

Prevalence of ABO, Rh and KELL Blood Group Types and Transfusion- Transmissible Infections (TTI) among Blood Donors in Najran City, Saudi Arabia

Osama M Alshehri¹, Mohammed H Nahari¹, Elhashimi E Hassan^{1*},
Musab F Alqahtani² and Turki H Awaji³

¹Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Najran University, Najran, Saudi Arabia.

²Department of Medical Laboratory, Armed Forces Hospital, Najran, Saudi Arabia.

³Department of Blood Bank, Armed Forces Hospital, Najran, Saudi Arabia.

*Corresponding author E-mail : alhashimihassan2018@gmail.com

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The knowledge of Red blood cells polymorphism and blood group antigens prevalence at the local and regional levels is necessary for safe blood transfusion services. This study was aimed to estimate the prevalence of significant blood group phenotypes like ABO, Rh, and Kell among the Najran people of Saudi Arabia. The transfusion transmittable infection (TTI) rate and blood abnormalities among various blood types were assessed to ensure safe blood transfusion. ABO and Rh blood prevalence (n=970) and Rh phenotype polymorphism were estimated in over 531 unrelated donors. The blood samples were screened for certain TTIs like AHBC- Anti-hepatitis B core, HTLV-1- human T-lymphotropic virus type I, HCV- hepatitis C virus, HBsAg- Hepatitis B antigen, HIV- Human immunodeficiency virus, SIC- Sickle cell, MP- Malaria parasite, and SYP- Syphilis. The selected samples were also observed for blood abnormalities by performing a complete blood count (CBC). Out of 970 subjects, 966 were males, and only 4 were females. The O>A>B>AB blood groups were identified with 46.89, 29.3, 9.1, and 2.38% prevalence among Rh-positive phenotype. While in the Rh system, 87.6% and 12.3% of Rh positive and Rh negative was observed. Among 953 samples, the prevalence of seropositive donors was approximately 5.66%. The screening showed about 5.036, 0.104, 0.314, 0.209, 2.18, 0.104, and 0.209% positivity for AHBC, HCV, HBsAg, HIV, SIC, MP, and SYP respectively. Results found that the frequency of D, C, E, c, and e were 99.9%, 67.98, 25.8, 77.9, 98.49%, respectively, in over 531 subjects. The e allele was more prevalent in Najran city. After observing the variations in the CBC parameters among the donors, it was perceived that about 28.78, 99.9, 29.41, and 31.6% of blood abnormalities were noticed for O, AB, B, and A blood groups, respectively. For blood banks and transfusion services, which play a significant role in the medical care of the patient, awareness of the distribution of the blood group is essential. Increasing consistency of blood donation programs would improve both donor satisfaction and motivation for potential donations of blood in near future.

Keywords: ABO; Blood Donation; Complete Blood Count (CBC); Kell; Rh Factor; Transfusion-Transmitted Infections (TTI).

The blood group antigens are inherited factors that are important for blood transfusion with different factors such as patterns of ancestry,

genetic abnormalities, vulnerability to diseases, and demographic movement¹. Until now, over 346 blood group antigens are present, which clusters

inside 36 blood group frameworks, according to the International Society of Blood Transfusion (ISBT) congress². In the development of blood transfusion, Karl Landsteiner's finding of the ABO blood groups was a significant accomplishment. He won the Noble Prize, preceded by his discovery of Rhesus (Rh) antigen by Wiener³.

To maintain blood banks and safe blood transfusion facilities efficiently, awareness of the delivery of ABO and Rh blood groups at the local and regional levels is necessary. The Rh is known as the world's leading blood group network, which differs across races^{4,5}. The two popular blood forms in transfusion medicines are ABO and Rh-positive (Rh (D))⁶. In the blood group structure for ABOs, people are divided into four significant groupings of blood, including A, B, AB, and O. They are sub-divided into Rh-negative and Rh-positive due to the existence of antigens and agglutinins on their cell surface⁷. However, these blood groups are strongly polymorphic, and their allelic frequency distribution differs between regions of the world⁸. There are 18 distinct phenotypes, with anti-D, C, c, E, and e, with eight potential haplotype arrangements, which results in 36 genotypes in line with ISBT 004⁹.

The monitoring of blood banks in healthcare institutions is known to be a mandatory procedure for minimizing the onset of transfusion-transmitted infections (TTIs)¹⁰. In addition, between different regions of the world, the incidence of TTIs among blood donors often differs dramatically¹⁰. Any blood bank's principal role is to provide patients with stable and compatible blood or blood supply on time. Any negligence of blood transfusion will endanger life with acute and delayed hemolytic transfusion (AHTR and DHTR) complications as well as in hemolytic disease in newborn (HDN)¹¹. Currently, 36 antigens with an explicit immunogenic K antigen nature in the KELL system are associated with AHTR, DHTR, and HDN (ISBT-006)¹².

A large proportion of TTIs is triggered by Human immunodeficiency virus (HIV) 1 and 2, hepatitis B virus (HBV), hepatitis C virus (HCV), syphilis, sickle cell anemia and human T-lymphotropic virus type I (HTLV-1)^{10,13}. HTLV-1 is a virus linked with inflammation, a high-risk factor in adult T-cell leukemia/lymphoma for lymphocytic involvement, which arises primarily

in Japan, South America, the Middle East, and other Austro-Melanesia countries¹³. The risk for transfusion-based Hepatitis B virus infection was subsequently reduced by introducing surface hepatitis B antigen (HBsAg) testing in blood donors¹⁴. Additional risk factors of TTI include inadequate surveillance systems, transfusion within family members, poor implementation of WHO rules and regulations, or the absence of reference screening tests such as Enzyme Immunoassays (EIA) or Nucleic Acid Testing (NAT)¹⁵. The new Minipol NAT technology (MP-NAT), which includes 96- blood donor data, is highly adaptive and has been embraced by several countries to eliminate "false reactive" outcomes¹⁶. However, TTI exposure in underdeveloped countries remains comparatively large, mainly because of the lack of infrastructure and funds to introduce sensitive testing techniques compared to developed countries¹⁷. ABO, Rh and KELL, and TTIs worldwide differed widely, and no reported data on this topic was available from Najran City, Saudi Arabia.

Donor selection is essential for protecting blood transfusion and eligibility policies and is intended to safeguard both the donor and recipients¹⁸. When blood is repeatedly donated, a person either achieves equilibrium at a lower level or becomes anemic¹⁹. Donors with comparatively low amounts of hemoglobin (Hb) or platelet count cannot contribute to blood donation²⁰. Hb screening is a conventional aspect of donor collection. However, Hb alone is ineffective in diagnosing donors of the blood that have an iron deficiency but who have no anemia²¹. The electronic hematology analyzers can calculate red cell indices such as mean cell hemoglobin (MCH) and mean cell volume (MCV) with a high degree of specificity and precision. A drop in MCV and MCH generally represents an increasing iron shortage²². Thus the goal is to evaluate various serological tests relevant for TTI-related blood donors and the percentage of different ABO and Rh antigens of Najran city. Moreover, this study focuses on analyzing the CBC of selected donors to assess an iron deficiency.

MATERIALS AND METHODS

The current study was conducted according to the Ethics Committee's guidelines

on Human Science at Najran University To engage in the research, all participants provided written consent.

Sample size, collection, and selection criteria

People from Najran City, Saudi Arabia, were among the population surveyed in our study. More than 966 unrelated people enrolled as donors to the Najran Hospital and took part in blood donation research. All the volunteers are from Saudi Arabia, where their details related to age, sex, clinical records were documented. The guidelines for blood transfusion were followed as per the National blood transfusion of Saudi Arabia. Blood donors have been estimated and recorded for infection history, complete blood picture (CBP), recurrent illnesses, and hemoglobin levels.

Blood group typing

The DiaMed method was used to conduct ABO and Rh D blood grouping by the gel column agglutination ID card process (ABO-Rh/Reverse Grouping, ID-Card, BIO-RAD, Switzerland). Blood of donors has been stored in EDTA anticoagulant tubes and processed further. Aluminum foil was removed from the selected microtubes as required by holding the ID-Card in the upright position. About 50 μ L of red cell suspension was added to ID-Card. Later, the ID-Card was centrifuged for 10 min at 1030 rpm, and the results were read and recorded. Blood group classification was based on the reverse and forward groupings and a negative control outcome. The presence of the poor D-phenotype has been further checked for RhD. Donors were also graded as D-negative in weak D testing signal.

Sample collection and screening for different blood types to detect TTIs

The blood serum samples of donors have been subjected to nucleic acid testing (NAT) (Cobas TaqScreen, MPX Test version 2.0, Roche, Switzerland). Further, screening for HIV, HCV, HTLV-I/II, HbsAg, Syphilis, and HBC II by ELISA method (ABBOTT, ARCHITECT i1000, Germany) using kit method supplied by ARCHITECT (Germany) for HIV Ag/Ab Combo, Anti-HCV, HTLV-I/II, HBsAg qualitative II, Syphilis TP, and Anti-HBc II reagent kit, respectively. For SIC, qualitative determination of hemoglobin S (Hb-S) in blood was followed by using the kit SAS SICKLE CELL TEST (USA). Immunochromatographic

membrane assay (USA) and rapid plasma reagin (RPR) method was followed to the detected malaria parasite and Syphilis, respectively.

Categorization of Rh phenotypes by antigen typing

For Rh expanded phenotyping, a unique single monoclonal antiserum, detected by Antigen D and Kell (Antigens C, c, E, e and K), and a Gel test was performed (Bio-Rad ID-Card, Switzerland). Each ID-Card was reviewed to ensure that the results were accurate and followed the same procedure as described for blood group typing¹².

Analysis of complete blood count (CBC)

The CBC analysis was performed using an automated blood cell counter analyzer (SYSMEX XN 1000, Japan). All the tests were carried out by following standard procedures by assuring blood sample quality²³.

Statistical analysis

The percentage prevalence of different blood group types and Rh antigens, and Kell blood groups was estimated. The figures were shown in percentages. Further, student t-test (unpaired) was performed for CBC data analysis. GraphPad Prism version 8 was used to calculate the prevalence percentage and to generate the graphs. Statistical difference was set at $p < 0.05$, and values of $p < 0.001$ indicate high significance.

RESULTS

Distribution of ABO and Rh types

A current survey of 970 volunteer blood donors was conducted in Najran City to determine the level of ABO and Rh-blood distribution among Saudi citizens. There were more male donors than female donors, with 966 and 4 respective members. Fig. 1A outline the prevalence of male vs. female donors. Our findings on the distribution of ABO blood types revealed that, O positive blood category was most prevalent with 46.89%, followed by A positive, B positive, O negative, A negative, AB positive, B negative, and AB negative with 29.3, 9.11, 7.45, 4.24, 2.38, 0.41, and 0.21%, respectively (Fig. 1B). The Rh (D) positive and Rh (d) negative subjects are shown in Fig. 1C, where a more prevalent Rh-positive (87.6%) than Rh negative (12.3%) was observed.

Distribution and comparison of TTIs among males and females

In this report, we have tested the blood donors for various blood-transfusing infections. In 953 samples, the prevalence of seropositive donors was approximately 5.66 percent, which includes AHBC, HCV, HBsAg, and HIV) (Fig. 2A). The NAT analysis showed positive reactions for only two patients for HBV, and none of the patients

tested positive for HIV and HCV. However, the serological screening showed about 5.037, 0.104, 0.314, and 0.209 seropositivity for AHBC, HCV, HBsAg, and HIV. Other TTI infection analysis for Sickle cell (SIC), malaria (MP) and syphilis (SYP) were found to be around 2.18, 0.104, and 0.209% positivity respectively. However, none of the studied donors showed positive for human T cell leukemia virus (HTLV) (Table 1). We also

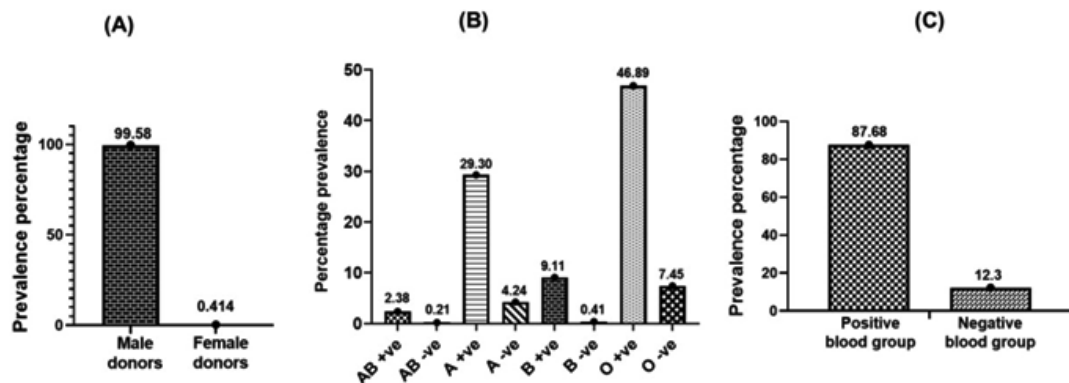


Fig. 1. Distribution of different blood groups (ABO and Rh) various donors, where the percentage prevalence of male and female volunteers is represented in (A), the significant difference of ABO blood groups with Rh positive and Rh negative is shown in (B) and total percentage of Rh positive and negative blood group among the analyzed samples (n=970) was signified in (C)

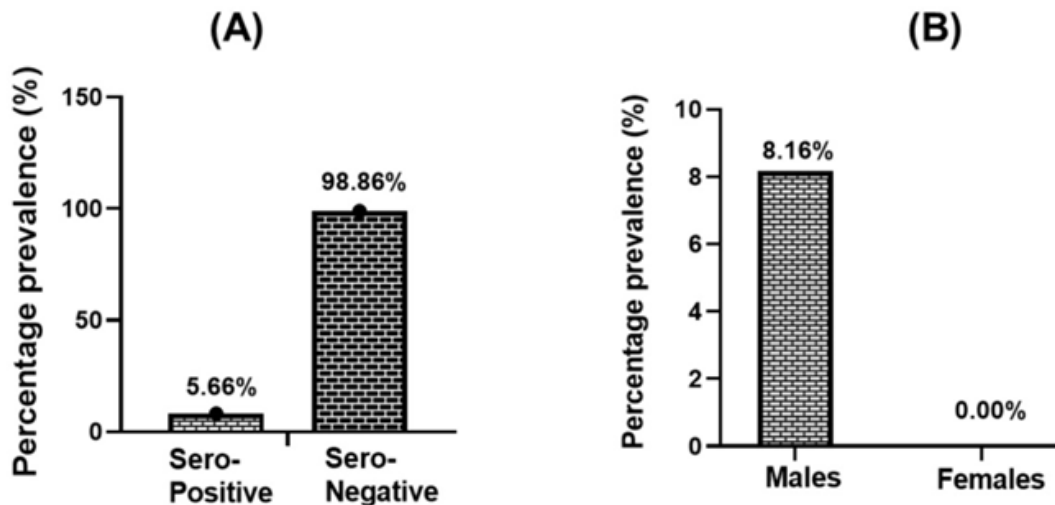


Fig. 2. Frequency of distribution of TTI positive percentage among selected donors. (A) Percentage prevalence of sero-positive among the tested donors and (B) Prevalence positive percentage among different male and female donors of Najran City

measured the percentage of seropositivity of TTIs in males as well as in females' donors, out of the total studied blood donors and observed that none of the females (n=4) possessed any TTI's, whereas 8.16% of males were found to be positive for the tested infections. Out of the total studied blood donors (n = 953), the percent TTIs in males (n = 78) were found to be appreciably in greater number when compared to females (n = 0) (Fig. 2B).

Prevalence of various Rh phenotypes

The distribution of alleles C, E, c, and e among the 531 donor volunteers has been calculated in the present analysis. Results found that the frequency of D, C, E, c, and e were 99.9%, 67.98, 25.8, 77.9, 98.49%, respectively (Fig. 3A).

Table 1. Percentage prevalence of various TTIs among the donors (n= 955) of Najran city

TTIs	Percentage of positive reaction	Percentage of negative reaction
AHBC	5.037	94.963
HTLV	0.000	100.00
HCV	0.105	99.895
HBs Ag	0.315	99.685
HIV	0.210	99.790
SIC	2.183	97.817
MP	0.104	99.895
SYP	0.209	99.790

Where, AHBC- Anti-hepatitis B core, HTLV-1- human T-lymphotropic virus type I, HCV- hepatitis C virus, HBs-Ag- Hepatitis B antigen, HIV- Human immunodeficiency virus, SIC- Sickle cell, MP- Malaria parasite, SYP- syphilis.

The prevalence of the K blood system was 12.05% in the sampled population. The most common Rh-positive haplotype frequency in the study was noticed for DCce with 33.40%, while in Rh D negative haplotype was observed for dce with 19.25% presence. The other forms of haplotypes identified in our analysis were DcEe, DCe, DCcEe, DCE, DcE, and DCee with 11.51, 21.51, 12.83, 1.13, 0.19, and 0.19% prevalence, respectively. The less common haplotypes noticed were DCE, DcE, and DCee (Fig. 3B). The haplotypes distribution among the Najran populace were found to be statistically different (p<0.05), with the highest prevalence of DCce type (33.4%) and least incidence of DcE and DCee (0.19% each) types.

Selection of donors based on complete blood count (CBC)

The donors who had shown positive reaction for TTIs were eliminated, and further analysis of CBC was performed for those donors whose Rh phenotype data is available. It was perceived that among 500 donors with different Rh haplotype profiles showed variation in their CBC analysis, the donors with any abnormalities and low or high values than the reference range were excluded from the study. Table 2 represents the overall prevalence of different blood group types based on the demographic feature of the age. The age group between 31-40 yrs was found to donate more blood in almost all the blood group types. The blood donation was more in O type followed by A, B, and least contribution was observed in AB blood group donors.

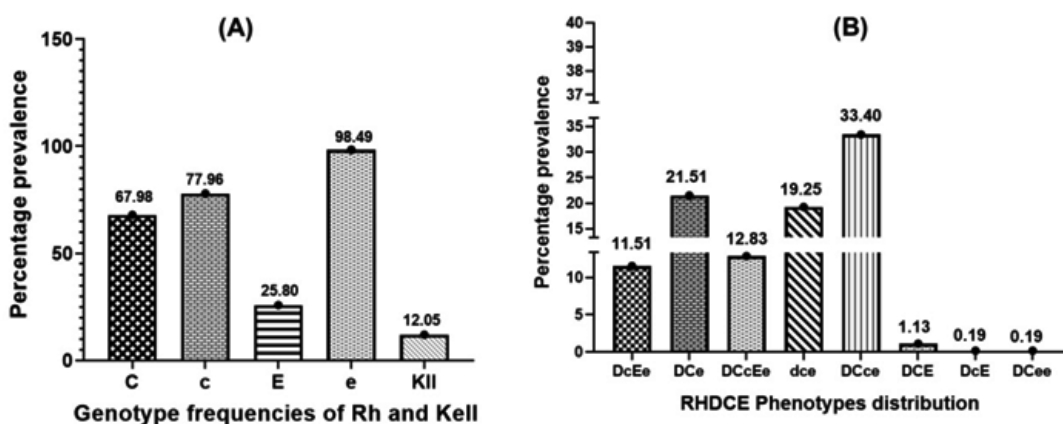


Fig. 3. The prevalence of different alleles and haplotypes among the Najran Saudi's population. The prevalence of different genotypes of Rh and kell (A) and RHDCE haplotypes (B) were represented

Table 2. Demographic distribution of donors based on age factor

Variables Age group	Donors (both Rh ⁺ and Rh ⁻) (n=500)				Frequency distribution (%)			
	O	AB	A	B	O	AB	A	B
<20	8	0	0	0	0.003	0	0	0
21-30	99	4	65	18	37.5	50.0	37.3	35.2
31-40	104	4	76	22	39.3	50.0	43.6	43.1
41-50	48	0	32	9	18.18	0	18.3	17.6
51-60	5	0	1	2	0.018	0	0.005	0.039
Overall Total	264	8	174	51	52.8	1.6	34.8	10.2

The Hb levels showed no variation ($p < 0.0001$) among the selected donors and were in the acceptable range, where the mean values were 15.44, 16.39, 15.44, and 15.65 (g/dL) for O, AB, A, and B blood groups, respectively (Table 3). Similarly, the WBC and RBC count was also in the normal range ($p < 0.0001$), except few abnormalities in WBC levels were noticed for few donors (n=63). The MCV and MCH levels were lower in few donors indicating few irregularities in the blood samples analyzed. The other parameters, like HCT, MCHC, and PLT, were in the acceptable range for all the donors. The values obtained for CBC did not show any significant variation ($p < 0.0001$), and R^2 values were around 0.9 for all the parameters, except for the WBC data for the AB blood group (Table 3). Further, since the data distribution was uneven, statistical analysis using student t-test (unpaired) was used to conclude statistical vs clinical significance. Accordingly, calculated t-value was significantly higher than the tabulated t-value at the level of significance used in all the cases except in WBC count of AB blood group. Hence, no significant ($p > 0.005$) association can be considered between the tested variables. After observing the variations in the CBC parameters among the donors, it was perceived that about 28.78, 99.9, 29.41, and 31.6% of blood abnormalities were noticed for O, AB, B, and A blood groups, respectively (Table 4). In AB group, out of 8 people analyzed, 7 were showing statistical varied WBCs count indicating significant abnormalities at the tested population and the level of significance. The abnormality level was high in this particular group. However the since the sample size is less, mean is very much affected

by the outliers. Thus the samples which showed TTIs and blood abnormalities can be eliminated for blood donation.

DISCUSSION

In terms of anthropology, forensics, and transfusion medicine, the knowledge of RBC polymorphism and antigen prevalence was essential, and any incompatibility could result in various forms of severity²⁴. Few reports on TTI seroprevalence among blood donors and the prevalence of ABOs and Rh blood in Saudi communities have been reported. However, our study focuses on selective donor screening with TTI and CBC trends along with Rh phenotype polymorphisms.

This analysis reveals how Saudi donors who donated blood to the blood transfusion service centers at Najran city in Saudi Arabia have spread ABO, Rh, and Kell phenotypes. In Tunisian, Riyadh, and Mauritania populations, the pattern of O>A>B>AB identified among their people was recorded^{10, 25, 26}. Our results are incompatible with the study indicated by Al-Noaemi and Daghiri²⁷. The O blood group in the City of Najran, Saudi Arabia, was large, and the Yam tribe was most prominent. In a blood transfusion, paternity, ethnicity allocation, and certain criminal proceedings, the Kell blood system plays a significant role. It was noticed that in areas like Riyadh, Sundanese, Caucasian, North India, East Asian Blacks, Japan, the prevalence of Kell blood system was 18.2, 5.6, 9, 4, 2, 0.0001, and 1.97%, respectively^{12, 28}. In our current research, the distribution of Kell blood system screening among

Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
B	3.3	4.38	12.2	38.8	71.5	21.5	30.1	205	
Min	13.08	7.03	17.4	54.9	93.8	31.5	35.4	527	
Max	9.78	2.65	5.2	16.1	22.3	10	5.3	322	
Range	6.95	5.70	15.65	47.85	84.28	27.64	32.76	295.6	
Mean	23.41	79.63	96.10	109.7	109.9	83.82	203.8	36.16	
t-value	50	50	50	50	50	50	50	50	
df	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	
p-value	0.916	0.992	0.995	0.996	0.996	0.993	0.999	0.963	
R ²	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	
Significant									

Reference values were obtained from Dean, [42]. In the above table WBC, RBC, HBG, HCT, MCV, MCH, MCHC and PLT stand for White Blood Cell, Red Blood Cell, Hemoglobin, Hematocrit, Mean Corpuscular Volume, Mean Corpuscular Hemoglobin, Mean Corpuscular Hemoglobin Concentration And Platelet count, respectively. While df is degree of freedom.

Table 4. Percentage of blood abnormalities among various donors

Blood group	No. of donors	Abnormalities (%)
O	76	28.78
AB	7	99.9
B	15	29.41
A	55	31.6

Najran’s populace was found to be 12.3% and suggests its importance for screening during blood transfusion²⁹. In the genetic, demographic analysis, the Kell blood community has a crucial position for the remedy and transfusion practices related to medical-legal problems²⁸. The Rh(D) positive was more in our study than Rh(D)-negative phenotype, and it was also documented in several areas with the ample existence of the Rh-positive phenotype [10, 26]. Rh-positive figures were reported as 95, 100, and 85%, respectively, in Africans, Americans, and Caucasians³⁰. In an emergency, some blood groups are more required than others. Hence the current study could have a substantial effect on the largest blood banks in Najran state.

The average accumulated frequency of TTIs in donor blood of our study was 5.66%. Our data suggested a high percentage of TTIs among male donors than Riyadh city of Saudi, which possessed only 1.002% TTI¹⁰. Few other countries also have strong TTI in places like Ethiopia, New Guinea, and Mozambique, with around 18 to 37 percent^{10,31}. The high TTI prevalence in the Najran population can be certified for detecting AHBC and SIC with 5.037 and 2.183 positivity. Sickle cell is a recessive blood disorder condition with a mutation at rs334 in α -globin gene leading to HbS type³². Individuals with HbS homozygous nature will encounter chronic anemia. Thus, the quantification of %HbS among Najran population indicated about 2% presence, this method was easy, rapid and low-cost effective, which improved the management of SIC³³. The seropositivity to HBV and HBsAg is also essential, as HBV can spread through transplantation procedures indicating the contagious HBV negative with HBsAg and anti-HBc positive donors, leading to the spread of infection³⁴. Hence, HBc antibodies must also be tested to minimize the likelihood of transfusion infection. The risk of HCV in the United Kingdom was decreased by 95% and HIV by 10% by NAT

analysis³⁵. NAT is an innovative and incredibly adaptive procedure, minimizing HBV, HCV, and HIV windows for 10.34, 1.34, and 2.93 days, respectively³⁶. None of the women (n=4) had shown some TTI. As the sample size for women is very less, we are encouraging more women to donate the blood, by providing more knowledge and bringing awareness about the blood donation through workshops and conferences. A minimal prevalence of HIV, HBV, HCV has been identified, and samples displaying seropositive behavior have been removed, and blood donation has not been suggested.

Saudi Arabia has different gene frequencies for each part of the world³⁷. In the current study, Rh phenotyping (D, C, E, c, and e) in over 531 donors revealed their frequencies of 99.9%, 67.98, 25.8, 77.9, and 98.49%, respectively. In Egypt, the prevalence of D>C>e>c > e was seen as 85.5, 70.4, 41.7, and 91.2 and 100%, respectively, in approximately 216 donor subjects¹². Swelem *et al.*¹² had displayed evidence regarding the Rh worldwide phenotypes distribution. The c antigen was more widespread, with 99% in Africans, whereas it is about 43% in East Asia³⁸. In our analysis, the e antigen is more in the studied Saudis, such prevalence of e allele has been reported in North India³⁹. The prevalence of different Rh alleles among various descendants or families suggests the gene flow. Such observation was reported for the Japanese Brazilian population, where C and E alleles were predominantly present²⁴.

Our analysis investigated hematological criteria for blood abnormalities among healthy donors. The age groups between 31-40 have actively participated in blood donation. Data showed further that the average values for hematology variables of donors in Najran City did not vary significantly except for a considerably higher WBC mean recorded by AB donors (p=0.0004). These anomalies in WBC have also been recorded in Nigerians with substantial variations between healthy blood donors⁴⁰. Subclinical bacteremia may be the cause of increasing overall WBC count⁴¹.

Moreover, it is also necessary to follow-up with both MCV and MCH, as any decline in MCV and/or MCH have been identified, the donors are requested to have iron supplements²¹. In our study, an abnormality of 28.78, 99.9, 29.41, and 31.6% in O, AB, B, and A blood types was observed,

and blood donation was not recommended for such donors. Results indicated that few donors had anemia and might be vulnerable to infections. Alternatively, the enumeration and evaluation of hypochromic cells percentage would offer adequate knowledge in decision-making on blood donation and suggest iron supplements' desirability to donors.

CONCLUSION

In creating a blood group database, the data collected from this study on the frequency of ABO and Kell blood phenotypic groups helps scan the available donors in emergencies without effort, especially when it is obscure to have any particular blood groups. The allelic patterns in the Rh phenotype would further strengthen the concept of blood donation. Our study also highlights the importance of TTIs and blood abnormalities as they play a significant role in the early detection of anemia or iron depletion. Extensive data and epidemiological studies would improve quality management in any location and play a vital role in avoiding TTI. Innovative research would offer quick detection methods that will attain significant advantages in the future. Given the numerous challenges, there have been essential improvements and cost-effective strategies for enhancing blood transfusions' safety. This continued dedication and transfusion service improvements would eventually guarantee the next steps and improve the efficiency and potential blood donation studies.

Limitations (not compulsory)

Blood donors typically delegate contagious risk, whereas the threat of part processing transmission is sometimes ignored. It is therefore highly technological, involving high-cost problems, dedicated services, supplies, consumables, and technical skills. Our study also had few limitations, where the serum ferritin levels were not assessed. Further, the data on Rh allele frequency distribution was not available for all the donors considered in the study. Lastly, the screening laboratories used for our research purpose was also found to be limited.

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Ethical clearance and consent to participate

The study was approved by the Ethics Committee in Najran University (Ethics board approval number: 23-10-3-19). The methods in the study were in accordance with the guidelines of the Declaration of Saudi Arabia. Written informed consent was obtained from all subjects participating in this research.

Conflict of interests

The authors claim no conflict of interest

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