related MSPs (5, 6). *MSP-3 β* gene encodes merozoite surface

proteins that are dominated by alanine-rich central domains strongly predicted to form coiled-coil tertiary structure, perhaps contributing to the structural complexity of the surface coat. The function of this family is unknown, although immune evasion has been raised as one possibility (6). Because of the utility of *MSP-3β* in future vaccine development against *P. vivax* (6,9), this study has carried out the first assessment of diversity in the *PvMSP-3β* gene by PCR/RFLP (polymerase chain reaction/restricted fragment length polymorphism) in isolates from southern and east-southern Iran, to reveal the extent of polymorphism in the gene.

Materials and Methods

The study was conducted in malarious areas of southern and east-southern provinces of Iran (Sistan and Baluchistan, Hormozgan, Kerman and Bushehr). Eighty five patients attending the clinics from August to September 2005 were recruited in the study. Sample collection was approved by the ethical committee of Tehran University of Medical Sciences, Iran and through informed consent of patients. Approximately 1000 μl of venous blood was collected in EDTA from *P. vivax* microscopy-positive samples with parasite density > 400/μl, parasite DNA was extracted by phenolchloroform method (10). All samples were rechecked by nested PCR using *Plasmodium* genus specific (primary PCR) and *P. vivax* and *P. falciparum* specific primers (nested) (10) that, all of them were negative with *P. falciparum* primers and positive with *P. vivax* specific primers (120 bp). Then, samples were tested by PCR for *PvMSP-3β* gene with specific primers: forward(5'-AACTTGAGAA-ACGGATG-3')/reverse(5'-TGCAGTGTTC-TATGCG-3'), which amplify a small *PvMSP-3β* fragment and bind at positions 102-118 and 1943-1961 of the Belem *PvMSP-3β* coding sequence (9). The polymerase chain reaction (PCR) was performed with an initial denaturation of 2.5 min at 95° C, followed by 35 cycles of 30 Sec at 95° C, 56° C for 30 sec and 68° C for 2.5 min (9). All PCR positive

samples were genotyped using restriction fragment length polymorphism (RFLP) by *Hha*I, in which approximately 5 μl of the PCR product was digested with *Hha*I and analyzed by electrophoresis on a 1.8% agarose gel. Major alleles were classified based on the differences in restriction banding patterns. For multiple infections, genotypes of the parasite were assigned to individual allele groups by comparing the RFLP patterns with those of single infections (11).

Results

All of 85 samples were successfully amplified in PCR process. Based on the size of PCR product of the gene, 7 genetically different types of parasite has been distinguished (Fig. 1). The biotypes were different in size, ranging from about 1.2 kb to 2.4 kb. Two alleles were simultaneously visible in 19% of the cases, indicating that some infections contained mixed genotypes. At least 15 allelic groups were identified in RFLP analysis (Fig. 2). Multiple infections have been found in 2.4% of cases (two isolates) (Fig. 2).

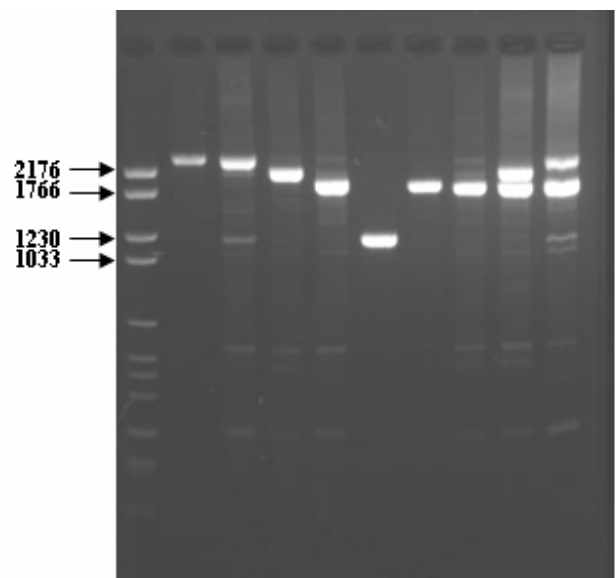


Fig. 1: Major 7 biotypes and 2 mixed genotypes (Lane 9 and 10) of *Plasmodium vivax* isolate from southern and east-southern using polymerase chain reaction of the *msp-3β* gene. Lane 1 contains DNA marker 6 (Roche).

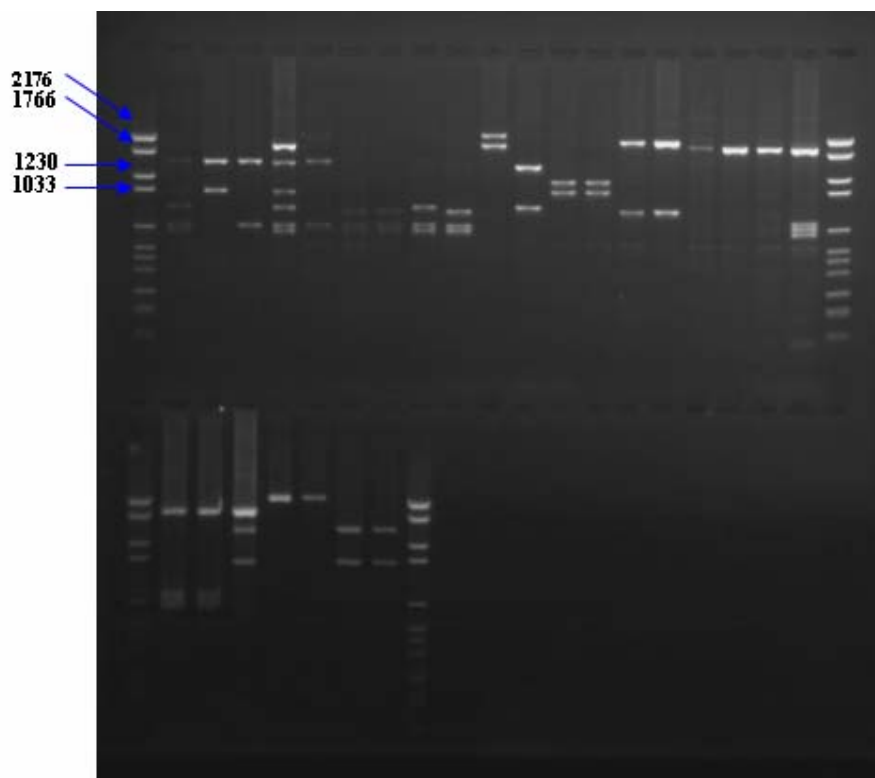


Fig. 2: Major alleles and mixed genotypes (Lane 10) of *Plasmodium vivax* isolate from southern and east-southern using PCR/RFLP of the *msp-3β* gene. Lane 1 and 21 of upper panel and 1 and 8 of lower panel contains DNA marker 6 (Roche).

Discussion

In 2005, 18966 cases of malaria was reported by malaria surveillance system in Iran, of which 88.3% were *P. vivax* infection and 91 % were occurred in southern and east-southern provinces of the country (Sistan and Bluchestan, Hormozgan, Kerman and Boushehr) (3). We investigated the genetic diversity of *P. vivax* population in isolates from malarious areas of Iran using the *PvMSP-3β* gene as a molecular marker. Merozoites are surrounded by a layer of proteins (merozoite surface proteins or MSPs) that are organized into a structurally complex coat (9). *PvMSP-3α*, *PvMSP-3β* and *PvMSP-3γ* are members of a multi-gene family of related MSPs (5, 6). The three encoded proteins share only 35-38% identity and 48-58% similarity in pair-wise comparisons. They all contains similar structures including signal sequences and are expressed on the merozoite surface, although they lake transmembran domains or GPI

attachment sites (5). All three proteins a large alanin-rich central domain spanning 60-70% of the amino acid sequence that is strongly predicted to form α -helix secondary and coiled-coil tertiary structures (6). As far as we know, only one assessment have been carried out about diversity, cloning and sequencing of full-length or near full-length *PvMSP-3β* gene fragments in fifteen *P. vivax* isolates originating in Asia, south America and Pacific, and, size polymorphism with five different alleles has been reported (9). In our study seven alleles were distinguishable by size of PCR products ranging from about 1.2 kb to about 2.4 kb and type C (about 1.9kb) was predominant (34%). We observed that PCR product of *PvMSP-3β* gene was not suitable polymorphic marker, because the variation in molecular weight of different alleles was not too much. We carried out the first assessment of diversity in the *PvMSP-3β* gene by RFLP method

in the world and identified at least 15 alleles based on HhaI enzyme digestion (Fig. 2) that indicates high degree of diversity in *P. vivax* population of malarious areas of Iran. We showed that, *PvMSP-3β* could be considered as a useful polymorphic locus for *P. vivax* population study in field setting, like *PvMSP-3α* gene (12- 15), even without sequencing. Extreme variations in *PvMSP-3β* gene sequences were also reported in previous study (9). Identification of 19% mixed genotypes, i.e. two alleles were simultaneously visible, in our study indicated that the *PvMSP-3β* gene could be useful marker for this purpose in addition to polymorphic analysis of *P. vivax* isolates. Multiple infections have been found in 2.4% of cases (two isolates) (Fig. 2). Multiplicity of infection can be determined by comparing the size of undigested PCR products with the sum of the size of the digestion fragments (15). The multiplicity of malaria infections, i.e. one person simultaneously infected by more than one parasite, is common in endemic areas and particularly high in holo-endemic areas. Multiplicity of infection can arise from co-infection, super-infection, or even somatic mutation during the course of infection (11). The differences in multiple infection rates in different malaria-endemic regions may reflect the difference in malaria transmission intensity (16).

Conclusively this preliminary analysis will serve as the foundation for future more detailed studies on population structure of *P. vivax* especially on structure and function of *PvMSP-3* gene family and their products in order to providing effective vaccine against the parasite. In our lab, we are continuing the study in order to reveal genetic structure of this gene based on sequencing.

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The authors declare that they have no conflict of Interests.

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