Journal of Blood Medicine

Dovepress
Taylor & Francis Group

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ORIGINAL RESEARCH

Phenotypic Identification of Blood Groups in Blood Donors: A Peruvian Multicenter Analysis

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Background: Red blood cell alloimmunization currently continues to be a significant problem during the blood transfusion process, where phenotypic identification plays a clinically relevant role in its prevention. The objective of the study was to carry out the phenotypic identification of blood groups in blood donors from three hospitals in Lima.

Methods: A cross-sectional study was conducted, including 20,141 blood donors in three hospitals in Lima, Perú during the period from January to June 2023. Red blood cell phenotyping was performed by the gel agglutination method using gel cards with the IH-500 automated system.

Results: A predominance of donors within the age group of 29 to 38 years (30.9%) was observed, with the majority being men (69.5%). Most donors were Peruvian (97.9%), and among foreign donors (2.1%), Venezuelans predominated (1.5%). In the distribution of the ABO and RhD blood groups, the O Rh+ phenotype predominated in 79% of the donors. In the phenotypic distribution of the Rh system, the presence of the D antigen was observed in 98.1% of the donors, with the c phenotype being the most frequent (76.4%). For the Kidd system, 70.7% of the donors presented the Jka antigen and 81.9% the Jkb antigen. In the Duffy system, 77.7% of the donors presented the Fya antigen and 50% the Fyb antigen. For the MNS system, 93.7% of donors had the S antigen and 76.1% had the s antigen. It was also found that 1.5% of donors are carriers of the Kell antigen, all of which are clinically important.

Conclusion: The phenotypic identification of blood groups in blood donors from three hospitals in Lima highlighted the clinical relevance of identifying less common antigens in the Kell, Kidd, Duffy, and MNS systems to prevent alloimmunization during blood transfusions.

Keywords: alloimmunization, phenotype, blood group antigens, blood donors

Introduction

Blood transfusion is a very common and vitally important procedure that plays a crucial role in the treatment of various health conditions. This procedure is performed when there is compatibility of the ABO and RhD systems between the donor and the recipient.[1] However, there are also other erythrocyte antigen systems that have been significantly related to the formation of alloantibodies and are clinically relevant.[2,3] Currently, 45 blood group systems are recognized, comprising a total of 362 red blood cell antigens. These 45 systems are genetically established by 50 genes.[4] Among the systems with clinical significance at risk of generating alloantibodies are the Kell, Kidd, Duffy, and MNS systems.[5]

Phenotyping of red blood cells is essential in transfusion medicine for the search for compatible blood, the treatment of various hematological diseases and surgical interventions.[6] This process allows identifying the presence or absence of specific antigens on the surface of red blood cells, which is essential to ensure blood compatibility in transfusions, prevent adverse reactions and minimize the risk of alloimmunization. The identification of phenotypic variations in red blood cells among individuals forms the basis for safe blood transfusion.[7]

Received: 14 June 2024
Accepted: 2 December 2024
Published: 30 January 2025

Alloimmunization is defined as the formation of antibodies in response to exposure to exogenous cell surface proteins or carbohydrates due to phenotypic incompatibilities in a blood transfusion, transplant, or, in women, by the fetus resulting from their pregnancy.[8–10] This immunological response presents clinically relevant consequences, such as the risk of having hemolytic reactions due to not having compatible blood, hyperhemolysis, and in women, it can also cause hemolytic disease of the fetus and newborn.[10,11]

The prevalence of general alloimmunization has been estimated to range from 1 to 5% in patients who receive a blood transfusion. In people with concomitant diseases such as sickle cell anemia, thalassemia, and myelodysplasia who require a greater number of blood transfusions (polytransfused), the prevalence of alloimmunization can reach up to 30%.[9,12] Furthermore, it has been observed that 1 in 80 pregnant women present erythrocyte alloantibodies that pose a high risk of causing hemolytic disease of the fetus and newborn, which, worldwide, affects around 1/300 to 1/600 live births.[8]

A systematic review and meta-analysis carried out in sub-Saharan Africa showed an red blood cell alloimmunization rate of 6.7 (5.7–7.8) per 100 transfused patients, where anti-E, anti-K, anti-C, and anti-D were the most common antibodies frequent.[13] Furthermore, an investigation carried out in Greece found a prevalence of 1.2% of alloimmunized people for one or more alloantibodies, observing that the most frequent antibodies were anti-K, anti-E, anti-D, anti-Jk^a, and anti-M.[14] In South America, this problem is also present. A study carried out in Brazil, which analysed 12,904 patient records, revealed that 7.5% of them had irregular red blood cell antibodies. Among these, the most common antibodies identified were anti-E (20%), anti-D (12%), anti-K (11%) and anti-C (8%).[15] In Chile, the incidence of red blood cell alloimmunization in transfused patients was 1.02%. The most frequent phenotypes were anti-E (30.8%), anti-K (26.9%), anti-D (7.7%) and anti-Fy a (5.8%).[16]

In polytransfused patients, alloimmunization represents a relevant complication due to the phenomenon of evanescence, which refers to the reduction in antibody concentrations below the detection limit that occurs in up to 70% of cases, causing false negatives in future determinations.[12]

In this context, red blood cell alloimmunization is considered a challenge in the field of transfusion therapy, especially for developing countries where the supply of compatible blood products for future transfusions is limited. Because very few hemotherapy centers and blood banks have the capacity to phenotypically identify donors, we consider it relevant to provide updated epidemiological information that allows us to have a more accurate picture of the antigenic dynamics in our population, addressing not only the study of the most relevant ABO and RhD systems but also the analysis of the Kell (k), Kidd (Jk^a, Jk^b), Duffy (Fy^a, Fy^b), and MNS (S, s) systems. Therefore, the objective of this research is to carry out a phenotypic identification of blood groups in blood donors from different hospitals in Lima.

Materials and Methods

Study Design and Population

A cross-sectional study was conducted, where information from blood donors in three national reference hospitals located in Lima was reviewed and collected. In this way, the general characteristics of blood donors were described, as well as the phenotypes of blood groups.

In the study, the population and sample consisted of a total of 20,141 records of blood donors in three hospitals in Lima, made up of the "Hospital Nacional Cayetano Heredia" (Hospital 1) located in the north of Lima with 4,955 blood donors, the "Hospital Nacional Guillermo Almenara Irigoyen" (Hospital 2) located in the center of Lima with 14,397 blood donors, and the "Hospital María Auxiliadora" (Hospital 3) located in the south of Lima with 789 blood donors who attended these hospitals during the period from January to June 2023 (Figure 1). Blood donor records with incomplete or incorrectly filled-out information and donor records in which phenotyping of antigens from the ABO-Rh System or other systems of clinical importance were not performed were excluded from the study.

Data Collection

For this study, the "Applicant Selection Form" was used as a research instrument validated by the PRONAHEBAS Quality Management System through Technical Regulation N°016-MINSA/DGSP-V.01. So, using the "Applicant

Figure 1 Geographic distribution of the three hospitals in Lima. (**A**) Hospital Nacional Cayetano Heredia (Hospital 1) located in the north of Lima. (**B**) Hospital Nacional Guillermo Almenara Irigoyen (Hospital 2) located in the center of Lima. (**C**) Hospital María Auxiliadora (Hospital 3) located in the south of Lima. Created using ArcGIS Desktop 10.8.2.

Selection Form" it was possible to collect sociodemographic information about the blood donor, such as age, sex, origin of the donor, place of birth, blood group, and phenotyping.

Data recording of blood group phenotype parameters was performed using the fully automated ID card process on the IH 500, which can process the full range of phenotypes and blood group gel cards. The system includes a 6-axis robotic transport arm that provides a high level of automation and reduces user operations. The IH-500 system also provides optimized safety controls, expanded reagent storage, and dynamic sample workflow management for additional efficiencies. In addition, the Neo Iris microprocessor-controlled device is used for the complete automation of in vitro diagnostic immunohematology tests of human blood, using commercial antisera with monoclonal antibodies for the establishment of phenotypes of the Rh, Kell system, and the extended phenotype; that is, clinically significant and is the same one found in each of the hemotherapy centers and type II blood banks of the "Hospital Nacional Guillermo Almenara Irigoyen", the "Hospital Nacional Cayetano Heredia", and the "Hospital María Auxiliadora", where each of the blood samples will be processed in 2023. It is relevant to mention that in these hospitals, the phenotyping of the ABO, Rh (CcEe), and Kell (K) blood groups is routinely performed for all donors. However, when discrepancies are detected in the results of these blood groups, extended phenotyping of the red blood cells is carried out. As a result, the number of donors varies for Kidd (n = 188), Duffy (n = 166) and MNS (n = 142) blood groups.

Phenotyping of red blood cells was performed using the automated IH 500 system (Bio-Rad, Switzerland), which extracts the appropriate amounts of sample, diluent and reagent. The system uses ID-Card gel cards (Bio-Rad, Switzerland). The identification system is based on the principle of column agglutination, ie the formation of antigen-

https://doi.org/10.2147/JBM.S475336

antibody complexes on the gel cards. The gel cards used contain 6 microtubules. The reaction is carried out on gel-filled microtubules. The gel contains specific antibodies or reagents, depending on the desired reaction. After the sample has been added to the analysis, the separation between agglutinated and non-agglutinated red blood cells can be seen with the naked eye in the gel column after centrifugation. Depending on the intensity of the reaction, the red blood cells penetrate the gel to a greater or lesser extent. In this way, the reaction can be classified into six levels: 4, 3, 2, 1, ± and -.

Statistical Analysis

All the information collected was tabulated in Microsoft Office Excel 2018 and then exported to the SPSS 26 statistical program, with which the statistical analysis and processing of data was carried out using central tendency statisticians (average), summations, and percentages, creating frequency tables and percentage distributions in relation to the stated objectives.

Ethical Approval

The Ethics Committee of the Research, Innovation and Entrepreneurship Unit of the Faculty of Medical Technology of the Federico Villarreal National University approved this research under "DICTAMEN N°PI2023-08", which allowed the execution of our research. Since it was a retrospective analysis, written informed consent was not obtained from blood donors. Therefore, the ethics committee did not consider informed consent as a requirement to carry out the research. In accordance with the Declaration of Helsinki, confidentiality and anonymity of blood donor information were respected in the three hospitals in Lima, and the researchers appropriately safeguarded this database.

Results

The research included a total of 20,141 (100%) blood donor records in three hospitals in Lima. The predominance of males was evident, with a total of 14,006 (69.5%) blood donors. The majority of blood donors belonged to the age group of 29 to 38 years with 6,237 (30.9%) blood donors and 2.7% of donors belonged to the age group of 59 to 65 years, where less frequency was found. It was observed that of all donors in the 3 hospitals, 97.94% were Peruvian nationals, while 2.1% were foreigners. Regarding the nationality of foreign donors, the majority were Venezuelan nationals (1.5%) and less frequently Brazilian nationals (0.1%) (Table 1).

The distribution of ABO antigens in blood donors showed that the majority belonged to blood groups O (80.4%) and less frequently to group AB (0.51%). Regarding the Rh antigen, it was found that the majority of donors were Rh positive (98.1%). Thus, a predominance of blood group O Rh+ was observed in 79% of donors, followed by group A Rh+ (13.2%) and a lower frequency of blood group B Rh- (0.2%) was found; in addition, no cases were observed with blood group AB Rh- (Table 2).

Table 1 General Characteristics of the Donors (N=20,141)

Variables	Category	Frequency (*f*)	Percentage (%)
Sex	Male	14,006	69.5
	Female	6135	30.5
Age range (years)	18 a 28	5368	26.7
	29 a 38	6237	30.9
	39 a 48	5062	25.1
	49 a 58	2936	14.6
	59 a 65	538	2.7

(*Continued*)

Table 1 (Continued).

Variables	Category	Frequency (*f*)	Percentage (%)
Nationality	Peruvians	19,724	97.9
	Venezuelans	306	1.5
	Ecuadorians	25	0.1
	Colombians	18	0.1
	Brazilians	15	0.1
	Others	47	0.3

Notes: *f*: Frecuency; %: Percentage.

Table 2 Distribution of ABO and Rh Antigens (N=20,141)

ABO	Rh				Total	
	D +		D -			
	f	%	*f*	%	*f*	%
O	15.901	79.0	286	1.4	16.187	80.4
A	2659	13.2	69	0.3	2728	13.5
B	1091	5.4	33	0.2	1124	5.6
AB	102	0.5	0	0	102	0.5
Total	19.753	98.1	388	1.9	20.141	100.0

Notes: *f*: Frequency; %: Percentage.

When analyzing the phenotypic distribution of the Rh system, the presence of the D antigen, which characterizes the positive Rh factor, was observed in 98.1% of blood donors from 3 hospitals in Lima. The majority of blood donors presented the c phenotype in 76.4% and, less frequently, they presented the e phenotype in 65.4% (Table 3).

The research found that 1.5% of donors are carriers of the K+ antigen, which is of clinical importance. Additionally, an expanded antigenic phenotyping was performed for the Kidd, Duffy and MNS systems. For the Kidd System, phenotypic identification was performed on 188 blood donors, where the majority had the Jk^b phenotype (81.9%). For

Table 3 Phenotypic Distribution of the Rh System (N=20,141)

Phenotype	Positive		Negative	
	f	%	*f*	%
D	19.753	98.1	388	1.9
C	14.630	72.6	5511	27.4
E	13.316	66.1	6825	33.9
c	15.395	76.4	4746	23.6
e	13.166	65.4	6975	34.6

Notes: *f*: Frequency; %:Percentage.

https://doi.org/10.2147/JBM.S475336

Table 4 Distribution of Antigens from the Kell System (K,K), Kidd (Jk^a and Jk^b), Duffy (Fy^a and Fy^b) and MNS (S and S)

System	Antigens	Positive		Negative		Total	
		f	%	*f*	%	*f*	%
Kell	K	309	1.5	19.832	98.5	20.141	100.0
Kidd	JK^a	113	70.7	75	29.3	188	100.0
	JK^b	154	81.9	34	18.1	188	100.0
Duffy	Fy^a	129	77.7	37	22.3	166	100.0
	Fy^b	83	50.0	83	50.0	166	100.0
MNS	S	133	93.7	9	6.3	142	100.0
	s	108	76.1	34	23.9	142	100.0

Notes: *f*: Frequency; %: Percentage.

the Duffy System, phenotypic identification was performed on 166 blood donors, where most had the Fy^a antigen (77.7%). Finally, in the MNS system, phenotypic identification was performed on 142 blood donors, where it was observed that the majority had the S antigen (93.7%), all of them of clinical importance (Table 4).

Discussion

In Peru there is a wide ethnic diversity, the result of a historical process of interbreeding and coexistence between different ethnic groups. We are descendants of pre-Columbian civilizations, such as the Incas and other Andean and Amazonian peoples. The majority of Peruvians identify themselves as mestizos, which implies a mixture of indigenous and European ancestry, mainly Spanish. This interbreeding is a direct result of Spanish colonization that began in the 16th century. There is also a significant Afro-Peruvian population. This community was formed from the arrival of Africans brought as slaves during the colonial era. Over the centuries, Peru has received a large number of immigrants from different parts of the world, including Italians, Spanish, Portuguese, and more recently, Chinese and Japanese. These communities have contributed to the cultural and ethnic diversity of the country.[17]

The present multicenter study analyzing the ABO system found a predominance of blood group O+ in 79% of donors. Similar results were reflected in Ethiopia where they found in the ABO system a higher frequency of blood group O in 41.6% of donors, followed by blood group A (28.7%), B (22.2%) and AB in 7.7% of blood donors.[18] A study carried out in 34 regions of China determined that the phenotypic distribution of ABO blood groups in the population is 34.2% for group O, 28.7% for group A, 28.2% for group B and 8.9% for group AB.[19] A research carried out in Venezuela observed that 57% of blood donor candidates were of blood group O, with blood groups A (30%), B (11%) and AB (2%) being the least frequent.[20] In Peruvian territory, a study carried out in a native community of Supayaku in Cajamarca, observed that 100% of the inhabitants had blood group O[21] and in Lima they found that 76% had blood group O, blood group A (18.6%) and B (4.9%).[22] Demonstrating in all the investigations a predominance of blood group O when analyzing the ABO system.

When analyzing the phenotypic distribution of the Rh system, our study observed the presence of the D antigen, which characterizes the positive Rh factor, in 98.1% of donors. It was also observed that the c phenotype was the most frequent (76.4%). In China showed that the majority (99.45%) of blood donors were RhD positive.[23] A study carried out in India, a country belonging to the Asian continent, that the phenotypic frequencies of the RhD blood groups in the investigated population were D (92.3%), C (87.6%), E (26.6%), c (51.1%) and e (98.4%).[24] Consequently, the e antigen was the most prevalent, while the E antigen was the least frequent among the different Rh types.

In South America, a study carried out in Ecuador, when analyzing the Rh system, reported a frequency of the D antigen in 97.7% of the cases, likewise the e antigen was present in 89.3% of the cases, the C antigen was present in 80.6% of the cases, the c antigen in 70.2% and the E antigen was present in 56% of the cases.[25] In Chile, another country

in South America, it was found that 96% of the samples analyzed showed the presence of the D antigen, e antigen (97.5%), C antigen (79%), c antigen (65.5%) and E antigen (35.5%).[26] When comparing our study with those from Ecuador and Chile, they all agree on the high prevalence of antigen D, which confirms the predominance of the positive Rh factor in South America. Our study showed a higher frequency of antigen C, while antigen E was less common compared to the other countries. Antigen E was more frequent in our sample, and antigen C had a lower relative prevalence. These differences could be linked to genetic variations between the populations of each country.

The research found in the identification of extended phenotypes of clinical significance in the three hospitals in Lima that only 1.5% of donors are carriers of the K+ antigen; for the Kidd system the majority had the Jk^b antigen present (81.9%); for the Duffy system the majority presented the Fy^a antigen (77.7%), for the MNS system the majority presented the S antigen (93.7%). A study carried out in China on blood donors showed that 0.14% of the participants were carriers of the K+ antigen, and the frequency of the Jk^a antigen was also found to be 68%, Jk^b (75.6%), Fy^a (98.7%), Fy^b (14.9%), s (99.5%) and S (9.99%).[27] A study of blood donors from Saudi Arabia found that the African population presented the K+ antigen in 2% of the cases, the majority had the Jk^a antigen (92%) and Jk^b was observed in 49% of the donors. Only 10% of the participants had the Fy^a antigen and the Fy^b phenotype was found in 23% of the cases. The majority of the donors had the s antigen (93%) and the S antigen was evident in 31%. When analyzing the phenotype of the Chinese population, it was observed that none of them were carriers of the K+ antigen, the frequency of the other antigens was Jk^a (68%), Jk^b (76%), Fy^a (100%), Fy^b (15%), s (100%) and S (10%).[28] Although some similarities are observed, such as the low prevalence of the K+ antigen and the high frequency of the s antigen in all the populations studied, there are notable differences in the Kidd, Duffy and MNS systems, which highlights the importance of considering the specific phenotypic characteristics of each ethnic and racial population when planning blood transfusion and donation programs.

An investigation carried out in Brazil evaluated the distribution of blood groups in blood donors from the south-western region of Paraná, located in the south of Brazil. They found in the Kell system the presence of the K+ antigen in 7.9% of cases, in the Kidd system a frequency of 74.9% was observed for the Jk^a antigen and 74.5% presented the Jk^b antigen, in the Duffy system it was found that 67.7% presented the Fy^a antigen and 78.1% had the Fy^b antigen.[29] This research conducted in South America reveals significant differences in the prevalence of certain antigens, such as the K+ of the Kell system, which is notably higher in Parana, Brazil. In the Kidd system, the frequencies of the Jk^b and Jk^a antigens are similar in both populations, while in the Duffy system, the Brazilian population shows greater genetic diversity. These differences can be attributed to factors such as genetic variability between populations, reflecting the particularities of genetic inheritance and migration history in South America. In this context, it is important to mention that during the 18th century, Brazil experienced a wave of European immigration, especially of Portuguese, but Africans also arrived as slaves and mixed with the indigenous population, giving rise to Brazil's current ethnic and genetic diversity.[30]

The present investigation has made it possible to better understand the distribution of frequencies of antigens and phenotypes of clinical importance, both of the Rh system and also of the Duffy, Kidd and MNS System, in donors from three national reference hospitals. It is relevant to mention that the distribution in the frequencies of multiple erythrocyte antigens in our population of blood donors differs from that observed in other international populations. Therefore, expanded red blood cell phenotyping is required to identify donors with blood groups uncommon in our population. This strategy can effectively cover blood transfusion needs in patients with rare or uncommon phenotypes, as well as prevent red blood cell alloimmunization in patients who receive multiple transfusions and require compatible red blood cells.

Conclusion

The results of our multicenter investigation carried out in three Peruvian national reference hospitals showed a low frequency of the Kell antigen and the detailed phenotypic analysis of the Kidd, Duffy and MNS systems reveals a high frequency of clinically significant phenotypes such as Jk^b, Fy^a and S in the donor population. These findings are of great relevance for transfusion management in Peru, since they allow a better selection of compatible blood units, reducing the

risk of hemolytic transfusion reactions and improving transfusion safety, especially in polytransfused patients or those with complex transfusion needs.

It is important to understand the phenotypic epidemiology of red blood cell antigens to create registries of donors typed by antigens of the aforementioned blood systems, which is a prerequisite for immunohematological assurance in the safety of blood component therapy. It is crucial to properly identify the blood group in haemotherapy, not only to avoid complications derived from incompatible transfusions, but also to optimise the use of units of blood products that present less common phenotypes. For this reason, we consider it important to create a national protocol that allows us to have a baseline of reference information at the national level in order to make an evidence-based decision to avoid alloimmunisation and increase transfusion safety that allows the appropriate use of phenotyped units.

Acknowledgments

We thank everyone who contributed to the research and helped collect the necessary information. We also thank the "Universidad Nacional Federico Villarreal" for financial support.

Disclosure

The authors declare no conflicts of interest.

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