

## Determination of FY\*BES allele in Iranian patients with sickle cell disease for enhanced matching blood transfusion

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### ABSTRACT

**Background:** Alloimmunization of red blood cells is a major challenge in sickle cell patient's dependent to transfusion. Accordingly, supplying of the appropriate antigen-negative blood units is a significant problem in blood transfusion for sickle cell patients. Duffy (FY) blood group genotyping is important in transfusion medicine because Duffy antibodies are involved in hemolytic transfusion reactions and hemolytic disease of the fetus and newborn. The aim of the present study was to evaluate the role of FY DNA typing as a tool in transfusion compatibility testing and enhanced matching RBC transfusion.

**Methods:** In this study, 135 blood samples from SCD patients from Southwest of Iran, were included. All samples were tested with anti-Fya and anti-Fyb using the hemagglutination technique, and 64 samples with the fy(a+b-) and fy(a-b-) phenotype were genotyped using DNA sequencing methods.

**Results:** The prevalence of alloimmunization in this population was 13.04%. Fy(a-b+) was the most common phenotype (37/135, 27.4%), followed by Fy(a+b-) (35/135, 26%), Fy(a+b+) (34/135, 25.2%); and Fy(a-b-) (29/135, 21.4%). Among the 64 Fy(a+b-) and Fy(a-b-) samples, 40 (62.5%) patients had FY\*BES allele. 21 out of 40 samples were FY\*BES/FY\*BES, 17 FY\*A/FY\*BES and 2 were FY\*B/FY\*BES.

**Conclusions:** The prevalence of GATA-1 mutation (FY\*BES allele), in fy (a-b-) and fy (a+b-) patients was reported 62.5%. Therefore, consistent with international protocols, these patients can be transfused by fyb+ RBC without any risk of alloimmunization.

## Introduction:

SCD is the second most common hemoglobinopathy after thalassemia in Iran. HbS polymerization is a pathophysiological event in sickle cell anemia, changes in the shape and physical properties of red blood cells, resulting leading to microvascular vasoocclusion, hemolytic anemia and acute pain crises. A key part of the management in SCD patients is RBC transfusion. Transfusion therapy decrease the ratio of circulating hemoglobin-S containing RBCs, vasoocclusive, and multiorgan failure syndromes [1-5] The most important challenge of transfusion is the alloimmunization to RBCs antigen [6] Alloimmunization leads to reducing the available pool of compatible blood for transfusion in subsequent crises. The incidence of alloimmunization to RBCs antigen reach to 13.6 percent among Iranian SCD patients who have received previous transfusions [7] This high rate is mainly caused by differences in the frequencies of RBCs antigen between blood donors and SCD patients. Alloimmunization is the source of the main problem to identification of appropriate antigen-negative RBCs for transfusion [8, 9]

Anti-Rh (D, Cc, and Ee) and Kell are the most common antibodies that has been reported in chronically transfused patients with SCD in Southwest of Iran (Khuzestan province). This has led to recommendations for patients with SCD to receive RBC transfusions matched for D, Cc, Ee, and K antigens. The antibody against Duffy blood group is also one of the antibodies reported in Khuzestan province patients up to 40% [7] Antibodies to Duffy antigens are clinically significant in transfusion medicine and they are involved in hemolytic transfusion reactions and hemolytic disease of the newborn [10]

Studies conducted in Khuzestan province have shown that Duffy negative Fy(a-b-) phenotypes in sickle cell disease patient are more than Duffy negative phenotypes in donors [11] Since one of the mechanisms of creating negative phenotypes of Fyb antigen is the presence of

FY\*BES allele or GATA-1(T33>C), not the absence of the Fyb gene, Fyb antigen is expressed in non-erythroid tissues. Therefore, this category of patients does not produce antibodies if they receive positive Fyb RBC [12] Therefore, FY genotyping and determining FY\*BES allele or GATA-1(T33>C) helps to better supply for compatible blood in SCD patients [10]

The silent allele FY\*BES occurs frequently in black Africans, particularly in malaria areas, but the prevalence of this allele in the south of Iran has not been studied so far. The aim of the present study was to investigate the prevalence of silent allele FY\*BES in SCD patients in Southwest of Iran (Khuzestan province), to be able to perform better compatibility tests and phenotype matched blood supply for transfusion.

## Materials and Methods:

**Ethic Statement:** This study was approved by the local medical ethics committee of High Institute for Research and Education in Transfusion Medicine. All participants read and signed written informed consent before enrollment. Demographic data of patients, transfusion and clinical history were collected by questionnaire that filled out by the nurse.

**Patient:** A total of 135 samples were collected from SCD and S $\beta$  patients admitted to Baqaei2 Hospital in Ahvaz (Khuzestan province) from 2021 to 2022. Ten ml of peripheral blood samples were collected in 2 separate tubes containing K2EDTA anticoagulant for serologic and molecular test.

**Serological Tests:** All samples were referred to immunohematology reference laboratories (IRLs), Tehran. The ABO and Rh typing, Direct anti-glubolin test (DAT), Antibody screening and identification and RBC phenotyping (Cc Ee, Kell, Kidd, and Duffy) was performed for all samples.

- **ABO and Rh typing:** ABO typing were performed by the conventional tube method using commercial monoclonal anti-A, anti-B, (Immundiagnostika, Germany), serum grouping was performed using in-house pooled A cells and B cells. The RhD antigen status was tested by two monoclonal anti-D: (IgM, clone RUM10, Immundiagnostika, Germany) and IgM/IgG blend anti-D (clone TH-28/MS-26, Immundiagnostika, Germany).
- **Antibody screening and identification:** Antibody screening test was carried out using three-cell panel (made at the IBTO) in 3 phases: IS, 37°C, AHG. The samples giving a positive reaction were further tested using 11 and 16 cell identification red cell panel (made at the IBTO), based on the IBTO standard procedure.
- **Minor blood group phenotyping:** The Rh (C, c, E, e), Kell, Jka, Jkb, and Fya, Fyb blood group phenotypes were determined using commercial blood group typing reagents (Immundiagnostika) according to the manufacturer's instructions for tube method. Previously determined phenotypic samples were also included with the test as positive and negative controls to ensure the expected reactivity of antisera to be used in the testing.
- **Direct anti globulin Test (DAT):** Since the Duffy blood group phenotype was determined in the AHG phase, direct antiglobulin test (DAT) test is performed to detect false positive results. DAT was done using poly-specific anti-human globulin (CE-Immunodiagnostika & Biotechnologie GmbH, Berlin, Germany) on all samples. That's why, DAT positive samples was treated with chloroquine solution (Gamma- Quin IMMUCOR)

by the method included in the kit and Duffy blood group phenotyping was performed again after IgG elution.

**Analysis of FY\*A, FY\*B and FY\*BES alleles by DNA sequencing:** DNA sequencing of exons 2 and promoter region was done using designed primers of Natukunda B et al. in 2012 (13) PCR amplification was performed with 60 ng of DNA, 1 µm primers, 2x Master Mix PCR (Yekta Tajhiz Azma, Iran) in a final volume of 25 µm reaction under the following conditions: 95 °C for 5 min, 30 cycles at 95 °C for 30 s, 63.3 °C for 40 s, 72 °C for 30 s, and the final extension at 72 °C for 5 min. DNA sequencing was done using Codon Company (ABI/3500). The results of sequencing were evaluated using Chromas software.

## Results:

**Patients' characteristics:** SCA (75%) and Sβ (25%) patients with a range of 2-72 years were genotyped for the Duffy blood group system. including 48% of patients were males and 52% were females. 59.7% of the patients had a history of taking drug. A history of pregnancy or abortion was reported in 14.6% of patients. 89.6% of patients had a history of at least one blood transfusion in their lifetime and 10% of patients had showed a history of blood transfusion reactions including itching and urticaria, hematuria, fever, chills and weakness, blurred vision and muscle pain, nausea, shortness of breath and heart palpitations, redness and swelling. The most common blood groups in this study were B+ (34%), O+ (32%) and A+ (18%). Only one patient had a positive direct Coombs result (IgG+).

**The frequency of alloantibodies:** Antibody screening results showed that 24 (17.7%) patients had alloantibodies. Antibody identification showed that 79% of alloantibodies were anti-Rh, and 16.6% were anti-Kell, and 40% of patients had developed more than one alloantibody. Autoantibodies

were not observed in any of the studied patients. The frequency of anti-Rh antibodies was as follows: anti-E (33%, n=8), anti-c (20.8%, n=5) and anti-D (16.6%, n=4), respectively. 21% of patients had developed more than one alloantibody. Anti-Jka, anti-Jkb, anti-S, anti-s and anti-Leb were the other encountered antibodies. Among the studied patients, only one person had Anti-Fya and one person had Anti-Fy5. There was a significant correlation between alloimmunization and gender and history of blood transfusion, while no such correlation was found with pregnancy history, and ABO blood group.

**The frequency of minor blood groups phenotype:** The phenotype results of the studied patients are shown in Table 1. In the Rh blood group system, DCcee was the most common phenotype (32.4%). In the Kell blood group system, K-k+ was the most common phenotype (90.9%) and the Kell positive phenotype was observed in 8.3% of patients. In the Duffy blood group system, Fy(a-b+) was the most common phenotype (27.4%), followed by Fy(a+b-) (26%), Fy(a+b+) (25.2%); and Fy(a-b-) (21.4%).

*Table 1: Phenotyping results of sickle cell patients*

Blood group	Phenotype	Prevalence percentage
Rh	DCcee	32.4
	DCcEe	20.4
	Dccee	8.3
	DCcee	19.4
	DccEe	7.4
	dccee	6.5
	DccEE	3.7
	DCCEe	0.9
	dCcee	0.9
Kell	K-k+	90.9
	K+k+	8.3
	K+k-	0.8
Kidd	Jk(a+b+)	30.0
	Jk(a+b-)	30.0
	Jk(a-b+)	29.1
	Jk(a-b-)	10.9
Duffy	Fy(a-b+)	27.4
	Fy(a+b-)	26
	Fy(a+b+)	25.2
	Fy(a-b-)	21.4
Ss	S-s+	47.3
	S+S+	33.9
	S+s-	15.2
	S-s-	3.6

### Genotyping of FY\*A, FY\*B and FY\*BES

**alleles:** Among the 64 Fy(a+b-) and Fy(a-b-) samples, 40 (62.5%) patients had FY\*BES allele. 21 out of 40 samples were FY\*BES/FY\*BES, 17 FY\*A/FY\*BES and 2 were FY\*B/FY\*BES. 8 out of 24 alloimmunized patients had fy(a+b-) and fy(a-b-) phenotype, of which 5 patients had GATA-1(T33>C) mutation in the promoter region.

### Comparison between genotype and phenotype:

Four patients showed discrepancies between genotype and phenotype in Duffy blood group systems. The first discrepancy was found in two patients with FY\*B/FY\*BES allele when serologically showed Fy(a-b-) phenotype. The second discrepancy was found in two patients with Fy(a+b-) phenotype with FY\*B/FY\*B and FY\*BES/FY\*BES alleles.

### Discussion:

Sickle cell disease is the most common genetic disease after thalassemia among patients with hemoglobinopathy in Southwest of Iran (Khuzestan province)[14, 15] Because, these patients may be transfused frequently, extra care must be taken to select closely antigen matched RBCs for transfusion to prevent RBC alloimmunization. The present study showed an alloimmunization rate of 13.04% in patients. Consistent with our studies, Jalalifar et al. in 2019 reported an alloimmunization rate of 13.8% in 104 sickle cell and sickle thalassemia patients of Khuzestan province [7]

In the present study, the most common antibodies (79%) were against the Rh antigens (E, c, D,) followed by anti-K, anti-S, anti-s, anti-jka, anti-jkb, anti-Leb, anti- Fya, and anti-Fy5. But in a study published by Vafaei et al. in 2016 in Khuzestan province, alloimmunization rate was reported 7.1%, and anti-K was a more prevalent antibody [11] In the present study, only 2 patients had antibodies against Fya and Fy5 antigens. In contrast to our results, Jalalifar

et al. had reported 40% antibodies against the Duffy blood group [7] In keeping with our results, Alkindi et al. demonstrated antibodies against Duffy system only in 2 patients [16]

One strategy for prevention of RBC alloimmunization is to provide RBCs that are matched for additional blood groups beyond ABO and RhD [17] In addition to identification of RBC antigens, genotyping of SCD patients allows assessment of the risk of alloimmunization against Duffy antigens due to regulation of antigen expression determined by the GATA-1 box [12] In the present study, it was observed that 72.4% of the patients with Fy(a-b-) phenotype had GATA-1 mutation in the promoter region of FYB gene (FY\*BES allele) and among the 64 Fy(a+b-) and Fy(a-b-) samples, 40 (62.5%) patients had FY\*BES allele. 21 out of 40 samples were FY\*BES/FY\*BES, 17 FY\*A/FY\*BES and 2 were FY\*B/FY\*BES. A very rare similar mutation (69T>C) in the promoter region of FY\*A has also been identified, which was reported by Zimmerman et al and Písačka et al [18, 19]

In our study, 4 patients showed discrepancies between genotype and phenotype. The first discrepancy was found in two patients with FY\*B/FY\*BES allele when serologically showed Fy(a-b-) phenotype. The second discrepancy was found in two patients with Fy(a+b-) phenotype with FY\*B/FY\*B and FY\*BES/FY\*BES alleles. In contrast with our results, Sarihi et al. had not observed FY\*BES alleles in any of discrepant sample with Fy (a-b-) phenotype and the FY\*B/ FY\*B genotype of the thalassemia patients from north of Iran [20] According to American 2020 guideline for transfusion in sickle cell anemia patients, since patients who have FY\*BES allele are not at risk of producing Anti-Fyb, they do not need to receive Fyb- blood and they can be transfused with Fyb positive units [21] In the present study, 8 out of 24 alloimmunized patients had fy(a+b-) and fy(a-b-) phenotype, of which 5 patients had FY\*BES allele and anti-fyb was not observed in any of these patients. In a study carried out by Marcia's et al, FY\*BES allele was

detected in all sickle cell patients with Fy(a-b-) phenotype, and they were able to receive Fyb+ blood without risking the production of anti-Fyb alloantibody [22] But in a study conducted by Cotorreule et al, only 13.8% of the patients with Fy(b-) had the FY\*BES / FY\*BES genotype, and they did not need Fy(b-) units for blood transfusion [23]

**Conclusion:** The prevalence of GATA-1 mutation (FY\*BES allele), in fy(a-b-) and fy

(a+b-) patients was reported 62.5%. Therefore, consistent with international protocols, sickle cell patients can be transfused by fyb+ RBC without any risk of alloimmunization. Therefore, the number of blood units available for blood transfusion to sickle cell patients increases and it is possible to use the genotypic information as a database to facilitate the process of searching and supplying better matched blood transfusion.

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