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Evaluation prevalence of Rh system major antigens (D, C, c, E, e) and their phenotypes in blood donors of Golestan province, Iran

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Abstract

Background: The understanding of blood group phenotypes is currently limited to the ABO and Rh blood group systems. This study aimed to determine the frequency of Rh system antigens (D, C, c, E, e) and the phenotypes of the system in blood donors. Identifying the blood group phenotypes of donors in any population is important for improving healthcare services and better serving patients.

Methods: This descriptive study was carried out on 575 donors (Turkmen and Fars) in blood transfusion centers in Golestan Province, Iran. A cell suspension (3-5%) from each sample was prepared in normal saline and exposed to Rh system antisera using the haemagglutination technique. The Rh phenotype was then determined based on the most common genotype.

Results: For the Rh system, the antigen frequencies of D, C, c, E, and e were 87.76%, 73.6%, 72.1%, 30.83%, and 93.59%, respectively. The most common phenotypes among the Turkmen and Fars donors were R1R1, R1r, and rr, respectively, while the least common phenotypes were R2Rz and ryry. The phenotypes r'r' and ryry were not detected in the Turkmen donors, and the phenotype r''r' was not identified in the Fars donors.

Conclusion: Identifying the prevalence of blood group antigens in donors from each region is crucial for organizing negative antigen blood units, preparing compatible blood for multitransfused patients, and preventing the development of alloantibodies in these patients.

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Introduction

Blood groups are determined by inherited characteristics of red blood cell (RBC) membrane antigens. Blood group systems consist of one or more antigens produced by one or more closely related genes. The International Society of Blood Transfusion has identified 43 blood group systems and more than 300 RBC antigens (1,2). The Rh blood group system is the second most important blood group system, after the ABO system, and consists of 50 defined blood group antigens. Among these, the five most important antigens are D, C, c, E, and e (3).

Antigen D is the most immunogenic of these antigens. The clinical significance of these antigens lies in the fact that if a person lacks one or more antigens and receives RBCs containing those antigens, antibodies are likely to be produced against them, leading to alloimmunization and hemolytic reactions. The group most at risk of these problems is multitransfused patients (4). Determining Rh phenotypes can play a crucial role in preventing alloimmunization in multitransfusion cases. In most blood banks, only the ABO and RhD blood group systems are matched for blood donors and recipients when RBCs are transfused (5). The rate of alloimmunization, or the production of antibodies that may potentially destroy foreign or donor RBCs, is significantly higher (8-76%) among patients receiving multitransfusions, such as those with sickle cell disease and thalassemia, and it increases with repeated transfusions (6). In countries where phenotyping is mandatory, such as France since 2002, post-transfusion alloimmunization has become rare (7).

A healthy and compatible blood supply is a critical issue for blood banks and hospitals, especially for patients with thalassemia major. Therefore, this study investigated the frequency and prevalence of major Rh blood group antigens in donors to provide healthy and compatible blood for recipients and reduce the incidence of alloimmunization and hemolytic reactions. This is the first step in determining the frequency of these antigens in the donor population and in creating a bank of negative antigen cells.

Methods

A descriptive study was conducted on 576 healthy blood donors (269 Turkmen and 306 Fars) with a 95% confidence level from June 2020 to July 2021 at the Golestan Blood Bank. Each donor was considered only once. The age of the blood donors ranged from 20 to 50 years, with an average age of 33 ± 7.7 years.

For antigen typing, a 6 mL blood sample was collected at the time of donation through the diversion pouch into a tube containing ethylenediaminetetraacetic acid. All collected blood units were phenotyped for the major Rh antigens (D, C, c, E, e). Units that tested positive for the Rh D antigen were labeled as Rh-positive. Units that tested negative for the Rh D antigen were further tested for weak expression of the D antigen. The pattern of reactions was evaluated, and based on the agglutination or non-agglutination of globular samples with antisera, the phenotype of each sample was determined.

For statistical analysis, data were analyzed using SPSS for Windows version 19. The frequencies of Rh antigens and phenotypes were calculated by dividing the number of samples with positive results by the total number of samples. Chi-square values were calculated to compare the frequency of antigens and the distribution of haplotypes between different ethnic groups. A p-value < 0.05 was considered significant. The findings of this study were also compared with similar studies conducted in other regions.

Results

A total of 575 blood donors were included in the study, and their blood samples were typed for antigens in the major Rh blood group system. The study included donors aged 20 to 50 years, with an average age of 33 ± 7.7 years. For the Rh blood group system, all samples were typed for the antigens D, C, E, c, and e. RhD typing, along with typing for other major Rh antigens, was performed on all donors. Of the samples, 87.76% were D positive and 12.24% were D negative. Among the five major antigens, the e antigen was found to be the most common at 93.59%, followed by C (73.6%), c (72.1%), and E (30.83%), which was the least common (Table 1). The most common phenotype observed was R1R1 followed by R1r and R1R2 among the total study population. The most common phenotype among Rh-positives was R1R1, while among Rh negatives it was rr. There were significant differences in the frequencies of the R1R1 (P-value = 0.0007), R0r (P-value = 0.002), and rr (P-value = 0.01) phenotypes between the Turkmen and Fars donors (Table 2).

Table 1. Frequency of rh antigens based on serological response in the population of the present study and other studies

Study population		D%	C%	E%	c%	e%	Ref
Country	This study (Iran)	87.76	73.6	30.83	72.1	93.59	-
	This study (Iran)	83.33	67.66	30.06	78.43	93.14	-
	This study (Iran)	92.19	79.55	31.6	65.8	94.05	-
	Iran	90.2	75.9	29.5	73.9	97.9	14
	Iran	87.1	71.29	32.9	74.19	95.16	9
	Iran	-	81.23	34.6	72.66	95.3	16
	Iran	92.88	73.71	43.69	71.44	91.34	12
	Pakistan	94.94	87.53	21.41	61.18	98.59	17
	China	98.94	88.81	50.78	58.43	92.28	13
	Iraqi	-	74.8	30.6	69.6	95.2	15
	Pakistan	95	89.6	22.6	62.8	97	18
	India	93.96	87	20	58	98	19
	India	84.34	81.74	21.74	56.52	100	20
	Morocco	89.81	69.67	17.18	87.07	99.14	11
Race	White	85	68	29	80	98	10
	Black	92	27	22	96	98	10

Table 2. Phenotype and frequency of possible genotype in the study population ethnics

Study population ethnics	Frequency		Phenotype		Results of reaction with antiserum					P value
	Total	Percentage			e	c	E	C	D	
Fars	67	21.89	DCe/DCe	R1R1	+	-	-	+	+	0.0007
Turkmen	93	34.57								
Fars	13	4.25	DcE/DcE	R2R2	-	+	+	-	+	0.59
Turkmen	14	5.2								
Fars	18	5.88	Dce/dce	R0r	+	+	-	-	+	0.002
Turkmen	3	1.11								
Fars	82	26.8	DCe/dce	R1r	+	+	-	+	+	0.75
Turkmen	69	25.65								
Fars	32	10.46	DcE/dce	R2r	+	+	+	-	+	0.27
Turkmen	21	7.8								
Fars	5	1.64	DCE/DCE	R2Rz	-	+	+	+	+	0.14
Turkmen	1	0.37								
Fars	39	12.74	DCe/DcE	R1R2	+	+	+	+	+	0.11
Turkmen	47	17.47								
Fars	2	0.65	dCe/dCe	r′r′	+	-	-	+	-	0.18
Turkmen	0	0								
Fars	37	12.09	dce/dce	rr	+	+	-	-	-	0.01
Turkmen	16	5.95								
Fars	1	0.33	dCE/dCE	ryry	-	-	+	+	-	0.34
Turkmen	0	0								
Fars	9	2.94	dCe/dce	rr′	+	+	-	+	-	0.13
Turkmen	3	1.11								
Fars	1	0.33	dcE/dce	r"r	+	+	+	-	-	0.92
Turkmen	1	0.37								
Fars	0	0	dce/dC	r"r′	+	+	+	+	-	0.28
Turkmen	1	0.37								

Table 3. Percentage of frequency of rh phenotypes in the present study and other studies

Study population		R1R1	R2R2	R0r	R1r	R2r	R2Rz	R1R2	r′r′	rr	ryry	r′r	r"r	r"r′	Ref
Country	This study	28.23	4.27	3.49	26.22	9.13	1	15.1	0.65	9.02	0.33	2.02	0.35	0.37	-
	This study	21.89	4.25	5.88	26.28	10.46	1.64	12.74	0.65	12.09	0.33	2.94	0.33	0	-
	This study	34.33	5.2	1.11	25.65	7.8	0.37	17.47	0	5.95	0	1.11	0.37	0.37	-
	Iran	25	1.7	4.2	31.8	9.6	0.4	16.5	-	8.3	-	1.21	0.2	0	14
	Iran	22.26	3.55	5.16	27.42	9.68	0.32	16.45	0.97	10	-	1.29	0.32	0.65	9
	Iran	26.9	3.4	3.7	28.2	10.6	0.001	16.3	-	5.7	-	1.21	0.4	0.001	12
	Iraqi	34	5.4	2.2	29.6	10.5	-	18.3	-	8.8	-	1.8	-	-	15
	India	44.6	0.8	1.3	32.6	5.9	-	14	-	0.07	-	-	-	-	21
	India	35.2	0.7	2.2	30.7	5.9	-	8.1	-	0.3	-	2.5	Rare	-	22
	India	35	2	-	30	8	-	13	-	7	-	2	-	-	23
	China	40.72	0.14	0.35	7.51	3.61	0.64	38.46	-	0.28	-	0.21	-	0.43	13
	Pakistan	37.7	-	-	33.4	5.2	-	19.4	-	4.3	-	-	0.43	-	18
Race	White	18.5	2.3	2.1	34.9	11.8	0.1	13.3	-	15.1	-	0.8	-	0.9	10
	Black	2	0.2	45.8	21	18.6	-	4	-	6.8	-	-	-	-	10

The lowest frequencies of phenotypes in Rh-positive and Rh-negative donors were associated with R2Rz and r"r', and r"r and ryry, respectively (Table 3). The phenotype r"r′ was not detected in the Fars donors, and the phenotype r′r′ and ryry were not identified in the Turkmen donors.

Discussion

Although blood transfusions can save lives, they are not without risk. The immune system may produce alloantibodies when exposed to incompatible RBCs, and these antibodies can then bind to donor cells, leading to hemolytic transfusion reactions. Patients with Rh alloantibodies should receive blood that lacks these antigens. This requires the determination of the immunological characteristics of both blood products and recipients through immunohematology analyses, such as phenotyping in the Rh blood group system.

Rh system antibodies play a crucial role in blood transfusion. The production of alloantibodies against foreign antigens can cause a range of reactions, from mild to severe, including hemolytic disease in the fetus and infant, as well as hemolytic reactions following blood transfusions (8).

In this study, 87.76% of donors (Fars and Turkmen) were Rh-positive, similar to findings in Isfahan (9), the white population (10), and Morocco (11).

Among the other Rh antigens (C, c, E, e), the e and C antigens had the highest frequencies, at 93.59% and 73.6%, respectively. The frequency of the e antigen aligns with studies in Isfahan (9), Khorramabad (12), and China (13), while the frequency of the C antigen is consistent with studies from northeastern Iran (14), Khorramabad (12) and Iraqi Kurdistan (15). Compared to other regions, the highest frequency of the e antigen was observed in Morocco and India, while the highest frequency of the C antigen was found in China and Pakistan (Table 1). The E antigen had the lowest frequency at 30.83%, which is consistent with studies from northeastern Iran (14), Isfahan (9), and Iraqi Kurdistan (15) (Table 1).

The most common phenotypes among Rh-positive donors in this study were R1R1 (28.23%) and R1r (26.22%), respectively, similar to results from studies in northeastern Iran (14), Khorramabad (13), and Isfahan (10). Among Rh-negative donors, the most common phenotype was rr (9.02%), which is consistent with findings from northeastern Iran (14), Isfahan (9), and Iraqi Kurdistan (15). The lowest frequencies were observed for the R2Rz (1%) and ryry (0.33%) phenotypes in Rh-positive and Rh-negative donors, respectively. The r'r" phenotype was not detected in the Fars donors, which is similar to the results from northeastern Iran (14). Moreover, the r′r′ and ryry phenotypes were not identified in the Turkmen donors (Tables 2 and 3). These findings suggest that the frequency of Rh system antigens and phenotypes varies across different geographical regions, highlighting the need for independent studies in each region. ABO and Rh(D) blood types are routinely tested at blood transfusion centers, the other four Rh antigens (C, c, E, e) are not. This gap means that if a recipient lacks one of these antigens, there is a risk of alloimmunization. This study was an initial effort to identify the major Rh blood group system among blood donors in Golestan Province to enhance healthcare services and better serve patients.

Conclusion

Outcomes of such studies can be used to determine the prevalence of antigens in each region, manage blood consumption, organize negative antigen blood units, maintain existing blood reserves, reduce blood transfusion reactions, minimize alloimmunization, and identify rare phenotypes.

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Ethical statement

All stages of the implementation of this study were carried out according to the instructions of the Iranian Blood Transfusion Organization. Ethics approvals and consent to participate were not applicable.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Author contributions

All authors contributed to one or more aspects of the study.

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