Iranian J Parasitol

Open access Journal at http:// ijpa.tums.ac.ir

Tehran University of Medical Sciences Publication http:// tums.ac.ir

Iranian Society of Parasitology http:// isp.tums.ac.ir

Original Article

Allelic Dimorphism of the *Plasmodium falciparum* Erythrocyte Binding Antigen-175 (EBA-175) Gene in the Southeast of Iran

*[*1]A Heidari, [2]H Keshavarz, [3]S Dittrich, [4]T Jelinek*

[1]Department of Medical Sciences, Ministry of Sciences (GPEF), Tehran, Iran
[2]Department of Medical Parasitology, School of Public Health, Tehran University of Medical Sciences, Iran
[3]Manchester Interdisciplinary Biocenter (MIB), The University of Manchester Manchester, UK
[4]Berlin Center for Travel and Tropical Medicine, Berlin, Germany

(Received 1 Oct 2008; accepted 15 Feb 2009)

Abstract

Background: The erythrocyte binding antigen 175 kDa (EBA-175) gene is located on chromosome 7. It encodes protein that binds to specific receptor glycophorin A on the erythrocyte surface during invasion. It has a dimorphic nature (FCR3 and CAMP). This study was designed to determine the distribution of EBA-175 alleles of *Plasmodium falciparum* in the southeast of Iran

Methods: We used the nested PCR method with specific primers, which improves the two fragments of the EBA-175 gene. Sixty eight microscopically positive blood samples were collected from the infected falciparum malaria subjects in the southeast of Iran.

Results: In this study which marks the first one in Iran, CAMP strains (714 bp) and FCR-3 strains (795 bp) were found in 14 (37.8%) and 23 (62.2%) in the originally Iranian subjects and in 10 (32.3%) and 19 (61.3%) Pakistani infected migrants respectively. Two migrant cases (6.4%) had mix CAMP/FCR-3 infection.

Conclusion: The two fragments of dimorphic EBA-175 gene were observed and the FCR-3 allele was more prevalent in Iran. There was no significant correlation between one of the EBA-175 alleles and the subject group in the mentioned region. This distributional pattern should be considered in designing to control *P. falciparum* malaria in the region.

Key words: *Malaria,* Plasmodium falciparum*, Erythrocyte binding antigen-175, Iran*

* **Corresponding Author**: Email: aliehsan2001@yahoo.com

Introduction

The asexual erythrocytic stage of the malaria parasite is responsible for all of the clinical and pathological manifestation of the disease (1). Antigens on the surface of the merozite and those released from apical organelles are potential targets of antibody-mediated immunity. The erythrocyte binding antigen 175 kDa (EBA-175) gene of *Plasmodium falciparum* is located on chromosome 7 (2). It encodes protein that localizes in the microneme organelles at the apical end of the merozites and binds to the specific receptor on the erythrocyte surface (3,4).

Its cysteine-rich region II can bind to sialic acid residues on glycophorin A on red blood cells to mediate invasion and it, therefore, acts as a bridge between the parasite and host red blood cells (5). There is a low amount of deduced amino acid polymorphism in this region among alleles of *P. falciparum* (6).

EBA-175 region III is located in the central part of the gene that there are highly divergent dimorphism segments of sequence as the F-fragment in the FCR3 strain and C-fragment in the CAMP strain (4, 5). The C and F fragments encode 114 and 141 amino acids respectively. *P. falciparum* merozoites, which are haploid, have either C or F but not both or neither (7).

Iran, located in the eastern Mediterranean region, is generally defined as a low to moderate endemic area. Although the rate of malaria transmission considerably has been decreased in Iran in recent years, local malaria transmission has been continued because of some technical and operational problems (8). Now, the southeast of Iran is a main center of the endemic *falciparum* malaria that is bordered by Pakistan and Afghanistan (9). Malaria transmission occurs almost in a year with two peaks, May-June and October-November (10). Both *P. falciparum* and *P. vivax* are found in the country. National Malaria Control Program reported 15,909 malaria cases in the year 2006 of which more than 83% were autochthonous. *P. falciparum* malaria cases are included nearly 11% of total malaria in Iran (11).

This study was designed to determine the distribution of EBA-175 alleles in the southeast of Iran and to analyze some potential protective association of the EBA-175 fragments in the region.

Material and Methods

Peripheral blood samples were collected from the 68 *P. falciparum* infected individuals participating in a descriptive cross sectional survey in Sarbaz district in the southeast of Iran in 2006.

Sarbaz, in (Iranshar) located in the Sistan and Baluchistan Province, (about 1982 Km southeast of the capital Tehran, Iran) was chosen for this study. In this part of the country, malaria is the oriental type; hence, it is more difficult to control than elsewhere in Iran. *P. falciparum* resistance to choloroquine and sulfadoxin-pyrimethamine and vector resistance to insecticides were reported in this area (8, 12, 13). Another tribulation encountered here is the importation of malaria, mostly *P. falciparum*, from Afghanistan and Pakistan.

Thirty one *P. falciparum* Pakistani infected migrants that moved to Iran during the eight days and 37 native subjects were included in this study. According to incubation period of *P. falciparum,* the migrant subjects had been infected to *P falciparum* in their own country. No history of anti-malaria treatment during a month before written informed consent was required for inclusion in this study. *P. falciparum* infected blood samples were collected from the symptomatic and asymptomatic cases attending the health care centers in the area and from subjects who were visited at home as well as the people who moved to the region. This study was approved by the Ethical Review Committee of Research in Tehran University of Medical Sciences, Iran.

Symptomatic patients suffered from fever (>37.5 ^{o}C) as well as chills and headache. Six of the subjects were asymptomatic. Their axillary temperature was below 37.5 ^{o}C on the day 0 and 4 days after sampling. Parasites were counted in the thick smears stained by the Giemsa method. DNA was isolated from

the blood samples by the QIAamP DNA mini kit (Quigen kit, Germany).
The sequence of primers consisted of EBA1 5-CAAGAAGCAGTTCCTGAGGAA-3 and EBA2 5- TCTCAACATTCATAT-TAACAATTC-3 for the outer PCR and EBA3 5-GAG-GAAAACACTGAAATAGCACAC-3 and EBA4 5-CAATTCCTCCA-GACTGTTGAACAT-3 for the nested PCR. The nested PCR method was applied as previously described (14). The product of PCR was electrophoresed on 1.5% agarose gel and stained with ethidium bromide. Ultraviolet light was used to visualize the stained DNA.
Chi-square test was applied to check for an association between fragment types and subject groups in the region.

Results

This study concluded 68 *P. falciparum* infected subjects aged between 2 and 45 year. Parasitemia on the day zero in subjects ranged from 50 to 30000 asexual parasites/mm^3. The median values of the parasitemia on day zero in the native group were 3200 and 3500 in subjects harbor FCR3 and CAMP fragments respectively. These amounts in the migrant group were 4100 and 4550 in subjects harbor FCR3 and CAMP fragments respectively. There was no significant correlation between parasitemia, gender and age of subjects with the two fragments of EBA-175 gene.
Both CAMP (714bp) and FCR-3 (795bp) strains of *P. falciparum* were observed in the two groups of the study (Fig. 1). There was no significant correlation between one of the two EBA-175 alleles and the subject group.

FCR-3 and CAMP strains were observed in 42 (61.8%) and 24 (35.3%) of our study subjects and FCR-3 allele was predominant in the endemic region of Iran for *P. falciparum*. Mixed infection was found in two samples (2.9%), both of which belonged to the migrant group. In mixed infection, at least, two single isolates were found in one subject. Therefore, 70 isolates were obtained from 68 samples (Table 1).

Table 1. Distribution of erythrocyte binding antigen -175(EBA-175) gene fragments of *Plasmodium falciparum* according the two groups in the southeast of Iran

Blood Samples	Fragments N (%)		
	FCR-3	**CAMP**	**Both** (FCR-3/CAMP)
Native Subjects (n=37)	23 (62.2)	14 (37.8)	0 (0)
Migrant Subjects (n=31)	19 (61.3)	10 (32.3)	2 (6.4)
Total	42	24	2

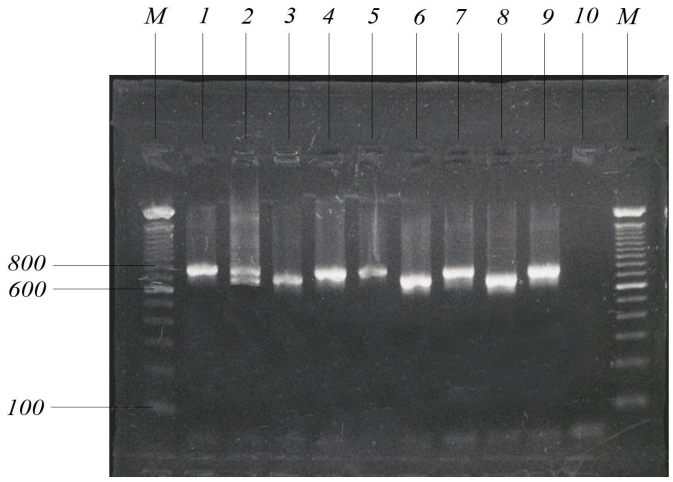

Fig. 1: Gel photograph showing PCR amplified products of EBA-175 from different *Plasmodium falciparum* infected isolates in the southeast of Iran. The DNA size marker is a 100 bp ladder shown on the left and right side. Lane2 and 10 are multiple infection and negative control respectively

Discussion

This study is the first to determine the EBA-175 allelic dimorphism in Iran. The CAMP and FCR-3 were both reported in our study, but the FCR-3 genotype was more prevalent (61.8%) which is similar to results obtained in the Lao PDR in Asia (7) and Gabon in Africa (15) but different results reported in Sudan (4).

Since there is a different population of *P. falciparum* in various parts of the world, the distribution of alleles of the EBA-175 gene might be different according to the geographical region (13,14). The genetic and immunologic differences in the host population for allele's selection could be considered as one explanation for this finding.

Our study shows that the prevalence of co-infection is lower in the Iranian infected subjects than that of the Lao PDR and Gabon (7, 15). However, the presence of either the F or C segment alone does not exclude the possibility of multiple infections, because in the previous study we showed 34% multiple infections in this area through MSP-1 and MSP-2 genotyping (16).

In this study, there was no significant correlation between two groups and fragment types. This finding is not consistent with the study performed in Lao PDR in which the distribution of EBA-175 alleles was different between the north and south provinces (7). The geographical proximity of the two groups of the study, which results in their contact, seems to be main factor in the similar allele distribution. While the median value of parasitemia was higher in subjects harbor CAMP fragment, there was no significant correlation between F and C fragments and parasitemia on day zero. Since only a single peripheral blood sample was obtained in this study, the total parasitic load was not determined and it needed a further detailed study. According to the study that conducted in Senegal, the parasite density had dramatic fluctuations in a few hours in most examined children (17).

All of the six asymptomatic subjects belonged to the migrant group. Although parasitemia ranged from 50 to 2000 asexual para-

sites/mm^3 in the subjects 4 days after sampling, they had no symptoms. The previous study conducted in the south of Iran has shown that Afghan immigrants harbor *P. falciparum* asymptomatic infection (18). It seems that most of the asymptomatic subjects have already been exposed to the current strain of malaria infection and acquired immunity. Some of them might become ill when exposed to the parasite, which represents a new antigenic variant (19). These asymptomatic infections have been suggested to represent a significant source of parasites for local mosquitoes and maintaining residual malaria transmission (20). There was no significant correlation between clinical outcomes and the EBA-175 fragments types in this study. More studies on larger populations are needed to understand the distributional pattern of EBA-175 alleles according to clinical outcomes of falciparum malaria in Iran.

We concluded that there were both FCR3 and CAMP fragments of EBA-175 gene of *P. falciparum* in the southeast of Iran of which the former is predominant. The significant correlation was not observed between EBA-175 fragments and two groups of the study. The molecular genotyping of the EBA-175 antigen gene showed the low co-infection in this area. According to our data, distributional pattern of the EBA-175 alleles is almost similar in Iranian native and Pakistani infected migrants.

Acknowledgements

We would like to thank those individuals from the malaria endemic region of Iran, who kindly contributed to this study and are grateful to Mr. Satvat, Mr. Seidzadeh and Mr. Akbarzadeh for their help in sampling. This study was supported financially by the Tehran University of Medical Sciences and the Institute of Tropical Medicine Berlin, Germany.

References

1. Tolia NH, Enemark EJ, Sim BK, Joshua-Tor l. Structural basis for the EBA-175 erythrocyte invasion pathway of the malaria parasite *Plasmodium falciparum*. Cell. 2005;122 (2):183-193.
2. Verra F, Chokejindachai W, Weedall GD, Polley SD, Mwangi TW, Marsh K, Conwy DJ. Contrasting signatures of selection on the *Plasmodium falciparum* erythrocyte binding antigen gene family. Mol Biochem Parasitol. 2006;149 (2):182-190.
3. Cramer JP, Mockenhaupt FP, Mohl I, Dittrich S, Dietz E, Otchwemah RN, Ehrhardt S, Bienzle U, Jelinek T. Allelic dimorphism of the erythrocyte binding antigen- 175 (eba-175) gene of *plasmodium falciparum* and sever malaria: significant association of the C-segment with fatal outcome in Ghanaian children. Malar J. 2004; 3:11.
4. Binks RH, Baum J, Oduola AM, Arnot DE, Babiker HA, Kremsner PG, Roper C, Greenwood BM, Conway DJ. Population genetic analysis of the *Plasmodium falciparum* erythrocyte binding antigene-175 (eba-175) gene. Mol Biochem Parasitol. 2001; 114 (1):63-70.
5. Mamillapalli A , Pattnaik P, Sharma M, Sharma SK, Tvaqi PK, Joshi H, Chitnis CE. Sequence polymorphisms in the receptor-binding domain of *Plasmodium falciparum* EBA-175: Implications for Malaria Vaccine Development. Mol Biochem Parasitol. 2006; 146 (1): 120-123.
6. Lianq H, Sim BK. conservation of structure and function of the erythrocyte-binding domain of *Plasmodium falciparum*,EBA-175. Mol Biochem Parasitol. 1997; 84 (2):241-5.
7. Dittrich S, Schwobel B, Jordan S, Jelinek T. Distribution the two forms of *Plasmodium falciparum* erythrocyte binding antigen-175 (eba-175) gene in lao PDR. Malar J. 2003; 2:23.

8. Edrissian GhH. Malaria in Iran:Past and present situation. Iranian J parasitol. 2006;1(1):1-14.
9. Rakhshani F,Ansari moghadam AR, Alemi R,Moradi A. Knowledge, perceptions and prevention of malaria among women in Sistan va Baluchestan, Islamic Republic of Iran. East Mediterr Health J. 2003; 9 (3):248-256.
10. Zakeri S, Afsharpad m, Raeisi A, Djadid ND. Prevalence of mutation associated with antimalarial drugs in *Plasmodium falciparum* isolates prior to the introduction of sulphadoxine-pyrimethamine as first –line treatment in Iran. Malar J. 2007;6 (148):1-8.
11. The work of WHO in the Eastern Medterranean region. Annual report of regional director-WHO Regional Office for the Eastern Mediteranian. 2006; Available from: http:www.emro.WHO.int.
12. Heidari A, Dittrich S, Jelinek T, Kheirandish A, Banihashemi K, Keshavarz H. Genotypes and Invivo Resistance of *Plasmodium falciparum* Isolates in an Endemic Region of Iran. Parasitol Res. 2007;100: 589-592.
13. Esmailli AR, Nateghpour M, Asmar M, Razavi MR,Kanbara H, Uemura H, Nadaf SR, Keshavarz H, Raeisi A, Mohebali M. Detection of K76 T mutation pfcrt gene as an applicable genetic marker for prediction of choloroquine resistant *falciparum* malaria in isolates from an endemic district of Iran. Iranian J Parasitol. 2008; 3(2):48-56.
14. Toure FS, Mavoungou E, Ndong JM, Tshipamba P,Deleron P. Erythrocyte binding antigen (EBA-175) of *Plasmodium falciparum* :improved genotype determination by nested polymerase chain reaction. Trop Med Int Health. 2001; 6:767-769.
15. Toure FS, Bisseye C, Mavoungou E. Imbalanced distribution of *Plasmodium falciparum* EBA-175 genotypes related to clinical status in children from Bakoumba, Gabon. Clin Med Res. 2006; 4(1): 7-11.
16. 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.
17. Jafari-Guemouri S, Boudin C, Fievet N, Ndiave P, Deloron P. *Plasmodium falciparum* genotype population dynamics in asymptomatic children from Senegal. Microbes Infect. 2006; 8 (7):1663-1670.
18. Podat A, Ladoni H, Reisi A. Probability factors in malaria situation in Bandarabass in Iran. J Hormozgan Medicine. 2007; 10 (2):101-110.
19. Nunes Mds, Ferreira Mu. Clinical spectrum of uncomplicated malaria in semi-immune Amazoinians: beyond the symptomatic vs asymptomatic dichotomy. Mem Inst Oswaldo Cruz. 2007; 102(3):341-347.
20. Coutra JR, Suarez-Mutis M, Ladeia-Andrade S. A new challenge for malaria control in Brazil: asymptomatic *Plasmodium* infection- A review. Mem Inst Oswaldo Cruz. 2006; 101(3): 229-237.