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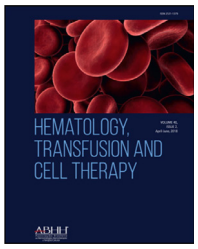

Original article

Frequency of Rh and K antigens in blood donors in Riyadh

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ABSTRACT

Objectives: Investigate the prevalence of Rh and the K antigens and their phenotypes in the red blood cells of blood donors in Riyadh, Saudi Arabia.
Methods: This is a retrospective study. The five principal Rh antigens (D, C, c, E, e) and the Kell antigen from the Kell blood group were tested in 4,675 random samples collected from four blood bank centers in Riyadh. Data were collected for seven weeks (from January 4, 2019 to February 28, 2019). Antigens were tested using the TANGO Optimo system.
Results: We found that approximately 86% of the donors had the D antigen, 66% had C, 78% had c, 26% had E, 97% had e and 14% had K. The most common Rh phenotypes were R1r (31%) and R1R1 (22%).
Conclusion: The differences in the results between the study population and other populations, such as Caucasian, Indian and African populations indicate the importance of establishing a population-specific database.

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Introduction

More than 350 antigens have been identified on the red blood cell (RBC) membrane and most of them have been assigned to 36 systems.[1] After the ABO system, the most important antigens in transfusion belong to the Rh system, followed by the Kell (K) antigen from the Kell system.[2] The clinical importance of RBC antigens is associated with their ability to induce alloantibodies, as well as the ability of these antibodies to cause RBCs destruction.

The antibodies of the ABO system occur naturally, while the Rh and Kell antibodies are immunogenic. The immune system may produce alloantibodies when it is exposed to incompatible RBCs and these antibodies can then bind to donor cells, leading to hemolytic transfusion reactions. Patients with Rh and Kell alloantibodies should be provided with blood lacking these antigens. These antibodies are also known to cause severe hemolytic disease in newborns. Thus, it is important to provide females of child-bearing age with compatible blood to minimize the chance of sensitizing them with these clinically important antigens. In Saudi Arabia (SA), there is a high prevalence of hemoglobinopathies, approximately 0.3% having sickle cell disease and 0.1%, thalassemia.[3] Alloimmunization has been detected in 13 to 18% of sickle cell disease patients[4,5] and 20% of thalassemia patients[6] and the most frequently detected antibodies in these patient groups are Rh and Kell, respectively. Furthermore, approximately 2% of women with a history of pregnancy were alloimmunized and the vast majority of them developed anti-D antibodies.[7]

The allele frequencies of the RBC antigens vary by race and there is a statistically significant difference in antigen

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prevalence among different populations. The D antigen, for example, is present in approximately 85% of Caucasians and 99% of the Chinese.[8] Knowing the antigenic frequencies in the population helps blood banks to improve the management of blood stocks. Moreover, since it is important to select specific antigen-negative blood when dealing with patients with alloantibodies, this information is necessary to predict the availability of the required units.

Several studies have been conducted to establish the antigenic frequencies of the RhD and ABO antigens in SA.[9–11] However, scant information has been found on the frequencies of the most clinically significant Rh antigens C, c, E and e, or the K antigen, and most of the studies were conducted in the eastern region of SA.[12,13] Although Riyadh is the capital city and has a number of the largest hospitals, not enough data were found regarding the prevalence of these antigens. Therefore, the present study aimed to determine the frequencies of the Rh and K antigens and their phenotypes in blood donors in Riyadh.

Methods

This retrospective study was approved by the Institutional Review Board, College of Medicine, King Saud University (19/0616). The inclusion and exclusion criteria depended on blood donation; donors of both sexes, Saudis and non-Saudis who had donated blood or one blood product were included, while those who did not complete the donation process were excluded. The criteria for the blood donors were recommended by the Saudi Central Board for Accreditation of Healthcare Institutions (CBAHI).[14]

The data of 4,675 voluntary donors were collected from four CBAHI-accredited blood bank centers in Riyadh to determine the frequencies of the five principal Rh antigens (D, C, c, E, and e) and the K antigen. A total of 5 ml of peripheral whole blood was collected in EDTA tubes from blood donors who provided informed consent. The D, C, c, E, e, and K phenotyping was performed using the TANGO Optimo Automated Blood Bank Analyzer System (Bio-Rad Laboratories, Hercules, CA, USA). Weak D samples were considered as D positive.

For statistical analysis, data were inputted and analyzed using the Windows version of Microsoft Excel 2010. The antigens and Rh phenotype frequencies were calculated by dividing the number of samples with positive results by the total number of samples. Allele frequencies of Rh and K and the Kell phenotype were calculated by the Ceppellini method[15] under the assumption of the Hardy-Weinberg equilibrium. The Fisher method[16] was used to find the variances of square root estimates of the Rh haplotypes frequencies, as previously described.[17]

Results

The samples from the 4,675 blood donors from four blood bank centers in Riyadh were tested. We determined the frequencies of the five principal Rh antigens (D, C, c, E and e) and the K antigen of the Kell blood group and calculated the maximum-likelihood estimates for *RH* and *KEL* allele frequencies (Table 1). The e antigen was the most frequently occurring Rh antigen (97.2%), while the E antigen was the least common one (25.8%). Approximately 14% of the tested donors had the K antigen. The phenotypes (K, K), (K, k), and (k, k) were calculated to be 0.5%, 13.4% and 86.1%, respectively, using the Hardy-Weinberg formula.

Table 1 – Antigenic and allelic frequencies of Rh (D, C, c, E, and e) and K.

Antigen	Observation, n	Antigen frequency, %	Allele frequency*, fraction
D	4,041	86.4	0.63
C	3,079	65.9	0.44
c	3,652	78.1	0.56
E	1,208	25.8	0.14
e	4,545	97.2	0.86
K	647	13.9	0.08

* Calculated using the Hardy-Weinberg formula.

The Rh haplotype frequencies were calculated (Table 2); The most common haplotype was R1 (0.45), followed by r (0.34). The Rh phenotype frequencies were also determined (Table 3); R1r was the most common one, with approximately 31% of the donors having this phenotype, followed by R1R1 (22%), R1R2 (12%) and rr (12%).

Discussion

The prevalence of blood phenotypes varies across populations. Data collected from local populations help blood bank centers manage their inventories more efficiently. Knowledge of antigenic frequencies also helps in cross-matching blood for patients with alloantibodies, as it is used to estimate the number of units that lack the corresponding antigens. If such data are not available, a blood bank center might resort to random cross-matching or rely on data from different populations, which may lead to inaccurate results. In this study, we determined the Rh and K antigen, allele, haplotype and phenotype frequencies in more than 4,600 donors in Riyadh.

The D antigen is the most clinically significant antigen in the Rh system. It is a highly immunogenic antigen; more than 25% of D-negative recipients have been found to develop anti-D when exposed to the antigen.[18] In this study, approximately 86% of the donors in Riyadh were D-positive, which is consistent with the findings of Elsayid *et al.*[19] Nomani *et al.*,[20] who studied Saudi donors in Riyadh, found an even higher incidence of the D antigen (approximately 91%). The prevalence of D is even higher in some regions, such as Jazan, where only 5% of donors lack the antigen.[21] The different percentages between our study and the previous two studies could be due to differences in donors' ethnicity. In our study, we investigated the prevalence in Riyadh donors, including Saudis and non-Saudis. It is expected that around 15% of the donors in Riyadh are foreigners.[22]

A few studies have investigated the frequencies of other major antigens of the Rh system in Saudi populations. In this study, we found that the e antigen had the highest frequency, followed by c. This is consistent with the findings of other studies conducted in Riyadh[19,20] and an eastern region of

Table 2 – Frequencies of Rh haplotypes.

Frequency	Haplotypes							
	DCe (R1)	DcE (R2)	Dce (R0)	DCE (Rz)	dCe (r')	dcE (r'')	Dce (r)	dCE (ry)
	0.4516	0.0733	0.1073	<0.0001	0.0157	0.0049	0.3472	<0.0001

Table 3 – Rh phenotype frequencies.

Rh phenotype	Most common genotype	Observation, n	Rh phenotype frequency, %
CCDEe	R1Rz	2	0.04
CCDee	R1R1	1,018	21.78
ccDEE	R2R2	127	2.72
ccDEe	R2r	486	10.40
ccDee	R0r	402	8.60
CcDEe	R1R2	574	12.28
CcDee	R1r	1,431	30.61
CcDEE	R2Rz	1	0.02
ccdee	rr	564	12.06
ccdEe	rr''	16	0.34
Ccdee	r'r	49	1.05
CCdee	r'r'	3	0.06
CcdEE	r''ry	1	0.02
ccdEE	r''r''	1	0.02

SA.[13] Our results are also in line with studies on Caucasian[23] and African populations.[24] In contrast, a study on a Chinese population reported a significantly higher prevalence of the C antigen and a lower prevalence of the c antigen (approximately 88% and 60%, respectively).[25]

The distributions of the Rh haplotype and phenotype differ between populations. We found that the most common haplotypes in Riyadh were R1 and r. This was reflected in the Rh phenotypes, the most frequent of which was R1r, followed by R1R1. Our results are consistent with those of Elsayid *et al.*[19] and with findings obtained from the eastern regions of SA.[12,13] Similar findings are expected in other regions of SA, as the vast majority of the population is of the same ethnic background (Arab).[26] The prevalence of these phenotypes is also similar to that in another Arab population[27] and in Caucasian populations.[23] In contrast, studies on an Indian[28] and an African[24] population reported that the most frequent phenotypes were R1R1 and R0r, respectively.

Although the Kell antigen is one of the most clinically significant RBC antigens, few studies have investigated its prevalence in SA. According to our results and the findings of other Saudi studies,[12,29] we expected that the prevalence of the k antigen would be more than 99%. A significantly higher percentage of heterozygous Kell (K, k) has been reported in Saudis[17,20] than in Caucasians (9%),[23] Africans (0%)[24] and Indians (3.5%).[28] In this study, we found that K was a polymorphic antigen carried by approximately 14% of donors from Riyadh. This result is consistent with those obtained from the central region of SA[29] and another Arab population.[30] On the other hand, a study in the eastern region reported a 6% lower prevalence,[12] which may have been due to the study's small sample size (100 participants).

Detecting variations in the expression of RBC antigens in a population can help assess the risk of allosensitization in recipients and pregnant women. Our results suggest that K-negative recipients and women of child-bearing age have a higher risk of developing anti-K than populations with a lower prevalence of the K antigen. Anti-K is one of the most frequently detected antibodies in multi-transfused Saudi patients.[4–6]

The detectable variations in the prevalence of some RBC antigens between populations highlight the importance of creating a population-specific database of the most clinically significant RBC antigens. This could also aid in finding appropriate selection panels for antibody screening and identification. Most Saudi blood bank centers conduct antibody screening and identification tests using commercially available panels prepared in Western countries. Although this study shows similarities in the Rh phenotype between our population and Caucasians, more studies should be conducted to investigate differences in other clinically important antigens and the efficiency of panels prepared from donors of different ethnicities.

This study has certain limitations. Although it determined the prevalence of many of the most clinically significant antigens from the Rh and Kell blood groups, it did not investigate important antigens from other systems, such as Kidd and Duffy. However, the most common alloantibodies detected in Saudi patients are against the antigens examined in this study.[6] Moreover, our study relied on results obtained from serological tests. Although these tests are more accurate and less complicated than molecular methods,[31] using molecular approaches can provide insight into the molecular base of Saudi blood group antigens, which has yet to be studied. Knowledge of the Rh genotypes can make it possible to predict the most common Rh genotype of the Saudi population, depending on the phenotype, rather than relying on results obtained from other populations.

Conclusion

We have presented refined frequency estimates of the five principal Rh antigens and the Kell blood group antigen in Riyadh, SA. We also established reliable estimates for the Rh and Kell phenotype, Rh haplotype and allele frequencies. Similarities in the results were found between our population and other SA populations, Arabs and Caucasians in contrast to remarkable variations observed with Africans, Chinese and Indians. The high incidence of K antigens in our population suggests the importance of providing recipients and women of child-bearing age with K compatible blood. We recommend establishing a population-specific database of the prevalence of clinically significant RBC antigens.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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