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Short Communication

Plasmodium vivax MSP-3ß Gene as a Genetic Marker for the Parasite Detection in Comparison with *Ssrrna* Gene

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Abstract

Background: The importance of accurate diagnosis of all of major diseases cannot be underestimated and efficient laboratory testing is vital to identifying and treating life-threatening illnesses including malaria. In this study, we compared the potential of one of merozoite surface protein genes, *PvMSP-3ß*, for detection of *Plasmodium vivax* in blood samples by PCR with routinely used marker, ssrRNA gene.

Methods: One hundred *P. vivax* microscopy-positive blood samples were simultaneously tested with two genetic markers, including *PvMSP-3ß* gene and *ssrRNA* gene by PCR and nestedPCR method, respectively, and their sensitivity and specificity in detection of *P. vivax* was compared.

Results: An important difference was seen in sensitivity between the 2 genetic markers, 100% in case of ssrRNA gene vs. 95% of *PvMSP-3ß* gene. The specificity of the two markers was same (100%). Microscopic diagnoses of thick and thin blood smears was used as "golden standard" method.

Conclusion: Due to critical importance of accurate detection of the parasite in malarious area, the *PvMSP-3ß* gene cannot be a suitable marker for detection of *P. vivax* in blood sample by PCR. More investigations are needed to find other valid markers.

Keywords: PvMSP-3ß, Plasmodium vivax, Ssrrna, *Iran*

Introduction

Plasmodium vivax causes most of the malaria morbidity in endemic regions of Central and South America, North Africa, and Asia (1). The broad and continuous occurrence of vivax malaria in some countries creates significant social and economic losses (2). Total malaria cases in Iran in 2005, were 18966, and about 88% of them caused by *P. vivax* (3). In the recent years the economy of four malaria endemic provinces of Iran (Sistan and Bluchestan, Hormozgan, Kerman and Booshehr) has sustained heavy losses during the malaria epidemics, and malaria control activities imposes a grave disbursement to the socio-economic development programs (3).

Prompt and accurate diagnosis is one of the most important aspects of effective management of any disease and a major strategy of the Global Malaria Control Strategy (4). Although, the careful examination of a well prepared and well stained blood film by an expert microscopist remains currently the "golden standard" for detecting and identifying malaria parasites (5), but there are several documents indicating misdiagnosis of malaria in different laboratories of health system in Iran (6-8), and there is an important need for applying an alternative method to control and confirm of the diagnostic results in malaria control system. Among several genetic markers that have been described for *P. vivax* detection and genetic diversity assessment, the small subunit ribosomal ribonucleic acid (ssrRNA) gene is the most familiar and is used in majority of studies in the world (5-9).

In this study, we assessed the value of a new marker, merozoite surface protein-3ß gene of *P. vivax*, for detection of the vivax malaria in comparison with molecular diagnosis with ssrRNA marker.

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Materials and Methods

One hundred symptomatic patients attending the malaria clinics in southeast of Iran from August to September 2005 were recruited in the study. Sample collection was approved by the Ethical Committee of Tehran University of Medical Sciences and through informed consent of patients. After primary detection of the disease through microscopic method by specialized staff, approximately 1000 µl of venous blood was collected in EDTA from *P. vivax* microscopy-positive samples. In Molecular Laboratory (Isfahan University of Medical Sciences, Isfahan, Iran) the microscopic diagnosis was rechecked by specialized laboratory staff, and parasite DNA was extracted by phenol-chlorophorm method (9). Primarily, samples were processed by nested PCR using plasmodium genus specific (primary PCR) and *p. vivax* species-specific primers for the small subunit ribosomal ribonucleic acid (ssrRNA) gene of *P. vivax* (9). Then, samples were tested by PCR for *PvMSP-3ß* gene with specific primers: forward (5`-AACTTGAGAAACGGATG-3')/ reverse (5'-TGCGAGTGTTTTATGCG-3'), which amplify a small *PvMSP-3ß* fragment and bind at positions 102-118 and 1943-1961 of the Belem *PvMSP-3ß* coding sequence (10). 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 (10).

For negative control we used 5 genomic DNA prepared from healthy individuals with no history of malaria living in non-malarious areas of the country and 4 genomic DNA prepared from *P. falciparum* positive samples maintained through permanent culture in malaria laboratory of department of Medical Parasitology and Mycology (School of Public Health of Tehran University of Medical Sciences). In the case of positive control, because of restrictions in laboratory maintenance of *P. vivax* isolates and lack of such an isolate, we used four positive PCR productions (Their positiveness had been proved through previous studies).

In this study, the golden standard was microscopic diagnosis of Geimsa-stained thick and thin blood smears by expert microscopist (5).

Results

All of samples diagnosed by microscopic examination of thick and thin blood smears and positive controls were positive through nested PCR by primers of *ssrRNA* gene. Negative controls were negative in this process. In PCR process with primers of *PvMSP-3ß* gene, 5 samples were negative. All of negative controls were negative and positive controls were positive with primers of *PvMSP-3ß* gene. Different types of *PvMSP-3ß* gene, based on the size of PCR products were observed (Fig. 1).

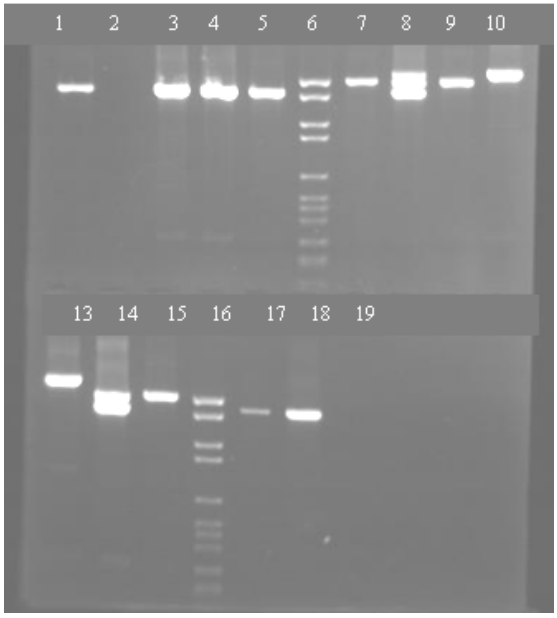

Fig. 1: PCR products of the msp-3β gene of Plasmodium vivax isolates. The gene had different genotypes with different sizes ranging from 1400 to 2400 bp. Lane 2 was microscopically positive and lane 19 was microscopically negative samples. Lanes 6 and 16 contain DNA marker 6 (Roche)

Dissuasion

Laboratory diagnosis of malaria currently is performed by detection of parasites by light microscopy of Geimsa-stained thick and thin blood smears. This procedure is cheap and simple, but is a labor-intensive procedure, which requires well-trained per-

sonnel (8). When parasitaemia is very low, the data of microscopy diagnosis is limited, and in some cases biased, by the inability to devote the necessary amount of time to the examination of blood smears (8).

Greater sensitivity and specificity of PCR method in comparison with thick blood films examination method have been shown by many studies. High sensitivity and specificity of PCR method, at low parasitaemia, has been reported earlier (11-17). The method described by Snounou et al. (9) is the most common one. The sensitivity of *Plasmodium* detection by PCR method is about 10 parasites per 5 micro liter of blood or 1 parasite in 200 microscopic fields (18).

In this study, we assessed the sensitivity and specificity of different genetic markers for detection of merozoite surface protein- 3ß of *P. vivax*. Merozoites are surrounded by a layer of proteins (merozoite surface proteins or MSPs) organized into a structurally complex coat (19). *PvMSP-3α, PvMSP-3ß* and *PvMSP-3γ* are members of a multi-gene family of related MSPs (19-21). The three encoded proteins share only 35-38% identity and 48-58% similarity in pairwise comparisons. All of them contain similar structures including signal sequences and are expressed on the merozoite surface, although they lack transmembran domains or GPI attachment sites (19). *MSP-3ß* gene encodes merozoite surface protein 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 gene is unknown, although immune evasion has been raised as one possibility (20).

In this study, we observed that the sensitivity of PCR with primers of *PvMSP-3ß* gene was 95% versus 100% with primers of *ssrRN*A gene of *P. vivax* and golden standard microscopic method. The specificity of both methods in comparison with golden standard microscopic method was the same (100%). Due to importance of prompt and accurate diagnosis as the key to effective malaria management, which is one of the main interventions of the Global Malaria Control Strategy and the first strategy in National strategy plan for malaria control of Iran (4), we can say that the sensitivity of *PvMSP-3ß* gene detected in our study, is not adequate for malaria control program in the malarious areas of the country, although this marker can detect different types of the parasite (10, 22). Previously, we showed that, *PvMSP-3ß* could be considered as a useful polymorphic locus for *P. vivax* population study in field setting (22), and the types of the parasite can be detected based on the various sizes of the PCR product of this marker (Fig.1).

Although the sensitivity of *PvMSP-3ß* marker in the diagnosis of parasite is less than *ssrRNA*, but for amplification of related part of *PvMSP-3ß* we did not follow the nested PCR procedures and we did it only by PCR method that was a major saving in time and cost in comparison with *ssrRNA* amplification (9).

In conclusion, detection of patients with low-grade parasitaemia and in carriers without any parasite in blood is vital for malaria laboratory diagnosis. Additionally, as there is no national standard malaria microscopic diagnosis quality control system in Iran and since it is only limited to occasionally issued circulars, and, whereas there are several documents indicating misdiagnosis of malaria in different laboratories of health system (6-8), it seems that the *ssrRNA* gene of *P. vivax,* would be useful until unknown future and more investigations is needed to introduce a more sensitive and specific marker.

Ethical Consideration

All Ethical issues (such as informed consent, conflict of interest, plagiarism, misconduct, co-authorship, double submission, etc) have been considered carefully.

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