Research Paper

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A Malaria Vaccine Candidate Protein, C-Terminuse Region, Block 17 of Pfmsp-1 (Plasmodium Falciparum Malaria Merozoite Surface Protein-1) From Field Isolates in South-East of Iran

Medical Science

KEYWORDS : Genetic Diversity, Merozoite Surface Protein-1, Plasmodium falciparum, nested PCR, C-terminal Region, Iran

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ABSTRACT

Merozoite surface protein 1 (MSP-1) is the most abundant protein on the surface of Plasmodium falciparum merozoites and is a leading candidate for malaria vaccine. The purpose of this study was to investigate genetic diversity of the carboxyl (C)-terminal region of MSP-1 gene in patients with falciparum malaria in Sistan and Baluchistan Province. A total of 94 P. falciparum-infected blood samples were collected from malaria patients residing in four regions of Sistan and Baluchistan Province between March 2012 to September 2013. Genomic DNA was extracted and genetic diversity of the C-terminal region of PfMSP-1 was investigated by semi-nested polymerase chain reactions (PCR).

In this study both MAD20 and K1 types were detected. MAD20 was the predominant allelic family identified in 77.5 % of the samples, whereas 22.5% of those were of K1 type. Multiple infection with two alleles was observed in any of the samples. The results of this study show that genetic diversity of the C-terminal region of PfMSP-1 is restricted in Sistan and Baluchistan province of Iran and All 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.

Introduction

Malaria is the most common infectious disease in tropical regions, according to the World Health Organization stats (WHO 2011) the incidence of its in the world is 216 million people and its overall mortality is estimated to be approximately 655 thousand people per year [1, 2]. This disease is caused by four species of protozoa of the genus Plasmodium, that the Plasmodium falciparum is the major cause of deaths [1]. In Iran, the majority of clinical cases of malaria (86%) are caused by Plasmodium vivax, and *Plasmodium falciparum*, is the remaining 14% of the clinical cases [3]. Merozoite Surface Protein-1 (MSP-1) is the most abundant antigen on the surface of *Plasmodium falciparum* merozoite and are an important candidate for Malaria vaccine [4]. This protein with a molecular size about 195 kDa, which synthesis during the shyzougoni stage, and the main objective for immune response in humans, a potential candidate vaccine for blood stage and is responsible for the parasite invade to red blood cells [1,5,6]. MSP-1 gene is located on chromosome 9, and based on the amount of amino acid polymorphism is divided into 17 blocks, contains blocks of self-protection, semi preservation and variables [7,8]. MSP-1 during merozoit maturation is processed to a 83 kDa, N-terminal polypeptide, two central polypeptide, with 30 and 38 kDa weight and a C-terminal polypeptide with kDa weight. The 42-kDa fragment before attacking erythrocytes is divided into two polypeptides of 19 and 33 kDa. 19-kDa polypeptide that is encoded by block 17, is the main aim of natural acquired immunity against Malaria [8,9]. This polypeptide containes of two damain, that are similar to epidermal growth factor (EGF). And it is the only piece that, after the invasion of red blood cells through the glycosyl phosphatidyl-inositol remains attached to the membrane of merozoit. Studies show that spatial epitopes, created by the EGF domain, are the aim of protecting antibodies [10, 11]. Iran is one of the countries in the Eastern Mediterranean region that there is malaria with low endemicity in some areas [12]. Sistan and Beluchestan, is an endemic region of falciparum malaria that is considered as eco-epidemiology of malaria in the eastern (13, 14). In this study using the Semi-nested PCR, was studied the genetic diversity in the C-terminal of merozoit surface protein -1 in malarious areas of Iran, Sistan and Beluchestan.

Materials and Metods

This cross - sectional study were conducted on 94 patients with falciparum malaria, seeking health and medical care in Chabahar, Iranshahr, Nikshahr and Sarbaz from March 2012 to September 2013. These cities are four districts of Sistan and Baluchestan province and there are the endemic foci of malaria in Iran. The 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 ethylene diamine tetra tube containing acetic acid and was maintained in -20°C for DNA extraction. Parasite DNA was extracted according to instruction of kit and also was maintained - 20 ° C until the PCR amplification. *PfMSP-1*, 19 kDa using semi-nested PCR and according to Sutton & colleagues instruction were duplicated [13]. The specific Primers sequences are presented in Table 1.

The reactions wad done in thermal cycler device (Biometra, Germany) in a final volume of 20 microliters. DNA purified from *Plasmodium falciparum*, which are the standard strains of them provided from Malaria Research Resources Center in Manzas, United States, were used as a positive controls. Then, 2% agarose gel containing ethidium bromide electrophoresis of PCR products was performed, and then observed by transluminator. For interpreting the fragments size, positive control and ladder marker 50 bp (formantase, litvani), were used.

Results

94 patients with *Plasmodium falciparum* in Sistan and Baluchestan, selected for this cross - sectional study. Five patients were excluded due to negative results of PCR assay. Alleles and variants observed in this block, were two cases, Both K1 and MAD20 allele, only the 450 bp fragment was observed. Overall, 69 patients (77.5%) belonged to the family MAD20 allelic, And 20 patients (22.5%) belonged to the K1 allelic family. The frequency distribution terms of the province and region under study are listed in Table 2, Infection with two alleles was not observed in

any of the samples (figures 1, 2, 3).

Table 1. Sequences of primers used for genotyping of *P. fMSP1*-19KD (13)

Primer	Primer sequences	Features of primers
MAD20F K1F UR	5′-GCAATATCTGTCACAATGG-3′ 5′-GCAG TAACTCCTTCCGTAATTG-3′ 5′-TTAGAGGAACTGCAGAAAATACCA-3′	Specific for MAD20- first stepPCR Specific for K1 – first step PCR First stepPCR- Universal Reverse
MAD20F K1F UR	5′-CCATAACGACTTCGAAGC-3′ 5′-CGTTGGAATTGCTGATTTATCAACAG-3′ 5′-TTAGAGGAACTGCAGAAAATACCA-3′	Specific for MAD20- second stepPCR Specific for K1- second step PCR Second setp PCR- Universal Reverse

Table 2. Distribution of allele frequency and variants in C-terminal *P fMSP-1* region, in terms of in sampling in different regions of Sistan and Beluchestan.

Region / allele	Province		Sarbaz		Nikshahr		Iranshahr		Chabahar	
	%	frequency	%	frequency	%	Frequency	%	frequency	%	frequency
MAD20	77.5	69	13.5	12	21.3	19	19.1	17	23.6	21
K1	22.5	20	4.5	4	4.5	4	5.6	5	7.9	7
Total	100	89	18	16	25.8	23	24.7	22	31.5	28

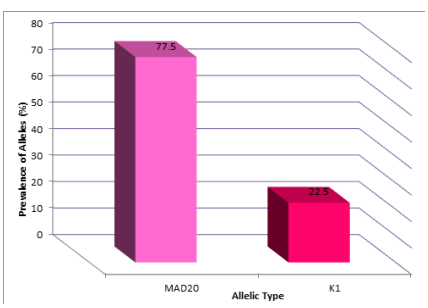

Figure 1: Allelic polymorphisms of the *PfMSP-1*, C-terminal region, block 17 in study area

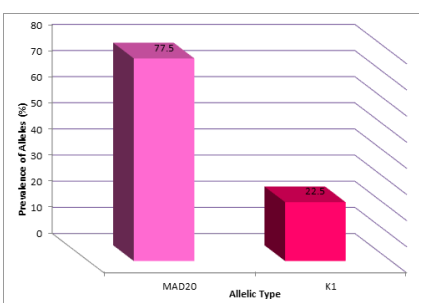

Figure 2: Schamatic presentation of *PfMSP1* block 17, MAD20 allele in 1.8% Agarose gel

(lane M, 50 bp ladder Marker, L2 Positive Co, 450 bp, L1 Negative Co, L6-7 Positive cases in study area

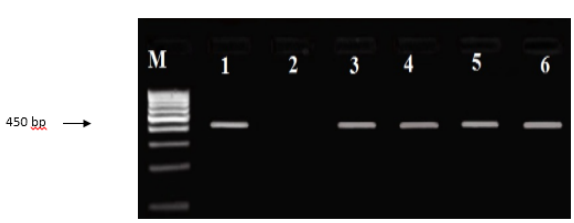

Figure 3: Schamatic presentation of *PfMSP1* block 17, K1 allele in 1.8% Agarose gel

(lane M, 50 bp ladder Marker, L1 Positive Co, 450 bp, L2 Negative Co, L3-6 Positive cases in study area)

Discussion

The structure of genetic population of *Plasmodium falciparum* play a major role in acquired immunity against malaria [15], so to control the disease and design effective vaccine against *Plasmodium falciparum*, its necessary to investigate the genetic structure of parasite population in clinical isolated in different endemic regions. The best candidates for malaria vaccine , which are against immunogen and conserved protein region and have limited genetic diversity. The 19 kDa C-terminal region of *PfMSP-1* candidate for vaccine. In Vitro and In Vivo Studies have shown, antibodies against the 19 kDa polypeptide inhibit the invasion of merozoites to red blood cells and blocked the parasite life cycle [14]. Therefore, to control malaria and designing vaccine for Plasmodium falciparum, the genetic diversity of this part of the protein in clinical isolates from different endemic areas should be studied. The transmission of malaria in southeastern Iran, is low and seasonal, and clinical signs of infection usually occurs in adults [16]. To study the C-terminal region of MSP-1 19 kDa polypeptide that is located in this area, using specific primers of MAD20 and K1 allelic family. Falciparum isolates were observed in both the family, allele family MAD20 had highest frequency (77.5%), and family K1 had lowest frequency (22.5%). These findings are similar to previous studies in Iran (Mehrizi 2008, and Zamani 2009) and India (Kumar 2005, Mamillapalli 2007). In that studies MAD20 allele in the C-terminal region, was predominant [1, 10, 17, 18]. However with the study conducted in Peru (Sutton 2010) where K1 allelic family was dominant, has been inconsistent [11].

Conclusion

Present study provided the information about genetic polymorphisms in the C-terminal *PfMSP-1*. This study showed that the genetic diversity of C-terminal region of this gene is limited in southeastern of Iran . And all infections are composed of a single clone, is consistent with an area with low malaria transmission. Comparison between this study with previous study in other geographic areas could be useful for designing an effective vaccine.

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REFERENCE

1. Zamani Z, Razavi MR, Sadeghi S, Naddaf S, Pourfallah F, Mirkhani F, et al. Sequence diversity of the C-terminal region of Plasmodium falciparum merozoite surface protein 1 in southern Iran. Southeast Asian J Trop Med Public Health. 2009;40(1):1-9. | 2. WHO. World malaria report. Geneva 2011; Available from: http://www.who.int/malaria/world_malaria_report_2011/WMR2011_factsheet.pdf. | 3. WHO. Iran (Islamic Republic of). 2012; Available from: http://www.who.int/countries/irn/en/. | 4. Cowan GJM, Creasey AM, Dhanasarnsombut K, Thomas AW, Remarque EJ, Cavanagh DR. A Malaria Vaccine Based on the Polymorphic Block 2 Region of MSP-1 that Elicits a Broad Serotype-Spanning Immune Response. PLoS ONE. 2011;6(10). | 5. Kang JM, Moon SU, Kim JY, Cho SH, Lin K, Sohn WM, et al. Genetic polymorphism of merozoite surface protein-1 and merozoite surface protein-2 in Plasmodium falciparum field isolates from Myanmar. Malar J. 2010;9(131). | 6. Hu J, Chen Z, Gu J, Wan M, Shen Q, Kieny MP, et al. Safety and Immunogenicity of a Malaria Vaccine, Plasmodium falciparum AMA-1/MSP-1 Chimeric Protein Formulated in Montanide ISA 720 in Healthy Adults. PLoS ONE. 2008;3(4). | 7. Zhong D, Afrane Y, Githeko A, Yang Z, Cui L, Menge DM. Plasmodium falciparum Genetic Diversity in Western Kenya Highlands. Am J Trop Med Hyg. 2007;77(6):1043-1050. | 8. Terrientes ZI, Vergara J, Kramer K, Herrera S, Chang SP. Restricted genetic diversity of Plasmodium falciparum major merozoite surface protein 1 in isolates from Colombia. Am J Trop Med Hyg. 2005;73(5):55-61. | 9. Chenet SM, Branch OH, Escalante AA, Lucas CM, Bacon DJ. Genetic diversity of vaccine candidate antigens in Plasmodium falciparum isolates from the Amazon basin of Peru. Malar J. 2008;7(93). | 10. Mehrizi AA, Zakeri S, Salmanian AH, Sanati MH, Djadid ND. Plasmodium falciparum: sequence analysis of the gene encoding the C-terminus region of the merozoite surface protein-1, a potential malaria vaccine antigen, in Iranian clinical isolates. Exp Parasitol. 2008;118(3):378-385. | 11. Sutton PL, Clark EH, Silva C, Branch OH. The Plasmodium falciparum merozoite surface protein-1 19 KD antibody response in the Peruvian Amazon predominantly targets the non-allele specific, shared sites of this antigen. Malar J. 2010;9(3) | 12. Heidari A, Keshavarz H, Rokni MB, Jelinek T. Genetic diversity in merozoite surface protein (MSP)-1 and MSP-2 genes of Plasmodium falciparum in a major endemic region of Iran. Korean J Parasitol. 2007;45(1):59-63. | 13. Mohammadi M, Ansari-Moghaddam A, Raiesi A, Rakhshani F, Nikpour F, Haghdost A, et al. Baseline results of the first malaria indicator survey in Iran at household level. Malar J. 2011;10(277). | 14. Zakeri S, Mehrizi AA, Zoghi S, Djadid ND. Non-variant specific antibody responses to the C-terminal region of merozoite surface protein-1 of Plasmodium falciparum (PfMSP-1 19) in Iranians exposed to unstable malaria transmission. Malar J. 2010;9(257). | 15. Farnert A, Williams TN, Mwangi TW, Ehlin A, Fegan G, Macharia A, et al. Transmission-dependent tolerance to multiclonal Plasmodium falciparum infection. J Infect Dis. 2009;200(7):1166-75. | 16. Zakeri S, Bereczky S, Naimi P, Pedro Gil J, Djadid ND, Farnert A, et al. Multiple genotypes of the merozoite surface proteins 1 and 2 in Plasmodium falciparum infections in a hypoendemic area in Iran. Trop Med Int Health. 2005;10(10):1060-4. | 17. Kumar SV, Ranjan S, Saxena V, Rajesh V, Roy SK, Kochar D, et al. Plasmodium falciparum: Genetic diversity of C-terminal region of MSP-1 in isolates from Indian sub-continent. Exp Parasitol. 2005;110(1):384-388. | 18. Mamillapalli A, Sunil S, Diwan SS, Sharma SK, Tyagi PK, Adak T, et al. Polymorphism and epitope sharing between the alleles of merozoite surface protein-1 of Plasmodium falciparum among Indian isolates. Malar J. 2007;6(95). |