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Frequencies of Diego Blood Group Alleles and Genotypes in Jazan Province of Saudi Arabia

Abstract: Background: Multiply-transfused patients are prevalent in Jazan region, Saudi Arabia. Alloimmunization may occur in case of red cell incompatibility. Therefore, extensive serotyping for other blood groups is essential. Two alleles of the Diego (DI) blood group system, *DI*A* and *DI*B* encode the main DI antigens Di(a+b–) and Di(a–b+), respectively. Anti-Di^{a} and anti-Di^{b} can be involved in transfusion reactions and pregnancies issues. This study aimed to investigate the allele and genotype frequencies of the DI blood groups in Jazan Province of Saudi Arabia. Methods: One-hundred-fifty samples was collected from Saudi blood donors living in Jazan Province. DNA was extracted and sequence-specific primers were designed to amplify the SNV region (rs2285644), distinguishes the *DI*A* allele from the *DI*B* allele. The resulting PCR amplicons were sequenced. Results: The frequency of *DI*B* alleles was 100%, while the *DI*A* allele was not observed. Therefore, the only detected genotype was *DI*B*/*DI*B* at 100%. Conclusions: This study reported the allele and genotype frequencies of the DI blood group system in Saudi Arabia. This study may assist to establish a national database for blood groups in Jazan region. Moreover, it may help to preclude the risk of alloimmunization by providing matching blood units.

Keywords: Diego blood group; blood group genotyping; red cell alloimmunization; blood transfusion;

1. Introduction

In 1955, an anti-Di^a antibody, which was produced to the first antigen in the Diego (DI) system, by a Venezuelan patient suffering from serious hemolytic disease of the fetus and newborn (HDFN) [1]. The Diego (DI) blood group system is considered as the tenth system (system symbol: DI, System number: 010) according to the International Society of Blood Transfusion (ISBT) [2].

The DI antigens are carried on a glycoprotein molecule that traverse the red cell membrane several times forming seven extracellular loops with both carboxyl and amino termini are intracellular. This glycoprotein is knowns as anion exchange 1 (AE1) and also denoted as band 3 [3]. Moreover, it is formed of 911 amino acid residues in length. It acts as an anion transporter and crucially interacts with cytoskeleton allowing it to maintain the structural integrity of the red cell membrane [4].

Twenty-three antigens have been identified until now, which belong to the DI blood group system. Three high prevalence antigens (Di^b, Wr^b, and DISK), while the remaining antigens are low prevalence. Six pairs of which are antithetical antigens, including Di^a/Di^b, Wr^a/Wr^b, Wu/DISK, Mo(a+)/Hg(a+), BOW+/NFLD+, Jn(a+)/KREP+ [2,5]. These antigens are represented by a single gene, *SLC4A1* gene, which is located on chromosome 17q21.31. The gene is composed of 20 exons, which span over 20 Kbp of the human genome [3]. The most important antigens are Di^a and Di^b, which are resulted from a single nucleotide variation (rs2285644). For example, in Di(a+b-) the nucleotide change c.2561C>T leads to amino acid substitution p.Pro854Leu [6].

The antibodies of the DI antigens, i.e. anti-Di^a and anti-Di^b, can involve in hemolytic transfusion reactions (HTR) as well as the HDFN. Anti-Di^a can cause mild to severe and fatal HDFN as well as severe immediate/delated HTR [7]. On the other hand, anti-Di^b may lead to mild HDFN with an observation of positive direct antiglobulin test and may show no symptoms. Regarding the blood transfusion, anti-Di^b has the ability to cause moderate and delayed HTR [8]. It has been reported that autoanti-Di^b in few cases [7].

The prevalence of the Di^a and Di^b antigens varies from one population to another. Regarding the Di^a antigen, it has been reported in 12% of Japanese, 11% of Chippewa Indians, 5% of Chinese, 1% of Hispanics, and 0.47% of Poles [9]. In addition, in South American Indians it varies from 2% in Caracas Indians to 54% in Kainganges Indians. Concerning the Di^b antigen, it is a high prevalence antigen that has been observed as 99% and 100% in native Americans and most population, respectively [9].

Given the importance of the DI blood group system and its clinical significance, this study aims to investigate the frequencies of the DI alleles and genotypes in Saudi population living in Jazan Province of Saudi Arabia.

2. Materials and Methods

2.1 Blood samples

This study was carried out between October 2023 to January 2024. Ethical approval was obtained from Jazan Health Ethics Committee (reference number: 2386) and was in compliance with the Declaration of Helsinki. Informed consent was taken from all subjects involved in the present study. All participants who contributed in the current study provided informed consent.

The sample size was calculated to a total of 143 samples and was rounded to 150 samples as previously described by Halawani et al. (2022) [10].

One-hundred-fifty blood samples were collected from the blood bank center at King Fahad Central Hospital, Jazan Province of Saudi Arabia. The samples were received in ethylene diamine tetraacetic acid tubes.

2.2 Inclusion criteria

Saudi citizens living in Jazan Province who were healthy and donated blood voluntarily. The age of the blood donors were 18 years old or above and free from any infectious diseases such as hepatitis.

2.3 Exclusion criteria

Any blood donors from other nationalities or unseccessful blood donation were excluded from this study.

2.4 DNA extraction

DNA extraction was carried out using a GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermo Fisher Scientific, Paisley, United Kingdom) according to the manufacturer's instructions. The DNA purity and quantification were assessed by NanoDrop 200 spectrophotometer (Thermo Fisher Scientific, Paisley, United Kingdom).

2.5 PCR primers

The preparation of the PCR was started by designing a primer pair using the National Center for Biotechnology Information primer BLAST tool in order to amplify a single nucleotide variation responsible for the *DI*A* and *DI*B* alleles (ID: rs2285644). Table 1 demonstrates the primer pair used to amplify the target of interest. The target size for the PCR product was 450 base pairs. The human growth hormone was used as an internal control and obtained from Touinssi et al. (2008) [11]. The primer pair was manufactured by Macrogen (Seoul, South Korea).

2.6 PCR setup

PCR experiments were conducted using 1X Phusion Green Host Start II High-Fidelity PCR Master Mix (Thermo Fisher Scientific, Paisley, United Kingdom), 50 ng of DNA template, and 0.5 µM of each forward and reverse primer. The cycling conditions were optimized as follows: initial denaturation at 98°C for 30 s, followed by 35 cycles of denaturation 98°C for 10 s; annealing 60°C for 30 s; and extension 72°C for 30 s. Then, the final extension at 72°C for 10 min followed by 4°C hold. Finally, the PCR amplicons were observed using 2% agarose gel electrophoresis.

2.7 Sequencing reactions

The PCR amplicons were shipped abroad for sequencing service (Macrogen, Seoul, South Korea). MacVector Software (Version 12.7) was assessed the sequencing electropherograms and evaluated the genotyping outcomes (MacVector, Inc., North Carolina, United States).

2.9 Statistical analysis

The data were analyzed in order to determine the rates of the alleles as well as the genotypes of the *DI*A* and *DI*B* and were demonstrated as percentages. Data comparison of the present study with other ethnic

backgrounds was carried out by calculating the *p*-values using chi-square test in order to demonstrate any statistically significant differences. The *p*-values < 0.05 and < 0.01 indicated significant and highly significant differences, respectively.

3. Results

One-hundred-fifty DNA samples were conducted to the PCR reactions followed by Sanger sequencing. The prevalence of the DI alleles is listed in Table 2. The only observed allele in Saudi population living in Jazan Province was *DI*B* at (n = 300, 100%). Regarding the genotyping, according to the allelic outcomes, the only genotypes seen was *DI*B/DI*B* (n = 150, 100%) as shown in Table 3. Data comparison of the present study with various ethnicities is demonstrated in Table 4.

4. Discussion

Jazan Province of Saudi Arabia is endemic with hemoglobinopathies, such as thalassemia and sickle cell disease [23]. Some of which are transfusion dependent patients, who require receiving blood transfusion regularly. Accordingly, extended phenotyping for such patients is crucial in order to preclude the risk of the red cell alloimmunization [24]. Because of that knowledge regarding frequencies of various blood group antigen is essential and may assist in prevention of such issues. Many research studies were conducted among Saudi Arabian population who live in Jazan Province. These include frequencies of ABO, RH, KEL, MNS, JK, FY, LE, LU and DO blood groups [25-30].

In this study, we identified the frequencies of DI blood group system. The frequency of the *DI*B* allele was determined to be 100% as it is a high prevalence antigen. Accordingly, the only detected genotype was *DI*B/DI*B*. On the other hand, there was no observation regarding the *DI*A* allele. These findings of the present study are in accordance with other reported studies investigating different ethnicities. For example, Italians in Naples [19], Esan in Nigeria, Gambian in Western Devision-Mandinka, British from England and Scotland, and Toscani in Italy [12].

However, very few studies have reported the incidence of the *DI*A* allele, which demonstrate variations in *DI*B* allele compared to the present study. Foe example, the frequencies of *DI*A* allele were reported in other ethnicities and it ranges from 0.6% in Native Alaska/Aleut to 6% in Chinese (Chengdu) [18] as demonstrated in Table 4.

Our findings demonstrate that there were statistically significant differences ($p < 0.05$) between the Saudis living in Jazan Province and Han Chinese in Beijing, Han Chinese South, Japanese in Tokyo, Chinese (Shen-Zhen), Colombian in Medellín, Brazilian Japanese descendants, and Southern Brazilians [12,15,20,22]. Interestingly, observations of high statistically significant differences ($p < 0.01$) were seen between the population of the current study compared to Han Chinese (Shanghai), Korean (Seoul), Korean, Chinese (Chengdu), Japanese, Mexican Ancestry in Los Angeles, and Peruvian in Lima [12,14,16-18].

Building an extended panel for the multiply transfused patients is crucial by investigating many alleles/antigens of different blood group systems. It is highly recommended for the healthcare providers in Saudi Arabia to switch to blood group genotyping especially for such patients. This protocol investigates the DNA alleles accurately compared to the conventional serotyping, and avoid mistyping especially in patients receiving recent transfusions [31]. In other words, those patients may suffer from red cell alloimmunization due to the wrong typing of the donor red cells in their bloodstream.

The limitations of this study may include that there were only two alleles were investigated regarding the DI blood group system. However, these two alleles are the main ones among the system and cause more clinically significant outcomes in case of mismatching antigens during blood transfusion or pregnancies.

5. Conclusions

This study reported the frequencies of the DI alleles and genotypes in Saudi blood donors living in Jazan Province. This may help to establish a national database and design the extending phenotyping panel to include blood groups. As a result, this will provide better transfusion practices and may assist in reducing the risk of the alloimmunization of the red cells.

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Institutional Review Board Statement: The study was approved by the Jazan Health Ethics Committee (H-10-Z-073, approval number: 2386). It was conducted following the guidelines of the Declaration of Helsinki.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data is unavailable due to ethical restrictions.

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Conflicts of Interest: The authors declare no conflict of interest.

Table 1. The primer pairs used to amplify the *DI*A* and *DI*B* alleles.

Primer	**5′ to 3′ sequence**	**Product size (bp)**	**Chromosomal location**
DI- rs2285644-F	ACACAGAGAAACAAGGCCCC	450	chr17:44250982+44251 431
DI- rs2285644-R	CTACGTCAAGCGGGTACAGG		
HGH-F*	GCCTTCCCAACCATTCCCTTA	429	chr17:61995373-61995801
HGH-R*	TCACGGATTTCTGTTGTGTTTC		

DI: Diego, Bp: base-pair, HGH: human growth hormone, F: forward, R: reverse

*The HGH was used for internal control.

Table 2. The frequencies of DI alleles among 150 Saudi Arabians living in Jazan Province

Allele	Predicted antigen	Observation (n)	Frequency (%)
*DI*A*	Di^a	0	0
*DI*B*	Di^b	300	100

DI: Diego

Table 3. The frequencies of the DI genotypes among Saudi Arabians in Jazan Province of Saudi Arabia.

Genotype	**Predicted phenotype**	**Observation (n)**	**Frequency (%)**
*DI*A/DI*A*	Di(a^+b^-)	0	0
*DI*A/DI*B*	Di(a^+b^+)	0	0
*DI*B/DI*B*	Di(a^-b^+)	150	100

DI: Diego

Table 4. Comparison of prevalence of DI alleles between the Jazan population (present study) and different ethnic backgrounds.

Population	**Count**	**Allele frequencies**		***p*-values¥**
		DI*A	***DI*B***	
Saudis (Current study)	150	0.0000	1.0000	-
Bengali in Bangladesh [12]	172	0.012	0.988	0.185
Chinese Dai in Xishuangbanna, China [12]	186	0.016	0.984	0.121
Han Chinese in Beijing [12]	206	0.029	0.971	0.035*
Han Chinese South [12]	210	0.029	0.971	0.036*
Japanese in Tokyo [12]	208	0.038	0.962	0.015*
Central Thais [13]	1,011	0.0183	0.9817	0.090
Southeast Asian [14]	942	0.0145	0.9855	0.132
Filipino [14]	1,333	0.0075	0.9925	0.287
Chinese (Shen-Zhen) [15]	1,766	0.0357	0.9643	0.018*
Han Chinese (Shanghai) [16]	403	0.0447	0.9553	0.008**
Korean (Seoul) [17]	116	0.0474	0.9526	0.005**
Korean [14]	1,033	0.0580	0.9420	0.002**
Chinese (Chengdu) [18]	300	0.0600	0.9400	0.002**
Japanese [14]	1,022	0.0430	0.9570	0.009**
African ancestry in Southwest United States [12]	122	0.008	0.992	0.266
Esan in Nigeria [12]	198	0.0000	1.000	-
Gambian in Western Division–Mandinka [12]	226	0.0000	1.000	-
British From England and Scotland [12]	182	0.0000	1.000	-
Toscani in Italy [12]	214	0.0000	1.000	-
Italians (Naples) [19]	225	0.0000	1.0000	-

American Native [14]	970	0.0110	0.9890	0.189
Colombian in Medellín [12]	188	0.037	0.963	0.016*
Mexican Ancestry in Los Angeles [12]	128	0.047	0.953	0.007**
Peruvian in Lima [12]	170	0.106	0.894	0.000**
Puerto Rican in Puerto Rico [12]	208	0.024	0.976	0.056
Alaska Native/Aleut [14]	621	0.0065	0.9935	0.324
Hawaiian/Pacific Islander [14]	522	0.0060	0.9940	0.352
Brazilian Japanese descendants [20]	209	0.0431	0.9569	0.010*
Brazilians [21]	4,326	0.0181	0.9819	0.097
Southern Brazilians [22]	373	0.0282	0.9718	0.033*

[¥]Chi-Square test

*Significant differences from Saudis,

**highly Significant differences from Saudis.

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