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ORIGINAL ARTICLE

Prevalence of Duffy Blood Group Antigens and Phenotypes among Saudi Blood Donors in Southwestern Saudi Arabia

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SUMMARY

Background: **Knowing the prevalence of blood group antigens in a given population is important to prevent hemolytic reactions. The Duffy blood group system (FY) has two main antigens, Fy^a and Fy^b. Antibodies binding these antigens can cause immediate/delayed hemolytic transfusion reactions as well as hemolytic disease of the fetus and newborn. In this study, frequencies of Fy^a and Fy^b antigen expression and FY phenotypes were determined in a cohort of Saudi blood donors.**

Methods: **For this study, 143 samples were collected from randomly selected volunteer Saudi blood donors living in Jazan Province. Serological analysis, using gel card technology, was performed to detect Fy^a and Fy^b antigens among the samples.**

Results: **The frequencies of Fy^a and Fy^b antigens were 12.58% and 11.18%, respectively. The numbers and frequencies of FY phenotypes were as follows: $Fy(a^+b^-)$, 15 (10.48%); $Fy(a^-b^+)$, 13 (9.10%); $Fy(a^+b^+)$, 3 (2.10%), and $Fy(a^-b^-)$, 112 (78.32%). The frequencies of the FY phenotypes were highly and significantly different in Jazan Saudis compared to other ethnicities (< 0.01).**

Conclusions: **This study reports the frequencies of the Fy^a and Fy^b antigens and phenotypes of the FY blood group system in the Kingdom of Saudi Arabia's Jazan Province. The null phenotype $Fy(a^-b^-)$ was the most prevalent among this population. This study highlights the importance of investigating FY alleles in different provinces of the Kingdom of Saudi Arabia.**

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INTRODUCTION

The Duffy blood group system (FY), which is designated by International Society of Blood Transfusion (number: 008), is also known as Duffy antigen receptor for chemokines (DARC), atypical chemokine receptor 1 (ACKR1) or CD234. A single gene (*FY* or *DARC*) encodes the 36 kD glycoprotein comprising the FY antigens and is located on the long arm of chromosome

1q21-q22 [1]. Several *DARC* gene alleles regulate FY antigen synthesis. The most common alleles of the FY system are *FY*A, FY*B, FY*X,* and silencing *FY* [2]. The FY antigens have two N-glycosylation sites and are assumed to traverse the red cell membrane seven times, producing three extracellular loops. These loops are numbered 1 to 3, starting with the extracellular N-terminal loop. Disulfide bonds are thought to link the N-terminal domain to the third loop and the first loop to the second loop. FY glycoprotein is composed of 336 amino acids [3].

The names ACKR1 and DARC suggest the function as a chemokine receptor. ACKR1 binds with various chemokines, including CXC acute inflammatory chemokines (interleukin-8 and melanocyte growth-stimulating activity) and CC chronic inflammatory chemokines (RANTES and macrophage chemoattractant protein-1) [4]. A well-defined role of these chemokine receptors on red cell surfaces is unknown. However, it is postulated that red cells having these chemokine receptors act as scavengers for locally released chemokines to reduce leukocyte activation [5,6].

Increased DARC expression in patients with renal disease has been reported. It was speculated that renal cell injury increases DARC expression to neutralize inflammatory chemokines and suppress renal inflammation [7]. The N-terminal ectoplasmic domain of FY antigens also serves as a receptor for *Plasmodium vivax*. This parasite infects red cells by binding with the FY-binding protein (PvDbp). Interestingly, it has been reported that the Fy(a^-b^-) phenotype (the null phenotype) is resistant to invasion by *Plasmodium Vivax* [5].

FY antigens can be detected on fetal red cells as early as the 6th week of gestation and are well developed at birth. The FY system has two major antigens: FY1 (Fy^a) and FY2 (Fy^b). Fy^a antigen differs from Fy^b antigen by only one amino acid substitution, in which aspartic acid is replaced by glycine at position 42 (Asp42Gly) [8,9]. The silencing *FY* allele results in the Fy(a^-b^-) phenotype, with no FY antigens being expressed on red cells. Indeed, this allele is a variant of the *FY*B* allele that results from a single nucleotide substitution (T>C) in the GATA promoter region. This substitution inhibits GATA-1-dependent *DARC* gene transcription in erythroid cells [10]. However, transcription of the *DARC* gene in other tissues is not affected, leading to the expression of Fy^b antigen on endothelial cells, epithelial cells, brain cells, thyroid glands, colon, and spleen [11]. This expression of Fy^b in other cells presents self-antigen. Therefore, individuals having a Fy(a^-b^-) phenotype do not develop anti-Fy^b antibodies. This null phenotype has been found most commonly in people of African descent. Conversely, this phenotype has not been found in Caucasians [12].

Alloimmune anti-Fy^a and anti-Fy^b antibodies usually develop following transfusion of FY phenotype-mismatched red blood cells or less frequently following pregnancy [13,14]. Anti-Fy^a can fix complement leading to immediate hemolytic transfusion reactions (HTR) [14]. Anti-Fy^b appears to be incapable of complement fixation and does not cause immediate HTR. However, both can lead to delayed HTR [15-17].

Given the importance of blood group antigens in HTR, our aim in this study was to investigate the frequency of the FY antigens, Fy^a and Fy^b, among the Saudi Arabian population in Jazan Province. Furthermore, the frequencies of FY phenotypes were determined and compared to other ethnic groups.

MATERIALS AND METHODS

Blood samples

For this study, 143 blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes at Prince Muhammad bin Nasser Hospital in Jazan Province of Saudi Arabia. Ethical approval was obtained from Jazan Hospital Institutional Review Board (No. 2017). These samples were obtained from voluntary Saudi blood donors living exclusively in the Jazan area. Those donors signed a consent form and completed questionnaires prior to blood donation. Participants underwent the donation procedure according to national blood transfusion guidelines. Blood samples were tested for infectious diseases, such as Hepatitis B and C.

Immunohematology

Serological analysis was performed using gel card technology with a commercially available kit; ID-Card Fy^a/Fy^b and ID-Anti-Fy^a/Fy^b antibodies (DiaMed GmbH, Cressier, Switzerland) according to the manufacturer's instructions. A total of 50 μL of 0.8% red cell suspension was added to ID-Card Fy^a/Fy^b, followed by adding a total volume of 50 μL of ID-Anti- Fy^a/Fy^b antibodies. Kits were incubated at 37°C for 15 minutes in an ID-incubator (DiaMed GmbH, Cressier, Switzerland). Finally, the ID-Card Fy^a/Fy^b was spun at 85 x g for 10 minutes in the ID-Centrifuge (DiaMed GmbH, Cressier, Switzerland).

Interpretation of results

A red line forming on the surface of the gel or a dispersed agglutination indicated positive results signifying the presence of the corresponding antigen. Conversely, a bottom pellet forming in the microtubes indicated negative results and the absence of the relevant antigen.

Sample size

The sample size was calculated using G*Power software Version 3.1.9.4 with a two-sided exact test for one proportion [18]. A population prevalence of the 'null value' of 0.66 was used based on the study conducted by Singleton et al. [19].

The 143-sample requirement was determined for appropriate statistical power using G* Power 3.1.9.4 software. This sample size had sufficient power to test the significance of the difference between the estimated and observed population prevalence with a 5% level of sig-

Table 1. The frequency of FY blood group antigens in Jazan Province, Saudi Arabia.

Antigen	Observation (n)	Frequency (%)
Fy^a	18	12.58%
Fy^b	16	11.18%

Table 2. FY phenotype frequencies in the Jazan population.

Phenotype	Observation	Frequency (%) n = 143
$Fy(a^+b^-)$	15	10.48
$Fy(a^+b^+)$	3	2.10
$Fy(a^-b^+)$	13 *	9.10
$Fy(a^-b^-)$	112	78.32
	143	100

*** - including one weak reaction.**

nificance, 11% allowable margin of error and 81% power.

Statistics

The frequencies of the FY antigens and phenotypes were presented and standardized as a percentage. A chi square test was used to compare phenotype frequencies between the Jazan population and other ethnic groups. p-values < 0.05 and < 0.01 indicated significant and highly significant differences, respectively.

RESULTS

The 143 samples were analyzed for the antigens of the FY blood group system. The results of serotyping of the FY antigens are presented in Table 1. The Fy^a antigen was found in 18 samples (12.58%), distributed among $Fy(a^+b^-)$ and $Fy(a^+b^+)$ phenotypes. Conversely, Fy^b antigen was found in only 16 samples (11.18%) among $Fy(a^-b^+)$ and $Fy(a^+b^+)$ phenotypes.

Table 2 shows the frequencies of the four phenotypes of the FY blood group system in the Jazan region according to our results. The $Fy(a^+b^-)$ phenotype was observed in 15 individuals (10.48%).

Three individuals were heterozygous for both Fy^a and Fy^b antigens, i.e., $Fy(a^+b^+)$, which accounts for 2.10% of the cohort. The $Fy(a^-b^+)$ phenotype was observed in 13 individuals (9.10%), with a weak reaction in a single sample. The silencing (null) phenotype was the most prevalent at 78.32%. Table 3 demonstrates phenotype frequencies of the FY blood groups system among the Jazan population compared to other ethnicities.

DISCUSSION

Knowing the prevalence of blood groups is crucial for preventing hemolytic reactions. In Jazan Province of Saudi Arabia, many patients require frequent blood transfusions, including those with thalassemia and sickle cell disease [20,21]. The incidence of alloimmunization may be higher in this population because of multiple transfusions [22,23].

The two main antigens of the FY blood group system, Fy^a and Fy^b, are polymorphic. In this study, serological analysis was conducted to obtain the frequencies of these antigens and phenotypes. The Fy^a antigen was detected in 18 individuals (12.58%). In contrast, Fy^b was observed in only 16 individuals (11.18%) as shown in Table 1. The frequency of the Fy^b antigen (11.58%) is similar to that observed in a Chinese population (10.8%) by Yan et al. [24]. In contrast, the Fy^a antigen was detected in 99% of this population.

In the current study, robust and highly significant differences in FY phenotypes were found between Saudis living in Jazan Province and other ethnicities, i.e., African, Caucasian [12], Chinese [24], and Thai populations [25], as shown in Table 3.

The $Fy(a^+b^-)$ phenotype was observed in 15 individuals (10.48%). The frequencies of this phenotype vary between ethnic groups, as shown in Table 3. In Asian populations, including Chinese and Thai, the frequencies were 89.2% and 88.5%, respectively. This is higher than other ethnic groups.

In the current study, the heterozygous phenotype $Fy(a^+b^+)$ was detected in three samples, making it the least prevalent phenotype (2.10%). Similarly, the frequency of $Fy(a^+b^+)$ in the African population was 2%. However, this phenotype's frequency was relatively high in the Caucasian population at 40% [12].

The homozygous phenotype $Fy(a^-b^+)$ was observed in 13 individuals (9.10%), which was greater than the observed frequencies in Asian populations (Chinese, 1%; Thai, 0.5%). Conversely, this frequency was relatively low compared to Caucasian (31%) and African populations (39%) [12,26].

Interestingly, the most common phenotype was the null phenotype $Fy(a^-b^-)$, which was observed in 112 individuals, accounting for 78.32% of this Jazan population. Remarkably, the frequency of $Fy(a^-b^-)$ was significantly higher in our cohort than in the African population (63%). This null phenotype is absent in Caucasian, Chinese and Thai populations. There may be a risk of anti-Fy^a and anti-Fy^b antibody reaction in the Jazan population receiving transfusions due to the lack of FY antigens. In addition, there may be increased risk of hemolytic disease of the fetus and newborn.

The observation of the null $Fy(a^-b^-)$ and $Fy(a^+b^+)$ phenotypes may result from the African origins of some individuals living in Jazan. The $Fy(a^-b^-)$ phenotype has a selective advantage for resistance to *Plasmodium Vivax* and *Plasmodium Knowlesi* infection [27]. Future work investigating the genotypes of *FY* alleles in this popula-

Table 3. FY phenotype frequencies in the Jazan population among different ethnicities.

Phenotype	Jazan (Saudi Arabia) (%) n = 143	Black (%) n = 100	White (%) n = 100	Chinese (%) n = 102	Thai n = 200
$Fy(a^+b^-)$	10.48	4	21	89.2	88.5
$Fy(a^+b^+)$	2.10	2	40 ***	10	11
$Fy(a^-b^+)$	9.10 *	31 **	39	1	0.5
$Fy(a^-b^-)$	78.32	63	0	0	0
p-values §		Jazan/Black p = 0.0000 §	Jazan/White p = 0.0000 §	Jazan/Chinese p = 0.0000 §	Jazan/Thai p = 0.0000 §

§ - highly significant by Chi square.
* - including one Fy^b weak reaction.
** - including one Fy^b weak reaction.
*** - including two Fy^b weak reactions.

tion is justified, including *FY*A, FY*B, FY*X, and FY* alleles.

In this study, we investigated the frequency of the main antigens of the FY blood group system, Fy^a and Fy^b, in Jazan province in the Kingdom of Saudi Arabia. Furthermore, we report the frequencies of the four FY phenotypes. Unexpectedly, the most common FY phenotype among the Saudi population living in Jazan Province of Saudi Arabia was $Fy(a^-b^-)$. It will be important to study FY antigens in different provinces within the Kingdom of Saudi Arabia.

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Declaration of Interest:
The authors have no conflicts of interest to declare.

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