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Duffy blood group genotypes among malaria *Plasmodium vivax* patients of Baoulch population in southeastern Iran

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ABSTRACT

Objective: To determine the distribution of Duffy blood group genotypes in Balouch population as a major ethnic group that living in a sub–tropical area in south East of Iran. **Methods:** In this study, the Duffy blood group FY phenotypes were determined using indirect anti–globulin technique and also genotype by PCR–RFLP in 160 vivax malaria patients and 160 control individuals. **Results:** The results showed that the most common Duffy genotype was FYA/FYB (46.6%) followed by FYA/FYA (15.3%), FYA/FYO (14.4%), FYB/FYO (11.9%), FYB/FYB (10%) and FYO/FYO (1.9%). In case individuals, frequency of FYA, FYB and FYO alleles were 0.471, 0.431 and 0.097, respectively compared to 0.444, 0.353 and 0.203, respectively in control (non–infected) group. **Conclusions:** This data provide evidence that individuals with the FYA/FYB genotype have higher susceptibility to malaria and there are significant associations between Duffy blood group variants and susceptibility or resistance to vivax malaria.

1. Introduction

Malaria is one of the most serious diseases to affect people in developing countries with tropical and subtropical climates. *Plasmodium vivax* (*P. vivax*) is the most widespread of the four human malaria species[1]. Though *P. vivax* is rarely lethal it can potentially lead to severe complication and have major deleterious effects on personal well–being, growth and development in endemic countries[1,2]. In Iran, about 65% of malaria transmission occurs essentially in the Sistan and Balouchistan Province located in south–East of the country[3]. Among malaria

species, *P. vivax* has been responsible for 80% to 90% of malaria cases in Iran. This disease is endemic in Sistan & Balouchistan province in southeastern Iran, shares the boarder with Afghanistan and Pakistan[4].

Innate resistance to malaria infections in humans is conferred by various blood group polymorphisms such as hemoglobinopathies (including thalassemias, HbS, HbC and HbE), erythrocyte polymorphisms and immunogenetic variants[5–7]. The Duffy protein has been identified as a scavenger on the red blood cell (RBC) surface eliminating excesses of circulating toxic chemokines, named Duffy Antigen Receptor for Chemokines (DARC)[8]. The Duffy blood group is locus located on the long arm of chromosome 1q21–22[9] and characterized by three main alleles: FYA, FYB and FYO. The FYA and FYB are co–dominant alleles which differ by a missense mutation in the major cDNA transcript (G125A>Gly42Asp)[10]. The corresponding anti–Fya and anti–Fyb antibodies define four different

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phenotypes; FY (a+b+), FY (a+b-), FY (a-b+) and FY (a-b-). The first three phenotype are common in European and Asian populations and the last phenotype is the predominant among Black population in many African ethnic groups and their descendants causes no obvious ill effect, and confers natural resistance against *P. vivax* infection[9-12]. In Fy(a-b-) phenotype, there is a-33 T→C point mutation on the FYO gene promoter, which abolishes the erythroid gene expression by disruption the GATA transcription factor binding site and results in which silences the gene encoding the Duffy system antigens in the RBCs[13; 14]. Distribution of Duffy blood groups polymorphisms are important in areas where *P. vivax* predominates, because this molecule on the surface of RBCs acts as a receptor for the human malaria *P. vivax*[15]. Therefore, genotyping methods are required to assess the potential impact of these quantitative differences in erythrocyte FY antigen expression on *P. vivax* susceptibility. Little is known on the frequency of erythroid polymorphisms that confer either partial or complete resistance against malaria in Balouch population that is the large ethnic group population is in the south of Sistan & Balouchistan province. Here we compared the distribution of Duffy blood group genotypes in Balouch population infected with *P. vivax* and those non-infected individuals as a control group.

2. Material and methods

2.1. General material

This case-control study took place in Iranshahr (27°, 12' 09" N; 60°, 41' 05" E) and Chabahar (25°, 17'31" N; 60°, 38'35" E) cities in south-east of Iran from May 2009 to August 2012. Balouch ethnic group make up the largest population of those cities[6] and all selected case and control individuals were Balouch from independent families born in this area. In patients (n=160), the *P. vivax* malaria was confirmed through thick blood film microscopically and they received anti-malarial treatment. The control group (n=160) was matched to the patients in respect to age, gender, ethnicity, place of birth. All control individuals had no malaria sign and symptoms at the initial interview and they

had never reported suffering from a malaria attack and also had negative results for thick blood film.

2.2. Methods

2.2.1. Peripheral blood samples collection

The protocol for this study was approved by ethical committee of Zahedan University of Medical Sciences and blood samples were collected in EDTA-contained tubes from all participants, after informed consent.

2.2.2. Phenotype determination

The FY phenotypes were determined within 24 hours of blood collection using indirect anti-globulin technique. Commercial Duffy antisera anti-Fya and Fyb (CE-Immunodiagnostika GmbH, Eschelbronn Germany) and anti-human globin were used according to manufacture's instruction. A positive and a negative control were performed in a parallel experiment, which obtained from the Iranian Blood Transfusion Organization.

2.2.3. Genotype determination

Genomic DNA was extracted from whole blood cells through the standard phenol-chloroform procedure. Genotypes of Duffy blood group were determined by using PCR-RFLP that was modified as described before. Each PCR contained 100 ng genomic DNA, 10 pmol each primer (Primers sequence, location and amplicon size are showed in Table 1), 2 nmol each dNTP, 1.0 U *Taq* DNA polymerase, and buffer, in a total reaction volume of 25 μL. The amplification of promoter region was done by using the FYN1 and FYN2 primers that flank the GATA box motif[16]. Mixture were incubated for 5 min at 95 °C, followed by 35 cycles, each lasting 60 s, with the following temperature: denaturation at 94 °C, annealing at 65 °C and extension at 72 °C and a final elongations at 72 °C for 5 min. Duffy RBC polymorphism was determined by FYAB1 sense and FYAB2 reverse sense primers were used that flank a segment of 392 bp in exon 2 that included the mutation 125 G>A[16]. The amplification profile was 94 °C for 5 min followed by 35 cycles, each of lasting 60 s, with the following temperatures: denaturation at 94 °C, annealing at 65 °C, and extension at 72 °C. The PCR products were run on 2% agarose gel to verify the fidelity

Table 1

Sequence of primers and PCR product allele primers sequence size.

Primer/direction	Sequences	Location	Size amplicon
FYAB /sense	5'- TCC CCC TCA ACT GAG AAC TC -3'	+63 to +82 bp	392 bp
FYAB/reverse	5'- AAG GCT GAG CCA TAC CAG AC -3'	+435 to +455 bp	
FYN1/ sense	5'- CAA GGC CAG TGA CCC CCA TA -3'	-153 to -133 bp	189 bp
FYN/ reverse	5'- CAT GGC ACC GTT TGG TTC TC -3'	+17 to 36 bp	

of PCR before restriction enzymes treatment. To detect the 125 G>A SNP, the amplification products were digested with endonucleases Ban1^[17]. The amplification of promoter region were digested by Sty1^[17] to determine the GATA box SNP at position –33 for 16 h at 37 °C and then separated on 12% acryl amid gel.

2.3. Statistical methods

Data were analyzed by the statistical package for social sciences (SPSS version 11). *Chi*-square test was used to compare the proportions of Duffy genotypes in control and cases groups and $P < 0.05$ was considered statistically significant.

3. Results

This study was performed on 320 unrelated subjects among Balouch population live in south-east of Iran. Of all, 160 individuals were infected with *P. vivax* (case group) and 160 subjects were uninfected with *P. vivax* (control group). There was no significant difference in the mean age of case (29.9±16.5) years and control (29.3±14.9) years groups.

3.1. Phenotype distributions

The serological assays indicated that the most common phenotypes in case and control groups were Fy (a+b+) with 51.9% and 41.3% respectively (P value = 0.057) (Table 2).

3.2. Genotype distributions

The most common Duffy genotype was FYA/FYB (46.9%) followed by FYA/FYA (15.3%), FYA/FYO (14.4%), FYB/FYO

(11.9%), FYB/FYB (10%) and FYO/FYO (1.9%) (Table 3).

3.3. Allele frequency

The frequencies of FYA, FYB and FYO alleles were 0.471, 0.431, and 0.097 respectively in the case compare to 0.444, 0.353 and 0.203 respectively in control group (Table 3).

4. Discussion

Malaria is one of the most important parasitic infections in the worldwide. The World Health Organization estimated that there were 219 million cases of malaria and 660 thousands deaths in 2010, which mainly happened in Africa, Asia and South America^[18]. *P. vivax*, is one of the public health challenges in Sistan & Balouchistan province (Iran) located in a sub-tropical areas sharing borders with Pakistan and Afghanistan. Prevalence of *Plasmodium* species in different regions can be related to genetic factors, including their Duffy blood group^[5–7]. The evolution of Duffy blood group polymorphisms has been used as a marker for ethnic composition. This study showed that there is a significant association between Duffy blood group variants and susceptibility or resistance to vivax malaria.

The current study results showed that the most common erythrocyte phenotype was Fy(a+b+), followed by Fy(a+b–), Fy(a–b+) and Fy(a–b–). Frequency of Duffy Fy (a+b+) genotype was 52% in patients compared to 41% in healthy participant (11% more). In two similar study Cavasini *et al* in Brazilian and Mohanty *et al* in India recorded an almost 8% and 23% higher prevalence of this phenotype in malaria patients compared to the healthy participants, respectively^[15,19]. Therefore, it seems that expression of the FYA and FYB genes in conditional heterozygote facilitate

Table 2

Comparison of Duffy phenotype using serological method in case and control groups in Balouch population in Southeast of Iran.

Erythrocyte phenotype	Number observe Case/control	Frequency, % Case/control	Over all (%)	<i>P</i> value
Fy(a+b–)	42/53	26.3/33.1	29.7	0.178
Fy(a–b+)	33/37	20.6/23.1	21.9	0.589
Fy(a+b+)	83/66	51.9/41.3	46.6	0.057
Fy(a–b–)	2/4	1.3/2.5	1.9	0.685

Table 3

Genotype and allele frequency of Duffy blood group in Balouch population in South-East of Iran.

Population	<i>n</i>	Genotype frequency						Allele frequency		
		FY*A/FY*A	FY*A/FY*O	FY*B/FY*B	FY*B/FY*O	FY*A/FY*B	FY*O/FY*O	FY*A	FY*B	FY*O
Case	160	26(16.3%)	16(10%)	22(13.8%)	11(6.9%)	83(51.9%)	2(1.3%)	0.471	0.431	0.097
Control	160	23(14.4%)	30(18.8%)	10(6.3%)	27(16.9%)	66(41.3%)	4(2.5%)	0.444	0.353	0.203
<i>P</i> value		0.641	0.026	0.025	0.006	0.057	0.685\$	0.563	0.170	0.012
Overall	320	49(15.3%)	46(14.4%)	32(10%)	38(11.9%)	149(46.6%)	6(1.9%)	0.458	0.392	0.150

\$: Fisher's Exact Test.

infection by *P. vivax* indicating that Fy(a+b+) individuals may be more prone to infection by this species of Plasmodium^[20]. Furthermore, lower quantity of DARC was detected in individuals with FYA and FYB alleles than heterozygote individuals. This may be led to more receptors available in later individuals for possible variations in the parasite protein which binds to the human erythrocytes^[20,21]. Therefore, susceptibility to infection may be not only dependent on the different levels of the expression, but also the specific conformation of the Fya and Fyb antigens.

The results indicated that homozygous FYA allele was more prevalent in the control group whereas heterozygous FYA allele was more prevalent in the case group and FYB allele results were almost similar. However, FYO linked with FYA and FYB alleles decreased the chance of infection by vivax malaria. Therefore, protection against this species of plasmodium may be supported by a combination of FYO/FYO genotypic with either FY (a+b-) or FY (b+b-). This hypothesis has been supported by In vitro studies^[7] so that significant reduction in cytoadherence of the parasite was reported in RBCs expressing those phenotypes than Fy(b+b+). Infection by *P.vivax* was reported to be significantly lower in Duffy negative heterozygotic individuals for the FYAnull allele^[22]. Albuquerque *et al* in study on 497 patients in the Amazonas State, Brazil, showed that FYA/FYB and FYA/FYA genotypes had higher levels of parasitism of *P. vivax* infection than FYB/FYO and FYA/FYO^[23]. A 50% reduction in the Duffy protein expression on the erythrocyte was also reported when FYO allele were present^[7,24]. Therefore, effect of the gene is dose-related, which may limit the invasion process of red blood cells by the parasite^[7,21].

In the current study, homozygous FYO allele was more frequent (2.1 times) among control group than case group ($P<0.05$). These findings may be a related to occurring mutation in a population as this phenotype favors resistance to the infection in areas where *P. vivax* is endemic, which is in accordance with some other studies^[7,13,25–28]. Although the absence of Fy antigen has not been reported to be exert any deleterious effect in many ethnic Negro groups, its presence causes resistance against infection by *P. vivax*^[12,26,29,30]. Thus, where *P. vivax* is predominates, the frequency of the Fyo allele is higher than expected level in the population, both in heterozygotes and homozygotes forms. This might lead to an increasing in the number of Duffy-negative individuals in the population as a result of natural advantages selection.

Transmission in homozygous for FYO allele has recently become evident in Africa, Brazil and Malagasy^[31–34] suggesting that this parasite is able to use alternative

receptors, apart from Duffy, to invasive erythrocytes, which may have an enormous impact in *P. vivax* current distribution.

In sum, our results support the hypothesis that individuals with the FYA/FYB genotype are more prone to malaria also increasing in number of FYO allele homozygote or heterozygote forms could be a result of an advantageous selection where *P. vivax* is endemic.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

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