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Analysis of *Plasmodium vivax* Merozoite Surface Protein-1 Gene Sequences

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Abstract

The emergence of *Plasmodium vivax* in Dashte Moghan in northwestern Iran has become a major concern for Iranian's health officials. Knowledge of genetic make up of the *P. vivax* populations in this area would give us an insight into the origin of the prevalent infections and the possible routes they are introduced. This paper reports the analysis of a variable region between the two interspecies conserved blocks (ICBs) of 5 and 6 of *MSP-1* gene in 18 isolates from Dashte Moghan. The results revealed that all the 18 isolates were similar to an Azari Belem-like type with 21 glutamine (Q) in the repeated residues. Our results may give a clue that the resurgent malaria in Dashteh Moghan might have primarily been introduced from Azerbaijan. However, much more molecular and epidemiological evidence are needed to confirm this hypothesis.

Keywords: *Malaria,* Plasmodium vivax, *Merozoite surface antigen* (MSP-1), *Belem allel (Bel), Iran*

Introduction

Plasmodium vivax was the most prevalent human malaria parasite throughout Iran in the past and accounted for the majority of 5 million cases reported annually in then 13 million populated Iran (Jalili Moslem GH, 1952, unpublished). With the beginning of malaria campaign at late 1950s this species was eradicated from the most northern parts of the country and confined to some areas in south and southeastern provinces, where it often co-occur with *Plasmodium falciparum*. The reemerge of *P. vivax* malaria In Dashte Moghan in northwestern Iran, which has been reported malaria-free since early 1960s (1) became a major concern for the public health officials. This area borders with Azerbaijan Republic, a newly independent state in central Asia with some experiences of *P. vivax* malaria outbreaks since early 1990s (2). Knowledge of the population genetic structure of the malaria parasite in Dashte Moghan would allow us to gain an insight into origin of the parasite and update our understanding of epidemiology of the disease, which would finally lead us to adoption of proper control measures. The growing evidences on capability of *MSP-1* in *P. falciparum* (*PfMSP-1*) as a useful genetic maker for discriminating variation within and among populations of this malaria parasite (3- 5) prompted many researches to use the same tool for studying genetic structure of *P. vivax* populations. *P. vivax* major surface protein 1 (*PvMSP-1*) like *PfMSP-1* exhibits antigenic polymorphism (6). Complete gene sequence of *PvMSP-1*, a 200 kDa polymorphic glycoptropein, was characterized in two isolated strains: Brazilian Belem (Bel) and Salvador (Sal1) (7, 8). Later, analysis of specific gene regions derived from parasite isolates from Srilanka (9) Thailand (10), and Colombia (11) revealed new sequence types of *MSP-1* polymorphic region, most of them resulting from recombination between the sequence of the two original Belem

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and Sal 1 strains (11). *PvMSP-1* gene consists of seven interallele conserved blocks flanked by six variable blocks. The variable blocks, particularly the one between interspecies conserved blocks 5 and 6 show extensive variation in repeats and nonrepeats unique sequences among *P. vivax* isolates (10). In particular, the Belem strain can easily be characterized by a variable poly-glutamine (poly Q) region absent in Sal 1 strain.

This study reports the genetic make up of *P. Vivax* population in Dashte Moghan by analyzing the variable block between the ICB5 and ICB6 of *MSP-1* gene.

Materials and Methods

Study area and sample collection Blood samples were collected from 18 febrile patients, who were seeking treatment from public health centers after their verbal consent. All the blood samples were shown to harbor *P. vivax* by both microscopy and rDNA-Polymerase chain reaction. After identification whole blood samples were frozen at -70 °C until used.

DNA extraction 300 µl of whole blood was mixed with three volumes of cold double distilled water (ddH_2O) and kept on ice for 10 min. The parasite and unlysed erythrocytes were recovered by centrifugation at 8000 rpm for 7 min. The supernatant was discarded and the pellet was resuspended in 1 ml cold PBS containing saponin to a final concentration of 0.05%. Immediately after the lysis was observed, parasites and white blood cells were recovered by centrifugation as above. DNA extraction from the pellet was accomplished using a DNA isolation kit (Amersham Bioscience, USA) according to the manufacturer's instructions. The recovered DNA was resuspended in 50 µl of ddH_2O.

Polymerase chain reaction and sequencing

The highly polymorphic region with the flanking ICB5 and ICB6 of *MSP-1* gene was amplified using Pv1 and Pv2 primers and PCR reaction conditions of Putaporntip et al., (10). These primers would allow amplification of a nucleotide region corresponding to basepairs 1813 to 2827 in Sal1 allel. 100 µl of each amplified sample was run on a 0.8% agarose gel and fragment of interest was cut and purified using a gel band purification kit (Amersham Pharmacia Biotech, Inc) according to manufacture's instructions. The concentration of recovered DNAs was estimated by running them on an agarose gel along with a known concentration of DNA molecular weight marker (MWM III, Roche). Sequencing of fragments was performed in both directions using an automated sequencer (SeqLab Laboratory, Germany).

DNA alignment was done using a Clustal X (version1.8) multiple sequence alignment program.

Results

PCR amplification of the variable region and the flanking ICB5 and ICB6 of the Pv*MSP-1* gene revealed a limited size variation among isolates ranging from 862 to 894.

The sequencing result of DNA and corresponding amino acids revealed a distinct Belem-like type in all 18 isolates. There was a consensus deletion of 6 nucleotides (CAACAA) in all isolates, corresponding to nucleotides 2334-2339 in Belem allel leading to two lesser number of Q in poly-Q repeats i.e. 21Q as compared to 23 in Belem allel. There were also some synonymous substitutions in the polymorphic region which could be classified into two types. Both types shared a substitution of ***g*** for ***a*** at position 2327, but type 2 had three more substitutions including ***t*** for ***c*** at position 2736, ***a*** for ***g*** at position 2738 and ***c*** for ***t*** at position 2747 (Fig. 1).

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************************************************************************************************ ****** ****************************************
Belem AATTGCTTAGAGGATCAAGCGAAGCAGCGACAGAGGTCACAACCAATGCGGTAACATCTGAAGTACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAGCAACAACAGCAACACAACAATCACAAGTAGTACCAG 150
Type1 AATTGCTTAGAGGATCAAGCGAAGCAGCGACAGAGGTCACAACCAATGCGGTAACATCTGAAGTACAACAACAACAACAACAACAACAACAACAACAACAGCAACAA------CAGCAACAACAGCAACAACAACAATCACAAGTAGTACCAG 144
Type2 AATTGCTTAGAGGATCAAGCGAAGCAGCGACAGAGGTCACAACCAATGCGGTAACATCTGAAGTACAACAACAACAACAACAACAACAACAACAACAACAGCAACAA------CAGCAACAACAGCAACAACAACAATCACAAGTAGTACCAG 144
ruler 1.......10........20........30........40........50........60........70........80........90.......100.......110.......120.......130.......140.......150
*****************************************************************************************************************************************************************
Belem CACCTGCAGGAGATGCCCAACAAGTAATCTCAACACAACCGACTAGTCAATCCGCAGCACCAGGCGTATCAGCCACACCAGCACCAACACCTGCTGCCGCAGCCGCCCCAGCACCAGCCATGTCCAACTGGAATACCTCGAAAAGCTCC 300
Type1 CACCTGCAGGAGATGCCCAACAAGTAATCTCAACACAACCGACTAGTCAATCCGCAGCACCAGGCGTATCAGCCACACCAGCACCAACACCTGCTGCCGCAGCCGCCCCAGCACCAGCCATGTCCAACTGGAATACCTCGAAAAGCTCC 294
Type2 CACCTGCAGGAGATGCCCAACAAGTAATCTCAACACAACCGACTAGTCAATCCGCAGCACCAGGCGTATCAGCCACACCAGCACCAACACCTGCTGCCGCAGCCGCCCCAGCACCAGCCATGTCCAACTGGAATACCTCGAAAAGCTCC 294
ruler .......160.......170.......180.......190.......200.......210.......220.......230.......240.......250.......260.......270.......280.......290.......300
*****************************************************************************************************************************************************************
Belem TTGATTTTTTAAAATCCGCTTACGCATGTCACAAGCACATCTTCGTAACCAACTCCACCATGGACAAGAAACTACTCAAAGAGTACGAACTTAACGCTGATGAGAAAACCAAAATTAATCAAAACAATGCGATGAATTGGACCTCCTAT 450
Type1 TTGATTTTTTAAAATCCGCTTACGCATGTCACAAGCACATCTTCGTAACCAACTCCACCATGGACAAGAAACTACTCAAAGAGTACGAACTTAACGCTGATGAGAAAACCAAAATTAATCAAAACAATGCGATGAATTGGACCTCCTAT 444
Type2 TTGATTTTTTAAAATCCGCTTACGCATGTCACAAGCACATCTTCGTAACCAACTCCACCATGGACAAGAAACTACTCAAAGAGTACGAACTTAACGCTGATGAGAAAACCAAAATTAATCAAAACAATGCGATGAATTGGACCTCCTAT 444
ruler .......310.......320.......330.......340.......350.......360.......370.......380.......390.......400.......410.......420.......430.......440.......450
********************************************** *********** ******** ******************************
Belem TCAATGTCCAGAACAACTTGCCAGCCATGTACTCCATATATGACTCCATGAGCAACGAGCTGCAGAATCTTTACATTGAGCTGTACCAGAAGGAAATG 548
Type1 TCAATGTCCAGAACAACTTGCCAGCCATGTACTCCATATATGACTCCATGAGCAACGAGCTGCAGAATCTTTACATTGAGCTGTACCAGAAGGAAATG 542
Type2 TCAATGTCCAGAACAACTTGCCAGCCATGTACTCCATATATGACTCTATGAGCAACGAACTGCAGAACCTTTACATTGAGCTGTACCAGAAGGAAATG 542
ruler .......460.......470.......480.......490.......500.......510.......520.......530.......540........
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Fig. 1: Nucleotide substitutions and deletions in the variable block (flanking by ICB5 and ICB6) of the Pv*MSP-1* gene among isolates from northwestern Iran in comparison with Belem allel (accession No. AF 435594). Dashes represent six nucleotide (CAACAA) deletions which are equal to two glutamine (Q). The lacks of astericx represent substitutions.

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Discussion

Dashte Moghan has been reported malaria-free since the implementation of an extensive national malaria eradication program throughout Iran beginning in 1958 (1). However this disease has reemerged in this area since 1994 with more than 3126 reported patients diagnosed with *P. vivax* up to 2004. Analysis of genetic make up of *P. vivax* populations in this area and their comparison with already published data available in Gebank from Iran and neighboring countries would gave us some clues on the origin of this resurgent malaria. In the neighboring country of Azerbaijan, analysis of the highly variable region between ICB5 and ICB6 of this gene showed that both Belem-like and Sal1-like types together with some recombinant types were prevalent in the five districts of the country (2). We studied the same fragment in 18 isolates collected from Dashte Moghan in Ardebil Province of Iran. This area borders with Azerbaijan from north and northeastern parts, where many people move between two countries without government control. Our study revealed two types similar to an Azeri Belem-like type in all the18 isolates, with the same poly-Q region of 21 repeats as compared to 23 in Belem allel. It is noteworthy that all the Belem-like types from Azerbaijan had also 21Q in the poly-Q repeats while the poly-Q repeats in the five Azari recombinant types ranged between 12-18 Q. Genetic analysis of 16 plasmodial isolates from southeastern Iran using the same region of *P. vivax MSP-1* revealed 12 Belem-like types with number of poly-Q repeats ranging from 17-23. The only isolate with 21 Q was proved a recombinant type (13).

Our results may give a clue that the resurgent malaria in Dashteh Moghan might have primarily been introduced from Azerbaijan. However, much more molecular and epidemiological studies are needed to confirm this hypothesis.

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