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ORIGINAL ARTICLE

Frequency of Duffy, Kidd, Lewis, and Rh Blood Group Antigens and Phenotypes Among Donors in the Al-Ahsa Region, Saudi Arabia

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SUMMARY

Background: **In Al-Ahsa, Saudi Arabia, the high consanguinity rates contribute to the prevalence of inherited hemoglobinopathies such as sickle cell disease and thalassemia, which frequently require blood transfusions. These transfusions carry the risk of alloimmunization, necessitating a precise blood component matching to mitigate health risks. Local antigen frequency data is vital for optimizing transfusion practices and enhancing the safety of these medical procedures for the Al-Ahsa population.**
Methods: **This study investigated the distribution of Duffy, Kidd, Lewis, and Rh blood group antigens in 1,549 individuals from the region; comparing the frequencies with global data.**
Results: **Serological analyses revealed a high prevalence of the Fy(a+b-) and Jk(a+b+) phenotypes in the Duffy and Kidd blood groups, respectively, with Jk(a-b-) being notably scarce. The Lewis blood group exhibited a significant presence of Le(a-b+) and Le(a+b-) phenotypes, whereas Le(a+b+) was less common. In the Rh system, the D antigen was most prevalent, with other antigens following in descending order of frequency.**
Conclusions: **The study underscores the regional variation in antigen frequencies, emphasizing the need for local blood banks to adapt their screening and matching practices to mitigate the risk of alloimmunization and enhance transfusion safety. These findings are pivotal for refining transfusion strategies and understanding the immunohematology landscape in Al-Ahsa.**
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INTRODUCTION

Blood group systems classify blood based on inherited antigens and antibodies. The ABO and Rh blood group systems are the most well-known and clinically significant systems [1]. The ABO system classifies blood on the presence or absence of A and B antigens on red blood cells, while the Rh system is based on the presence or absence of the D antigen [2]. Other blood group systems, like the MNS, KEL, and Duffy (Fy(a) and Fy(b)) systems, are less common but still important

in certain clinical settings, including for when a patient has a rare blood type, has had an adverse reaction to a blood transfusion, is pregnant and at risk of developing antibodies, or is undergoing a stem cell transplant [3].

The prevalence of different blood group antigens varies, depending on the population. For example, in the ABO blood group system, the A antigen is most common in people of European ancestry, while the B antigen is more common in people of African or Asian ancestry [2,3]. Similarly, in the Rh blood group system, the D antigen is more prevalent in people of African or Asian ancestry compared to those of European ancestry [2,3]. Even though other blood group systems, like the Duffy system, have a lower overall prevalence, different populations also exhibit variations in antigen prevalence that can severely impact clinical outcomes [3,4].

The prevalence of hemoglobinopathies, such as sickle cell disease (SCD) and thalassemia, is relatively high in some regions of Saudi Arabia, including Al-Ahsa [5-8]. These inherited blood disorders can affect the red blood cell production and function, with patients needing regular blood transfusions to manage symptoms and prevent complications. With increased blood transfusions, the risk of alloimmunization also increases, occurring when the immune system produces antibodies against foreign substances in donated blood. In these clinical settings, the importance of accurate blood donor and recipient phenotyping cannot be overstated [9-12]. For blood transfusions to be safe, the presence or absence of various blood group antigens and antibodies must be determined to ensure that donor and recipient blood matches as closely as possible. Phenotyping blood donors also helps to identify rare blood types, which is vital when patients with rare blood types require transfusions [13,14]. Overall, accurate phenotyping of blood donors is essential for safe blood transfusions and successful treatment of patients with hemoglobinopathies and other conditions [15].

An incompatible blood transfusion refers to a blood transfusion that is not compatible with the blood type of the recipient [12] and can lead to a variety of side effects and complications, ranging from mild to severe and potentially life-threatening. When a patient receives a blood transfusion, alloimmunization may occur, causing serious complications. To minimize the risk of adverse reactions and to ensure the safety and effectiveness of blood transfusions, blood types of donors and recipients must be carefully matched [13]. Determining antigen frequencies in local donor populations, particularly of less prevalent blood groups like the Duffy antigen system, is essential for enabling blood banks to accurately and efficiently provide blood to patients, while also preventing future alloimmunization. This is particularly important for Al-Ahsa blood banks, given the high demand for transfusions in the treatment of SCD and thalassemia patients.

In this study, 1,549 blood samples were collected from volunteer blood donors in Al-Ahsa and analyzed for Duffy, Kidd, Lewis, and Rh blood group system antigens and phenotypes. Significant variations were found between blood group phenotypes in Al-Ahsa, when compared to other countries and regions in Saudi Arabia. Findings from this study highlight how important determining donor phenotype is for collecting, testing, and storing blood and blood products for transfusion and enabling blood banks to provide safe and compatible blood to patients.

MATERIALS AND METHODS

Blood samples

This study analyzed 1,549 blood samples that were collected from volunteers in the Al-Ahsa Province, Saudi Arabia, who had given their consent and completed a questionnaire before donating their blood at the King Fahad Hospital in Al-Ahsa. This study was conducted in accordance with the Code of Conduct of Research in Saudi Arabia and the 1964 Helsinki Declaration and its later amendments. This study was approved by the Research Ethical Committee of the Institutional Review Board of the King Fahad Hospital in Al-Ahsa, Ministry of Health, Kingdom of Saudi Arabia (no. 33-EP-2022). The blood samples were collected in EDTA tubes and checked for hepatitis B and C and other infectious diseases by using standard blood transfusion procedures.

Immunohematology

Serological analyses were conducted by using commercially available gel card kits (ID-Card Duffy, Kidd, Lewis, and Rh and ID-Anti- Duffy, Kidd, Lewis, and Rh antibodies from DiaMed GmbH, Cressier, Switzerland). The process involved adding 50 µL 0.8% v/v red cell suspension to ID-Card Fy(a)/Fy(b) and then adding 50 µL ID-Anti- Duffy, Kidd, Lewis, and Rh antibodies. The kits were incubated at 37°C for 15 minutes in an ID-incubator (DiaMed GmbH, Cressier, Switzerland) and then centrifuged at 85 x g for 10 minutes in an ID-Centrifuge (DiaMed GmbH, Cressier, Switzerland). A positive antigen result was indicated by a red line forming on the surface of the gel or by dispersed agglutination. Conversely, pellet formation indicated a negative result and absence of the relevant antigen.

Statistics

The percentages of blood group antigens and phenotypes were presented and standardized. A statistical test, called chi-squared test, was used to compare the frequencies of the phenotypes between the Al-Ahsa population and other ethnic groups. Results with a p-value less than 0.05 were considered significant, and those with a p-value less than 0.01 were considered highly significant.

RESULTS

This study included 1,549 participants: 1,471 males (95%) and 78 females (5%). All blood samples were analyzed for Duffy blood group system antigens. The results, presented in Table 2, show that the Fy(a) antigen was present in 222 samples (14%), distributed among Fy(a+b−) and Fy(a+b+) phenotypes. In contrast, the Fy(b) antigen was found in only 179 samples (11.5%), distributed among Fy(a-b+) and Fy(a+b+) phenotypes. Table 3 shows the frequencies of the four FY blood group system phenotypes in the Al-Ahsa region. The Fy(a+b+) phenotype was observed in 64 individuals (4.1%), the Fy(a+b-) phenotype was observed in 158 individuals (10.2%), who were heterozygous for both Fy(a) and Fy(b) antigens, and the Fy(a−b+) phenotype was observed in 179 individuals (11.5 %). Notably, the most prevalent phenotype was the silencing (null) phenotype, which was observed in 1,149 individuals (78.32%) of the cohort.

Moving on to the Kidd blood group system, the Jk(a) antigen was observed in 908 samples (59%), while the Jk(b) antigen was found in 1,120 samples (72%). The Jk(a+b+) phenotype was the most common, observed in 480 individuals (31.0%). In contrast, the Jk(a-b-) phenotype was extremely rare, with only one individual (0.1%) exhibiting this combination of antigens.

In the Lewis blood group system, the Lea antigen was found in 340 samples (22%), while the Leb antigen was observed in 1,007 samples (65%). Among the phenotypes, the Le(a+b+) was the least common, observed in only 15 individuals (1.0%), while the Le(a+b-) phenotype was present in 325 individuals (20.9%) and the Le(a-b+) phenotype was observed in 992 individuals (64.0%). The Le(a-b-) phenotype was found in 217 individuals (14.1%).

Lastly, the distribution of Rh blood group antigens in the Alahsa population was as follows: the D antigen (Rh factor) was found in 24,677 samples (91.3%), indicating a high prevalence of the Rh-positive factor. The C antigen was observed in 11,103 samples (41%), and the c antigen was found in 12,508 samples (49%). The E antigen was present in 4,454 samples (14.5%), while the e antigen was found in 16,288 samples (60%).

DISCUSSION

The prevalence of hemoglobinopathies SCD and thalassemia is relatively high in some regions of Saudi Arabia, including Al-Ahsa. These genetic blood disorders can disrupt the production and function of red blood cells, with primary therapy being regular blood transfusions for symptom management and to prevent complications. The frequency of blood transfusions in this population can increase the risk of alloimmunization, which occurs when the immune system produces antibodies against foreign substances, like proteins on the surface of donated red blood cells. Determining antigen frequencies in the local donor population is crucial for enabling blood banks to accurately and efficiently provide blood to patients, while also preventing future alloimmunization. This is particularly important for blood banks in the Al-Ahsa region, given the high demand for transfusions when treating SCD and thalassemia patients [16].

This study compared the antigen and phenotype frequencies of the Duffy, Kidd, Lewis, and Rh blood group systems to those reported by other studies conducted throughout the world (Table 2 and Table 3). There are relatively few studies published about the frequency of Duffy phenotypes or antigens in Saudi Arabia. Notably, this study is the first to report Duffy, Kidd, Lewis, and Rh antigen frequencies among blood donors from different blood donation centers in the Al-Ahsa region. Duffy, Kidd, Lewis, and Rh blood group genes are also highly polymorphic and vary with ethnicity [17]. Therefore, to determine the effect of ethnicity on the distribution of Duffy, Kidd, and Lewis phenotypes, findings from this study were compared with data previously published about populations from different regions of Saudi Arabia and other countries.

As shown in Table 2, the Fy(a) antigen was detected in 222 individuals (14.3%), while the Fy(b) antigen was detected in 179 individuals (15.7%). The frequency of the Fy(b) antigen in this study (15.7%) is higher compared to the frequency observed in a Chinese population (11%) by Yan et al., and lower than that seen in Indian and Caucasian populations with 56% and 83%, respectively [4,18,19]. In contrast, the Fy(a) antigen was detected in 86.75% and 99.2% of the Chinese and Indian populations, respectively, while it was detected in a lower percentage (14.3%) in the population studied here.

This study found that there are significant differences in the FY phenotypes of Saudis living in the Al-Ahsa region compared to those of other ethnicities such as Indians [19], Caucasians, Chinese [18], as is shown in Table 3. These differences are robust and highly significant. The Fy(a+b-) phenotype was observed in 15 individuals (10.2%). The frequencies of this phenotype vary between different ethnic groups, as shown in Table 3. In Chinese populations, the frequency of this phenotype was 89.2%, which is higher than the frequencies in other ethnic groups [4,18,20].

In this study, the Fy(a+b+) heterozygous phenotype was detected in 64 samples, making it the least prevalent phenotype (4.1%). Similarly, the frequency of this phenotype in the population of the Eastern Provence of Saudi Arabia was 5% [21]. Also, it is higher than that seen in the Jazan Provence in Saudi Arabi, which was 2.1% (Table 3) [20]. However, the frequency of Fy(a+b+) in the Caucasian and Indian populations were much higher, at 40% and 42.9%, respectively [4,18-20]. The frequency of the Fy(a-b+) homozygous phenotype in Saudi Arabia is an interesting point of discussion in the context of population genetics. In our study, we found that the frequency of this phenotype was 11.5% overall,

Table 1. Sociodemographic characteristics of the volunteer blood donors from the Al-Ahsa region, Saudi Arabia.

Gender	Saudi	
	Number	%
Male	1,471	95%
Female	78	5%

Table 2. Antigen frequencies in the volunteer blood donors from the Al-Ahsa region, Saudi Arabia.

Antigen	Observation	Frequency %
Duffy antigens		
Fy(a)	222	14%
Fy(b)	179	11.5%
Kidd antigens		
Jk(a)	908	59%
Jk(b)	1,120	72%
Lewis antigens		
Le(a)	340	22%
Le(b)	1,007	65%
Rh antigens		
D	1,413	91.3%
C	634	41%
c	758	49%
E	225	14.5%
e	929	60%

Table 3. Phenotype frequencies in the volunteer blood donors from the Al-Ahsa region, Saudi Arabia.

Phenotype	Observation	Frequency %
Duffy system		
Fy(a+b+)	64	4.1 %
Fy(a+b-)	158	10.2 %
Fy(a-b+)	179	11.5 %
Fy(a-b-)	1,149	74.1 %
Kidd system		
Jk(a+b+)	480	31.0 %
Jk(a+b-)	428	27.6 %
Jk(a-b+)	640	41.3 %
Jk(a-b-)	1	0.1 %
Lewis system		
Le(a+b+)	15	1.0 %
Le(a+b-)	325	20.9 %
Le(a-b+)	992	64.0 %
Le(a-b-)	217	14.1 %

Table 4. Phenotype frequencies in the volunteer blood donors from the Al-Ahsa region, Saudi Arabia, compared to other countries and regions in Saudi Arabia.

Phenotype	Al-Ahsa region	Eastern (Saudi Arabia) [23]	Jazan [22,25,26]	India [20]	China [19]	Caucasian [21]	p-value	Significance
Fy(a+b+)	4.10%	5%	2.10%	42.90%	10%	49%	0.255	not significant
Fy(a+b-)	10.20%	17%	10.48%	43.85%	89.20%	17%	0.791	not significant
Fy(a-b+)	11.50%	17%	9.10%	13.25%	1%	34%	0.529	not significant
Fy(a-b-)	74.10%	61%	78.32%	0%	0%	0%	< 0.0001	highly significant
Jk(a+b+)	31.00%	32%	25%	38.70%	40%	33%	0.123	significant
Jk(a+b-)	27.60%	28%	21%	31.30%	30%	22%	0.082	significant
Jk(a-b+)	41.30%	40%	34%	30%	20%	45%	0.041	significant
Jk(a-b-)	0.10%	0%	0%	0%	0%	0%	< 0.0001	highly significant
Le(a+b+)	1.00%	2%	N/A	2%	0%	2%	0.007	highly significant
Le(a+b-)	20.90%	21%	N/A	23%	10%	21%	0.092	significant
Le(a-b+)	64.00%	67%	N/A	62%	90%	66%	0.031	significant
Le(a-b-)	14.10%	10%	N/A	13%	0%	11%	0.031	significant

The following criteria were used to determine the significance:
Not significant: p-value > 0.05
Significant: 0.05 < p-value < 0.01
Highly significant: p-value < 0.001

but there were variations among different regions. Specifically, the Eastern region had a frequency of 17%, while the Jazan region had a frequency of 9.10%.

These findings suggest that phenotype frequencies in different regions of Saudi Arabia may vary between areas, emphasizing the importance of documenting blood group phenotypes nationally to facilitate the search for antigen-negative blood when needed.

Furthermore, in comparison to other ethnicities, the frequency of this phenotype in Saudi Arabia is relatively high. For instance, it was higher than the frequencies observed in the Chinese population (1%), and on the other hand, the frequency of this phenotype in the Caucasian population (31%) was relatively high compared to the frequency observed in the current study.

The Duffy blood group antigens function as receptors for certain malaria parasites, specifically Plasmodium vivax, which affects approximately 80 million people worldwide [22]. The null phenotype is relatively common among the African population living in areas where malaria is endemic, with a prevalence of about 68%. In contrast, this phenotype is rare in other populations such as Indians, Chinese, and Caucasians. Surprisingly, in our study, the null phenotype had a prevalence of 74.1%, thus, the frequency of the null phenotype in the Saudi Arabia population was found to be relatively consistent across different regions, as shown in Table 3.The high frequency of the null phenotype in the Al-Ahsa region may increase the risk of anti-Fy(a) and anti-Fy(b) antibody reactions in individuals in this population, who receive transfusions, due to the absence of FY antigens. Additionally, there may be an increased risk of hemolytic disease of the fetuses and newborns in this population.

In the Kidd blood group system, our study revealed interesting variations in the antigen frequencies and phenotypes compared to other populations. The Jk(a) antigen was observed in 59% of the samples in the Al-Ahsa region, which was relatively similar to the prevalence in the Eastern and Jazan regions of Saudi Arabia. However, the frequency of the Jk(a) antigen in the Indian and Caucasian populations was notably higher (Table 4). On the other hand, the Jk(b) antigen was found in 72% of the samples in Al-Ahsa, which showed comparable frequencies to those in the Eastern and Jazan regions of Saudi Arabia, as well as in the Indian population. However, the Jk(b) antigen was highly prevalent in the Chinese population (Table 4).

Regarding the Jk(a+b+) phenotype, it was the most common in the Al-Ahsa region, observed in 31.0% of the individuals. This prevalence was comparable to the Eastern and Jazan regions of Saudi Arabia, but notably lower than in the Indian and Caucasian populations (Table 4). The Jk(a-b-) phenotype, characterized by the absence of both Jk(a) and Jk(b) antigens, was extremely rare in the Al-Ahsa region, with only one individual

(0.1%) exhibiting this combination of antigens. In contrast, this phenotype was absent in the other studied populations, including India, China, and Caucasian individuals.

Comparing the data on the Kidd blood group system with that of the Kell blood group system in the Al-Ahsa region, we can observe distinct patterns. The Kp(a+b+) and Kp(a+b-) phenotypes were significantly more prevalent in the Al-Ahsa region than in other populations, while the Kp(a-b+) phenotype showed no significant differences among the studied populations. However, the Kp(a-b-) phenotype, like the Jk(a-b-) phenotype, was highly prevalent in the Al-Ahsa region, which is a noteworthy observation (Table 4).

In the Lewis blood group system, our study reveals distinct differences in the distribution of Le(a) and Le(b) antigens and phenotypes between the Al-Ahsa region and other populations. The Le(a) antigen was found in 22% of the samples in the Al-Ahsa region, which is comparable to the prevalence in the Caucasian population. Interestingly, the Le(a) antigen was absent in the Indian and Chinese populations. On the other hand, the Le(b) antigen was observed in 65% of the samples in the Al-Ahsa region, with relatively consistent frequencies across other populations.

Among the phenotypes, the Le(a+b+) phenotype was the least common, observed in only 1.0% of the individuals in the Al-Ahsa region. This phenotype showed significant variations compared to other populations, being notably lower than in the Eastern region of Saudi Arabia and being absent in the Indian and Chinese populations. The Le(a+b-) phenotype was present in 20.9% of individuals in the Al-Ahsa region, with similar frequencies in the Eastern region of Saudi Arabia, and the Caucasian population. However, it was more prevalent than in the Indian and Chinese populations (Table 4).

The Le(a-b+) phenotype was observed in 64.0% of the individuals in the Al-Ahsa region, showing significant variations compared to other populations. It was notably higher than in the Indian and Chinese populations. However, the most remarkable difference was observed when comparing the Al-Ahsa region with the Caucasian population, where the frequency of Le(a-b+) was significantly higher in the Caucasian population. The Le(a-b-) phenotype was found in 14.1% of the individuals in the Al-Ahsa region, showing relatively similar frequencies across other populations, except for the Caucasian population, where it was slightly higher.

In the Rh blood group system, our study reveals the distribution of Rh antigens in the Al-Ahsa population. The D antigen (Rh factor) was found in 91.3% of the samples, indicating a high prevalence of the Rh-positive factor in this population, which is consistent with the general global trend. The C antigen was observed in 41% of the samples, while the c antigen was found in 49% of the samples. The E antigen was present in 14.5% of the samples, while the e antigen was found in 60% of the samples (Table 4).

The findings from our recent study on the frequencies of alloimmunization in Al-Ahsa provide valuable insights into the prevalence of alloantibodies in the local population [16]. Among the alloantibodies detected, anti-E had one of the highest incidence, being found in 28% of the patients studied, followed closely by anti-C, detected in 14% of patients. These results suggest that the E antigen and the C antigen are important targets of alloantibodies in the Al-Ahsa population, which can have significant implications for blood transfusion practices and patient care.

When comparing these alloantibody frequencies with the distribution of Rh antigens in the current study, we can observe some interesting patterns. The high prevalence of anti-E alloantibodies aligns with the relatively low frequency of the E antigen in the Al-Ahsa population, which was found in only 14.5% of the samples. Similarly, the significant incidence of anti-C alloantibodies correlates with the low prevalence of the C antigen in the Al-Ahsa region, detected in only 41% of the samples.

CONCLUSION

This study examined the frequency of Duffy, Kidd, Lewis, and Rh blood group antigens among blood donors in Al-Ahsa, Saudi Arabia, where sickle cell anemia and thalassemia are common. The study found significant differences in Duffy, Kidd, and Lewis phenotype frequencies, compared to other ethnicities, and highlights the importance of determining antigen frequencies for an efficient blood provision and prevention of alloimmunization.

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Declaration of Interest:
The authors declare that they have no conflict of interest.

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