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Genetic Polymorphism of the Merozoit Surface Protein-1 (*PfMSP-1*)in the Block 4 regionin *Plasmodium falciparum* Malaria Isolates from South-East of Iran

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ABSTRACT

Malaria is still a public health problem in Iran especially in Sistan and Baluchestan Province. Many malarial proteins have been proposed for use as vaccine candidate antigens, but merozoit surface protein-1 (MSP-1) is the most used. The aim of the present study was to investigate the polymorphism in block 4 of MSP-1 gene among Plasmodium falciparum isolates in Iran. A total of 94 P. falciparum infected persons were included in this descriptive cross-sectional survey. Genomic DNA was extracted and genetic polymorphism of PfMSP-1 block 4 was investigated by nested polymerase chain reactions(PCR).All four alleles of MSP1 block 4 genotypes were detected, although MKwas the predominant type (25.8%) and KM was the least frequent (16.9%). Multiple infections with at least two genotypes were detected in 11.2% of samples. The results of this study showed that PfMSP-1 polymorphism is relatively low in Study area and most of infections are composed of one clone, which is consistent with an area of low malaria transmission. These data are useful for malaria prevention and control in Iran.

KEYWORDS : Genetic Polymorphism, Block4, Merozoit Surface Protein-1, *Plasmodium falciparum* , Nested-PCR

Introduction

Malaria is one of the most dangerous parasitic disease that affects approximately 216 million people annually and causes the death of 655,000 people worldwide [1, 2]. This transmitted via mosquito disease, caused by four species of protozoa of the genus Plasmodium that *Plasmodium falciparum* cause severe malaria [1, 3]. One of the most commonly used markers for geno typing of *Plasmodium falciparum* , is Merozoit surface protein -1 (MSP-1). most abundant antigen on the surface of Plasmodium falciparum and its an important candidate for Malaria Vaccine [4,5]. Merpzpit surface protein -1 of *Plasmodium falciparum* is a glycoprotein with a molecular weight of 190-200 kDa and synthesis during the shyzogoni and to be placed on the surface of Plasmodium falciparum . Because this protein is the target of immune response, so it's a strong candidate for vaccine (6). MSP-1 during maturation of merozoit, is proceed by protease into a 83 kDa N-terminal polypeptide, two central polypeptide with 30 and 38 kDa weight and a 42 kDa C-terminal polypeptide. This gene is located on chromosome nine. And based on the amount of amino acid polymorphisms, they classified into 17 blocks, including blocks of self-protection, semi-preservation and variable [8,9]. Each block has K1 or MAD20 allelic dy-morphism. Excep for block 2 that has additional allele RO33 and block 4 that can be exist into one of four modes such as: MK, KK, MM, KM [9]. Variable Block 4 located in the N-terminal region of gene and in 83-kDa polypeptide, and include of two part, 4A and 4P [10,11]. Iran is one of the countries in the Eastern Mediterranean region with little endimicity of malaria in some areas [12]. Sistan and Baluchestan is endemic region of falciparum malaria that is considered as eastern eco- epidemiology of malaria [13]. In the present study, using nested-PCR, was studied the genetic diversity of Block 4 of MSP-1 gene in malarious areas of Iran, Sistan and Baluchestan.

Materials and Method

This cross - sectional study were conducted on 94 patients with falciparum malaria, health and medical in care in Chabahar, Iranshahr, Nikshahr and Sarbaz from March 2011 to September 2012., these cities are the functions of Sistan and Baluchestan province and there are the endemic foci of malaria in Iran. Selection criteria were as follows: stay on target areas for more than 6 months, no previous treatment with anti-malarial drugs in the past month, patient satisfaction, and in the case of children, satisfaction of parents. 2 ml of venous blood was obtained from each patient. In order to confirm infection with *Plasmodium falciparum* . Four drops of each sample was used for thick and thin smear, after preparation, were stained with Giemsa and examined by light microscopy. The remaining of each sample were collected in tube containing ethylene diamine tetra acetic acid (EDTA) and was maintained in -20°C to collect DNA extraction. Parasite DNA using a DNA extraction kit (Fermentas, Lithuania) and was extracted according to instruction of kit and also was maintained at - 20 ° C until the PCR time.

Variable block 4 of the P.FMSP gene was reproduced with nested-PCR method and based on Kaneko and colleagues guidelines [14]. The first stage PCR using C3F and C5R olygo nucleotide, and the second stage was performed using specific primers K1 and MAD20 allelic family. Primer sequences are presented in Table 1.

Primer	Primer sequence	Features of primer
C3F	5′-TTCGTGCAAATGAATTAGACGTAC-3′	A member of block3- first stagePCR
C5R	5′-GGATCAGTAAATAAACTATCAATGT-3′	A member of block 5- first stagePCR
K4AF	5′-AATGAAATTAAAAATCCCCCACCGG-3′	
M4AF	5′-TTGAAGATATAGATAAAATTAAAACAGATG-3′	Specific for K1- second stage PCR Specific for MAD20- second stage PCR
K4PR	5′-TCCTCGATTTTTTTGTTCTTATCAAG-3′	
M4PR	5′-TCGACTTCTTTTTTCTTATTCTCAG-3′	Specific for K1- second stage PCR Specific for MAD20- second stage PCR

Table 1. Sequences of primers used for genotyping of block 4 of *PfMSP-1* gene (14).

The reactions wad done in thermal cycler[1] device (biometra, Germany) in a final volume of 20 microliters using Accu Power TLA PCR Premix (Baunir, Korea). That would include the following materials:

Enzyme TLA DNA polymerase (1 U), Desoxy nucleotide triphosphate (250 µM), buffer 10X (2 µl), stabilizers and color tracer. After adding 1.5 microliter of extracted DNA from samples, and 0.75 microliters of each diluted primer into tube that containing Premix, Using sterile distilled water to make a final volume of 20 microliters. To reduce transmission of first stage PCR primers to the second stage, only 1 microliter of the primary PCR product was used as template in the second reaction. DNA purified from Plasmodium falciparum , which are the standard strains of them was bought from Malaria Research Resources Center in Manzas, United States, were used as positive controls. 2% agarose gel containing ethidium bromide electrophoresis of PCR products was performed, and then observed by trans luminator. For interpreted the fragments size, were used positive control and ladder marker 50[2]bp (formantase, litvani).

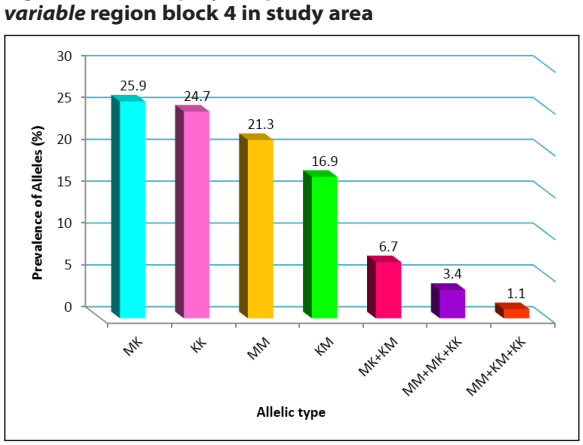
Results

94 patients with Plasmodium falciparum in Sistan and Baluchestan, enter this discriptive cross - sectional study. Five patients were excluded because of negative results of PCR. In this block, four modes MM, KK, MK and KM were observed. In genotype of MM, 97 bp fragment, in KK, 80 bp fragment, in MK, 94 bp fragment and in KM, 83 bp fragment were observed. MK genotype in 25.8 percent, KK on 24.8 percent, MM at 21.3%, and KM in 16.9% of the samples was observed (Table 2). Multi-clonal infections in 11.2% of the samples were found. Therefore, despite the total number of samples was 89. But the frequency of fragments in these patients was 103, which indicate that some patients have infection with two or more genotype.

Genotype	Frequency	%
MK	23	25.8
KK	22	24.8
MM	19	21.3
KM	15	16.9
MK+ KM	6	6.8
MM+ MK+ KK	3	3.3
MM+ KM+ KK	1	1.1
Total	89	100

Table 2. Distribution of genotype frequencies in Block 4 of PfMSP-1 gene in Sistan and Baluchestan Province

Figure 1: Allelic polymorphisms of the *PfMSP-1 , semi-variable* region block 4 in study area



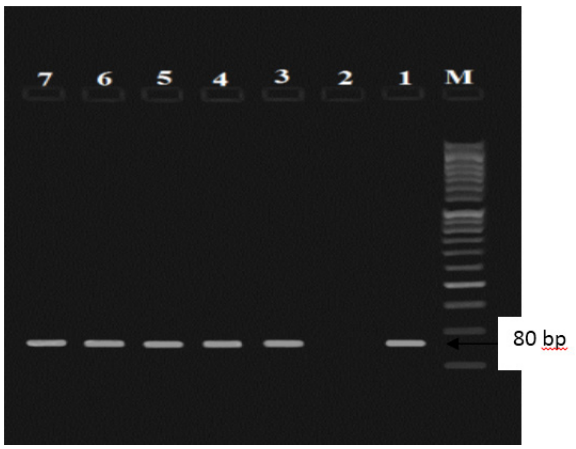


Figure 2: Schamatic presentation of *PfMSP1* block 4, KK allele in 1.8% Agarose gel

(lane M, A 50bp ladder Marker, L1 Positive Co, 80 bp, L2 Negative Co, L3-7 Positive cases in study area)

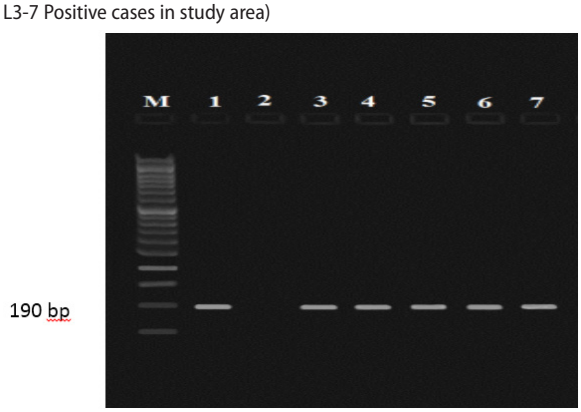


Figure 3 : Schamatic presentation of *PfMSP1* block 4, MM allele in 1.8% Agarose gel

(lane M, A 50 bp ladder Marker, L1 Positive Co, 190 bp, L2 Negative Co, L3-7 Positive cases in study area)

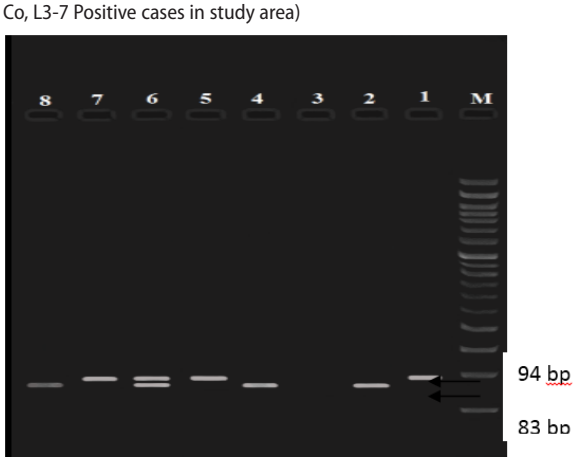


Figure 4 : Schamatic presentation of *PfMSP*1 block 4, MK and KM alleles in 1.8% Agarose gel

(lane M, A 50bp ladder Marker, L1 Positive Co, for MK allele 94 bp, , L2 positive Co, for KM allele 83bp, L3 Negative Co, L4-8 Positive cases in study area)

Discussion

The structure of genetic population of *Plasmodium falciparum* play a major role in acquired immunity against malaria [15]. In the present study was used nested-PCR method to investigate the genet-

ic diversity in block 4 of *PfMSP-1* gene in southeastern of Iran. Studies have shown that the sensitivity and specificity of this method is up to 94 percent [16]. Compared with other PCR methods in the field of our study, can produce more product and is very affordable unlike sequencing. In the present study, all four Blocks 4 (MK, KK, MM and KM) were observed in Sistan and Baluchestan Province. MK genotype had the highest frequency and lowest frequency was for KM. These findings are similar to previous studies conducted in Iran, Vietnam and Brazil In which the predominant genotype was MK and KM had the lowest prevalence [11, 17, 18, 19]. However, with studies in the Solomon Isands and Columbia, kk had lowest frequency that is in contrary with our finding. Also, MM genotype was dominant in the Solomon Islands [9, 20].

In this study, 11.2% of the samples contain multi-clonal infections, this value compared to the multi-clonal infections were observed in Iran, eith 80% in 5 'region of the PfMSP-1 gene, Philippines with 33% , Colombia with 5.79% , Thailand with 5.60% and Vietnam with 43% showing less value. But towards the island of Vanuatu with 7.0%, indicating a higher rate [9, 11, 14, 17, 20, 21]. Differences in the epidemiology of malaria among that countries and Iran can be one of the reasons, for low multi-clonal infections in this region. This study confirms the occurrence of recombination in Block 4. As we know recombination cause the increasing in diversity in *PfMSP-1*.

Conclusion:

This research provide an information about genetic polymorphysm of *PfMSP-1* in Iran. This study showed that genetic variation in this block 4 of gene is relatively low in the southeast, of Iran. And most infections are composed of a clone and consistent with an area of low malaria transmission. The study Comparison between this study and other previous studies in other geographic areas in designing an effective vaccine for malaria could be useful.

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