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Diversity of Merozoite Surface Protein-3α Gene of *Plasmodium vivax* in Isolates from Sistan and Bluchestan Provinces in Iran

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Approximately 90 million people are suffered from vivax malaria annually. This disease imposes a heavy economic cost in endemic areas of Asia and the Americas. This study was design and implemented to reveal the extent of polymorphism of the *Plasmodium vivax* Merozoite Surface Protein -3α gene. 37 blood samples (20 Iranian and 17 non-Iranian) collected from patients attending to malaria clinics in the Sistan and Baluchistan province. The DNA was amplified by nested PCR and the products were digested with HhaI enzyme through PCR/RFLP technique. Three biotypes of the gene based on the size of PCR products, including A (about 1900bp), B (about 1400bp) and C (about 1100bp) and 10 allelic groups after digesting of PCR products with HhaI enzyme was observed. A significant difference between the two groups of patients was not detected.We observed that *P. vivax* isolates of Sistan and Bluchestan were extremely diverse, and the results are almost compatible with the results of other studies performed previously. Based on the results of our study RFLP method using HhaI (small fragments from 50-500bp) is suitable technique for describing diversity of the gene.

Key words: *Plasmodium vivax*, Diversity, Iran, Sistan and Bluchestan.

In the recent years, *Plasmodium vivax* has been distributed significantly in the world in many regions and the number of the reported cases is increasing. Nowadays, *P. vivax* is the most prevalent species of human malaria parasites (*P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*), although, *P. falciparum* is responsible for most malaria deaths[1]. Approximately 90 million people are suffered from debilitating vivax malaria annually. The disease imposes a heavy economic cost in endemic areas of Asia and the Americas[2]. More than 90% of all malaria cases in 2010 in Iran caused by *P. vivax* and the east-southern provinces of the country (Sistan and Bluchestan, Hormozgan, Kerman and Boushehr) encounter with the transmission of the disease[3]. In recent years many investigations have been performed on the diversity of plasmodium genetic structure using polymorphic markers for revealing intra species differences of the parasite that is necessary for designing and producing of vaccines or drugs to combat the parasite. For example, markers such as merozoite surface protein-1(MSP-1), MSP-2, glutamate- rich protein, and microsatellites have been assessed for *P. falciparum* diversity[4]. There are some important markers that have been used for assessing the polymorphism of field isolates of P. vivax. Some of these markers are including PvCSP

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(P.vivax Circumsporozoite protein), PvMSP-3α (P. vivax Merozoite Surface Protein -3α), PvMSP-3β (Plasmodium vivax Merozoite Surface Protein - 3β) and PvMSP-3β *(Plasmodium vivax* Merozoite Surface Protein - 3γ)[1, 4, 5-9]. PvMSP-3α, PvMSP-3β, and PvMSP-3γ are members of MSPs multi-gene family. Immune evasion may be one possibility for the function of this family[7-9] The (PvMSP-3α) is a genetic marker that has been used recently for population genetic studies of *P. vivax* and is a potential vaccine candidate[4, 8-14]. It has been proved that PvMSP-3α is highly polymorphic with three types including types A, B, and C that are detectable through molecular techniques[8-14] Obviously, immune responses adopted against one type of an antigen may not necessarily be protective against parasite strains expressing other types of the antigen. So, genetic diversity should be addressed as a great challenge in designing MSP-based vaccines and before any investment in this field the extent of genetic diversity of candidate antigens must be evaluated[1]. Because of the importance of MSP-3α in future in order to vaccine production against *P. vivax,* and to reveal the extent of polymorphism of the gene in the gene this study was design and implemented.

MATERIALS AND METHODS

37 blood samples (20 Iranian and 17 non-Iranian) collected from patients attending to malaria clinics in the Sistan and Baluchistan province. Approximately 1000 ¼l of venous blood was collected in tubes containing EDTA. DNA was extracted by Q1Aamp® DNA blood mini kit (50) (Germany) according to its guideline. Sample collection was performed after obtaining informed consent from each subject and approved by the ethical committee of Tehran university of medical sciences. All samples were rechecked by PCR using *P. vivax* and *P. falciparum* species specific primers (15). The DNA was amplified by nested PCR using primers bind at positions 111-131 and 2286- 2305 (in primary PCR) and positions 205-227 and 2078-2100 (nested) of the Belem laboratory strain (15): P1-5/ CAGCAGACACCATTTAAGG3; P2-5/ CCGTTTGTTGATTAGT, TGC3/; N1-5/ GACCAGTGTGATACCATTAAC C3/; N2-5/ ATACTGGTTCTTCGTCTTCAGG 3/. PCR was performed, based on previously described protocol[15], with an initial denaturation of 3 min at 94 C°, 35 cycles of 30 sec at 94 C°, 56 C° for 30 sec and 68 C° for 2.5 min. Nested PCR was performed with 30 cycles of 94 C° for 30 sec, 30 sec at 57C°, 68C° for 2.5 min. *HhaI* enzyme was used for digesting of PCR products in RFLP procedure. Approximately 5 µl of the PCR product was digested with HhaI and analyzed by electrophoresis on 1.8% agarose gel[15]. Products were visualized under UV illumination by electrophoresis on 1.5% agarose gels containing 0.25 µg/ml of ethidium bromide. Major alleles were classified based on the patterns of bands.

RESULTS

We found three biotypes of the parasite, based on the size of PCR products, including A

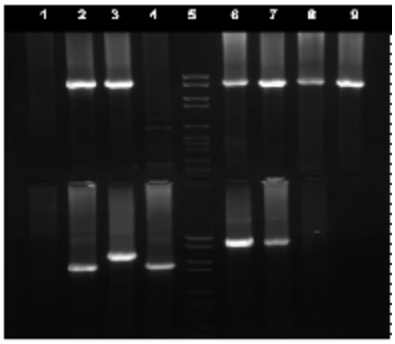

Fig. 1. Major 3 biotypes of *PvMSP-3α* of *Plasmodium vivax* isolate from Sistan and Bluchestan province using polymerase chain reaction of the msp-3α gene. Lanes 2,3,6,7,8 and 9 upper and 6 and 7 lower are biotype A, lane 3 lower is biotype B and lanes 2 and 4 lower are correspond to biotype C. Lane 5 (upper and lower rows) contains DNA marker 6 (Roche)

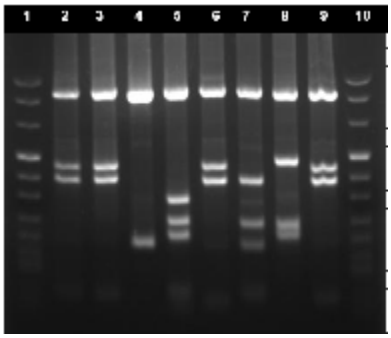

Fig. 2. Some allelic patterns of *PvMSP-3α* of *Plasmodium vivax* isolate from Sistan and Bluchestan province using RFLP/PCR with *Hha1* enzyme. Lanes 1 and 10 contains DNA marker 6 (Roche)

(about 1900bp), B (about 1400bp) and C (about 1100bp) (Fig.1). Biotype A of PvMSP-3α was more frequent in both Iranian (65%) and non-Iranian (82%) patients (73% in total). Type B and C accounted for 2.7% and 24.3% of the parasite genotype, respectively (Table 1). No significant difference was observed between the two groups of patient in terms of biotype of PvMSP-3α. After digesting of PCR products with HhaI enzyme, we observed 10 allelic groups of PvMSP-3α (Fig. 2), that, 75.7% , 10.8 % and 13.5% of isolates correspond to biotypes A, B and C, respectively (Table 2). We did not observe significant difference between the two groups of patient in terms of RFLP patterns of PvMSP-3α.

Table 1. Frequency of biotypes of PvMSP-3± in *P. vivax* isolates from Sistan and Bluchestan, Iran

Nationality	Biotype A		Biotype B		Biotype C	
	Number	%	Number	%	Number	%
Iranian	13	65	1	5	6	30
Non Iranian	14	82	0	0	3	18
Total	27	73	1	2.7	9	24.3

Table 2. Frequency of alleles of *PvMSP-3*± after *digestion with HhaI* in *P. vivax* isolates from Sistan and Bluchestan, Iran

Pattern in RFLP	Nationality					
	Iranian		Non Iranian		Total	
	Number	%	Number	%	Number	%
A1	2	10	1	5.9	3	8.1
A2	3	15	5	29.4	8	21.6
A3	4	20	1	5.9	5	13.5
A4	1	5	0	0	1	2.7
A5	1	5	1	5.9	2	5.4
A6	3	15	4	23.5	7	18.9
A7	0	0	1	5.9	1	2.7
B1	2	10	1	5.9	3	8.1
B2	1	5	1	5.9	2	5.4
C	3	15	2	11.8	5	13.5

DISCUSSION

As regards several studies have been conducted on protein-coding genes candidate for malaria vaccine, not much research has been done in this area in Iran. At the present study, we have investigated the genetic variation of the PvMSP-3α gene of *P. vivax* that has recently been considered by malaria researchers. Recent studies addressing genetic polymorphism of *P. vivax* merozoite antigens have indicated significant levels of genetic diversities in wild population of the parasite[1].We observed that *P. vivax* isolates of Sistan and Bluchestan were extremely diverse, and the results are almost compatible with the results of other studies performed previously[4, 10]. Even, reported diversity of PvMSP-3α gene from some countries is less than our observation in this study[8, 16]. Based on the results of our study RFLP method using HhaI (small fragments from 50-500bp) is suitable technique for describing the biotype A of PvMSP-3α gene. Totally, we could detect 10 distinct alleles of the gene in non Iranian and Iranian patients, but there was not one of the alleles in each group, so that alleles A4 and A7 were not found in non Iranian and Iranian patients, respectively. But we could not found any significant differences in distribution of alleles in studied population (Table 2).As it has been described before[10,12,16], where the sequencing is not applicable, PvMSP-3α can be considered as a useful polymorphic locus for *P. vivax* population

study and cross-species interactions between malaria parasites in humans in field setting. In order to providing vaccines and drugs with high efficacy and effectiveness, the structure and function of molecular markers, such as PvMSP-3 gene family that is known to be a potential candidate for vaccine development and as an epidemiological indicator[13], should be studied carefully and completely. In conclusion, we hope the results of this preliminary study will serve as the foundation for more detailed studies in future on population structure, function and dynamics of *P. vivax* and other species of malaria parasite.

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REFERENCES

1. Mascorro, C.N., Zhao, K., Khuntirat, B., Sattabongkot, J., Yan, G., Escalante, A.A., Cui, L. Molecular evaluation and intragenic recombination of the merozoite surface protein MSP-3± from the malarian parasite *Plasmodium vivax* in Thailand. *Parasitol*., 2005; **131**:25-35.
2. Gomez, J.C., Namara, M.C., Bockarie, D.T., Baird, J.M., Carlton, J.K., Zimmerman, J.M. Identification of a polymorphic *Plasmodium vivax* microsatellite marker. *Am. J. Trop. Med. Hyg*., 2003; **69**(4):377-79.
3. Diseases Management Center of MOH, I.R. Iran. Annual Reports of Malaria, 2010.
4. Cui, L., mascorro, C.N., Fan, Q., Rzomp, K.A., Khuntirat, B., Zhou, G., Chen, H., Yan, G., Sattabongkot, J. Genetic diversity and multiple infections of *P. vivax* malaria in western Thailand. *Am. J. Trop. Med. Hyg*., 2003; **68**:613-619.
5. Rosenberg, R., Wirtz, R.A., Lanar, D.E., Sattabongkot, J., Hall, T., Waters, A.P., Prasittisuk, C. Circumsporozoite protein heterogeneity in human malaria parasite *Plasmodium vivax*. *Science*., 1989;**245**:973- 976.
6. Arnot, D.E., Barnwell, J.W., Tam, J.P., Nussenzweig, V., Nussenzweig, R.S., Enea, V. Circumsporozoite protein of *P. vivax*: gene cloning and characterization of the immuno-dominant epitope. *Science*., 1985; **230**: 815-818.
7. Qari, S.H., Collins, W.E., Lobel, H.O., Tylor, F., Lal, A.A., A study of polymorphism in the Circumsporozoite protein of human malaria parasite. *Am. J. Trop. Med. Hyg.*, 1994;**50**:45-5.
8. Kim, J.R., Imwong, M., Nandy, A., Chotivanich, K., Nontprasert, A., Tonomsing, N., Majia, A., Addy, M., Day, N.P, White, N.J., Pukrittayakmee, S. Genetic diversity of *P. vivax* in Kolkata, India. *Malaria J*., 2006; **5**: 71.
9. Bruce, M.C., Galinski, M.R., Barnwell, J.W., Snounou, G., Day, K.P. polymorphism at the merozoite surface protein-3α locus of *plasmodium vivax*: Global and local diversity. *Am. J. Trop. Med. Hyg.*, 1999; **61**: 518-25.
10. Bruce, M.C., Galinski, M.R., Barnwell, J.W., Donnelly, C.A., Walmsley, M., Alpers, M.P., Walliker, D., Day, K.P. Genetic diversity and dynamics of *Plasmodium falciparum* and *Plasmodium vivax* populations in multiply infected children with asymptomatic malaria infections in Papua New Guinea. *Parasitol.*, 2000; **121**:272-5.
11. Galinski, M.R., Ingravallo, P., Corredor-Medina, C., Al-Khedery, B., Povoa, M., Barnwell, J.W. *Plasmodium vivax* merozoite surface proteins - 3β and 3γ share structural similarities with *P. vivax* merozoite surface protein-3α and define a new gene family. *Mol Biochem Parasitol*., 2001; **115**: 41-53.
12. Bruce, M.C., Donnelly, C.A., Alpers, M.P., Galinski, M.R., Barnwell, J.W., walliker, D., Day, K.P. Cross-species interactions between malaria parasites in humans. *Science*., 2000; **287**: 845-848.
13. Rayner, J.C., Corredor, V., Feldman, D., Ingravallo, P., Iderabdullah, F., Galinski, M.R., Barnwell, J.W. Extensive polymorphism in the *Plasmodium vivax* merozoite surface coat protein MSP-3± is limited to specific domains.*Parasitol.*, 2002; **125**: 393-405.
14. Zakeri, S., Barjesteh, H., Djadid, N.D. Merozoite surface protein-3± is a reliable marker for population genetic analysis of *Plasmodium vivax*. *Malaria J*., 2006; **5**:53.
15. Snounou, G., Pinheiro, L., Goncalves, A., Fonseca, L., Dias, F., Brown, K.N., Rosario, V.E. The importance of sensitive detection of malaria parasites in the human and insect hosts in epidemiological studies, as shown by the analysis of field samples from Guinea Bissau. *Trans. Roy. Soc. Trop. Med. Hyg.*, 1993; **87**:649-53.
16. Mueller, I., Kaiok, J., Reeder, J.C., Cortes, A. The population structure of *Plasmodium falciparum* and *Plasmodium vivax* during an epidemic of malaria in the Eastern Highlands of Papua New Guinea. *Am. J. Trop. Med. Hyg.*, 2002; **67**(5):459-64.