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Determining the *FY*BES* Allele in Iranian Sickle Cell Disease Patients to Enhance Matching Blood Transfusion

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ABSTRACT

Background: Duffy antibodies play a significant role in hemolytic transfusion reactions and hemolytic disease of the fetus and newborn, Duffy(*FY*) blood group genotyping an essential part of transfusion medicine. The purpose of this study was to assess the importance of Duffy (*FY*) DNA typing in conducting transfusion compatibility testing and improving Red Blood Cell matching during transfusion.

Materials and Methods: In this study, 135 blood samples from SCD patients from the 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-) phenotypes 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 were *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, it is possible to use the genotypic information as a database to facilitate the process of searching and supplying better-matched blood transfusion.

Keywords: Sickle cell disease; *FY*BES* allele; Duffy

INTRODUCTION

Sickle Cell Disease (SCD) ranks second in frequency of hemoglobinopathies after thalassemia in Iran. HbS polymerization is a pathophysiological event in sickle cell anemia (SCA), changes in the shape and physical properties of red blood cells, resulting leading to micro vascular occlusion, hemolytic anemia and acute pain crises. Red blood cell transfusions along with medicine that lessen symptoms, gene therapy and blood and bone marrow transplant are crucial parts of SCD treatment. Transfusion therapy decrease the ratio of circulating hemoglobin-S containing red blood cells, vasoocclusive, and multiorgan failure syndromes[1-5]. The most important challenge of transfusion is the alloimmunization to red blood cells antigen[6]. Alloimmunization leads to reducing the available pool of compatible blood for transfusion in subsequent crises. The incidence of alloimmunization to red blood cells 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

red blood cells antigen between blood donors and SCD patients. Alloimmunization is the main problem in identifying appropriate antigen-negative red blood cells for transfusion[8, 9].

For multi-transfused thalassemia patient identification of alloantibodies must be done for transfusion of well-matched donor (lacking specific antigen against which the antibody developed) for desire rise of hemoglobin level and thereby reduces the transfusion frequencies as well as increase transfusion interval.(10) Anti-Rh (D, Cc, and Ee) and Kell are the most common antibodies reported in chronically transfused patients with SCD in the Southwest of Iran (Khuzestan province). As a result, recommendations have been made for patients with SCD to have RBC transfusions that are 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 %[1]. Antibodies to Duffy antigens are clinically significant in transfusion medicine and they are involved in hemolytic transfusion reactions and hemolytic disease of the newborn[2].

The extended red cell antigen profile may be determined by genotype or serology. c Extended red cell antigen matching (Jka /Jkb , Fya /Fyb , S/s) may provide further protection from alloimmunization. c Patients who have a GATA mutation in the ACKR1 gene, which encodes Fy antigens, are not at risk for anti-Fyb and do not require Fyb negative red cells[11].

Molecular genotyping was found to be an accurate reliable method for minor antigen typing and should be used for providing antigen-negative or antigen-matched blood units to multi transfused patients[12]. While in our country, genotyping for minor blood groups is not routine even in multi transfused patients with allo-antibodies and difficulty in finding matched blood units and is only performed for research purposes.

Studies conducted in Khuzestan province have shown that Duffy negative fy(a-b-) phenotypes in SCD patient are more than Duffy negative phenotypes in donors[1]. 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 red blood cell[2]. Therefore, FY genotyping and determining *FY*BES* allele or *GATA-1(T33>C)* helps to better supply for compatible blood in SCD patients[3].

The silent allele *FY*BES* is prevalent among black Africans, particularly in malaria-prone areas, but it has not been studied in the south of Iran yet. 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 the High Institute for Research and Education in Transfusion Medicine. Written informed consent was read and signed by all participants before enrolling. Demographic data of patients, transfusions, and clinical history were collected through a questionnaire filled out by the nurse.

Patients

A total of 135 samples were collected from SCD and Sβ patients admitted to Baqaei2 Hospital in Ahvaz, Khuzestan province, from 2021 to 2022. Ten ml of peripheral blood samples were collected in two separate tubes containing K2EDTA anticoagulant for serologic and molecular testing.

Serological Tests

All samples were referred to Immunohematology reference laboratories (IRLs), Tehran. ABO and Rh typing, direct Anti-glubolin test (DAT), antibody screening and identification, and red blood cell phenotyping (Cc Ee, Kell, Kidd, and Duffy) were performed for all samples.

ABO and Rh typing

ABO typing was performed using the conventional tube method using commercial monoclonal Anti-A and Anti-B antibodies (Immundiagnostika, Germany). Serum grouping was performed using in-house pooled A cells and B cells. IgM (clone RUM10,

Immundiagnostika, Germany) and IgM/IgG blend anti-D (clone TH-28/MS-26, Immundiagnostika, Germany) were used to test the status of the red cell antigens.

Antibody screening and identification

Antibody screening test was carried out using a three-cell panel (made at the IBTO) in three phases: IS, 37°C, and AHG. The samples that had a positive reaction were further tested using 11 and 16 cell identification red cell panels (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. Positive and negative controls were incorporated into the test using previously determined phenotypic samples to ensure the expected reactivity of the antiserum used in the testing.

Direct anti globulin Test (DAT)

Since the Duffy blood group phenotype was determined in the AHG phase, a direct antiglobulin test (DAT) is performed to detect false positive results. DAT was performed on all samples using poly-specific Anti-human globulin (CE-Immunodiagnostika & Biotechnologie GmbH, Berlin, and Germany). 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.(4) 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 sequencing results were analyzed using Chromas software.

RESULT

Patients' characteristics

Sickle Cell Anemia (SCA) (75%) and Sickle-Beta (Sβ) (25%) patients with a range of 2-72 years were genotyped for the Duffy blood group system. 48% of patients were males, while 52% were females. 59.7% of the patients had a history of taking drugs. 14.6% of patients had a history of pregnancy or abortion. 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%). A positive direct Coombs result (IgG+) was only found in one patient.

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%).

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.

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

DISCUSSION

Sickle cell disease is the most common genetic disease after thalassemia among patients with hemoglobinopathy in Southwest of Iran (Khuzestan province)[5,6]. Because, these patients may be transfused frequently, extra care must be taken to select closely antigen matched red blood cells 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[1]. In the present study, only two 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 two patients[8].

One strategy for prevention of RBC alloimmunization is to provide RBC that are matched for additional blood groups beyond ABO and RhD[9]. In addition to identification of RBC antigens, genotyping of Sickle Cell Disease (SCD) patients allows assessment of the risk of alloimmunization against Duffy antigens due to regulation of antigen expression determined by the *GATA-1* box[2]. 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 two 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[13, 14].

In our study, four 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[15].

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[11]. In the present study, eight out of 24 alloimmunized patients had fy(a+b-) and fy(a-b-) phenotype, of whom five 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[16]. However, 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[17].

CONCLUSION

The prevalence of *GATA-1* mutation (*FY*BES* allele), in fy(a-b-) and fy (a+b-) patients was reported 62.5%. 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.

REFERENCES

1. Jalali Far MA, Oodi A, Amirizadeh N, et al. The Rh blood group system and its role in alloimmunization rate among sickle cell disease and sickle thalassemia patients in Iran. Mol Genet Genomic Med. 2021;9(3):e1614.

2. Talano JAM, Hillery CA, Gottschall JL, et al. Delayed hemolytic transfusion reaction/hyperhemolysis syndrome in children with sickle cell disease. Pediatrics. 2003;111(6 Pt 1):e661-5.

3. Vafaei M, Keikhaei Dehdazi B. Frequency of red blood cell alloimmunization in sickle cell patients and healthy donors: The influence of racial and antigenic pattern differences. Int J Community Med Public Health. 2017;4(7):2226-9.

4. Castilho L. The value of DNA analysis for antigens in the Duffy blood group system. Transfusion. 2007;47(1 Suppl):28S-31S.

5. Natukunda B. Red blood cell alloimmunization and antigen matching in sickle cell disease–the African perspective. ISBT Sci Ser. 2012;7(1):129-33.

6. Amini A, Nadali F, Dereikvand G. Prevalence determination of sickle cell trait by premarital screening at isfahan thalassemia prevention center. J Pathol Res. 2005;7(2);19-22.

7. Zandian KM, Pedram M, Hamadi H. An analysis of clinical and laboratory findings of hemoglobinopathies and their distribution in khozestan provience. Jundishapur Sci Med J . 2005;0(43):7-14

8. Alkindi S, AlMahrooqi S, AlHinai S, et al. Alloimmunization in patients with sickle cell disease and thalassemia: experience of a single centre in Oman. Mediterr J Hematol Infect Dis. 2017;9(1):e2017013.

9. Wilkinson K, Harris S, Gaur P, et al. Molecular blood typing augments serologic testing and allows for enhanced matching of red blood cells for transfusion in patients with sickle cell disease. Transfusion. 2012;52(2):381-8.

10. Hazra S, Parveen PA, Lyngdoh B, et al. Importance of screening and identification of alloantibodies in multi-transfused patients of thalassemia major. Int J Hum Health Sci. 2021;5(2):230-4.

11. Chou ST, Alsawas M, Fasano RM, et al. American Society of Hematology 2020 guidelines for sickle cell disease: transfusion support. Blood Adv. 2020;4(2):327-55.

12. Kulkarni S, Choudhary B, Gogri H, et al. Molecular genotyping of clinically important blood group antigens in patients with thalassaemia. Indian J Med Res. 2018;148(6):713-720.

13. Zimmerman PA, Woolley I, Masinde GL, et al. Emergence of FY* A null in a Plasmodium vivax-endemic

region of Papua New Guinea. Proc Natl Acad Sci U S A. 1999;96(24):13973-7.
14. Písačka M, Marinov I, Králová M, et al. FY* A silencing by the GATA-motif variant FY* A (− 69C) in a Caucasian family. Transfusion. 2015;55(11):2616-9.
15. Sarihi R, Oodi A, Dadkhah Tehrani R, et al. Blood group genotyping in alloimmunized multi-transfused thalassemia patients from Iran. Mol Genet Genomic Med. 2021;9(7):e1701.
16. Novaretti MC, Bonifacio SL, Medeiros VR, et al. Rapid Duffy Genotyping in Sickle Cell Disease Patients Using PCR-SSP Is a Valuable Tool for Blood Component Selection. Blood. 2007; 110(11):2901-2901.
17. Cotorruelo C, Biondi C, Racca L, et al. Duffy genotyping facilitates transfusion therapy. Clin Exp Med. 2009;9(3):249-51.