Introduction:

In today's challenging healthcare environment, transfusion medicine specialists are motivated by the self-satisfaction of fulfilling their professional responsibilities in providing optimal services for the health and well beings of their patients. Thus, it is critical to identify and invest in the areas of transfusion services that lead to the maximum impacts and results.

In order to ensure a safe and effective blood supply, the field of immunohematology as a specialized area of transfusion medicine tries to effectively address difficulties that arise when individuals are exposed to the red blood cells of others, either through transfusion or pregnancy. In such cases, an individual's immune system can begin to produce antibodies in order to combat against antigens that are foreign to the individual's system. To identify and properly treat immunohematologic diseases such as hemolytic transfusion reactions (HTR), autoimmune hemolytic anemia (AIHA), or hemolytic disease of fetus and newborn (HDFN), experts in the field of immunohematology and those operating in hospital blood banks must attain specialized education. They must be fully trained in laboratory procedures to resolve observed incompatibilities and address unusual test results. Effective academic education and training, participation in national and international seminars, research activities, and the exchange of experiences through scientific writing can provide excellent opportunities for improving and advancing the field of immunohematology.

The educational curriculum of clinical laboratory colleges and the training programs of hospital blood bank laboratories in Iran can benefit from increased expertise in this area. A comprehensive educational curriculum that provides training opportunities in universities and

hospital blood banks that specialize in advanced immunohematology sciences need to be developed. New university graduates need to be trained and competent in executing complex immunohematology methods.

This publication aims to contribute to the field of knowledge of immunohematology by providing diverse content and perspectives through a catalog of articles that examine facets of immunohemotogy and rare blood topics. Individuals interested in the field of immunohematology are thus provided with ready access to published articles in international scientific journals.

The continued exchange of ideas in this field can help to ensure optimal services in blood transfusion facilities. I hope that transfusion medicine communities, including physicians, clinical laboratory medicine students, and technologists, can continue to gain more knowledge and expanded access to current immunohematology subjects in Iran.

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ORIGINAL REPORT

Distribution of blood groups in the Iranian general population

E. Shahverdi, M. Moghaddam, A. Talebian, and H. Abolghasemi

We report the first study of antigen and phenotype prevalence within various blood group systems in the Iranian general population. In this retrospective study, samples from 3475 individuals referred to the Immunohematology Reference Laboratory of the Iranian Blood Transfusion Organization, Tehran, Iran, for paternity testing from 1998 to 2008 were additionally tested for red blood cell (RBC) antigens in the Rh, Kell, Kidd, Duffy, MNS, Lutheran, P1PK, and Xg blood group systems. The antigen testing was performed by the tube method, and the phenotype prevalences were expressed as percentages. Of 3475 (1857 male and 1618 female) blood samples, 1268 samples were typed as group O (36.49%), 1115 as group A (32.09%), 823 as group B (23.68%), and 269 as group AB (7.74%). In our sample population, 3152 (90.71%) samples were D+ and 323 (9.29%) were D−. Analysis of Rh antigen typing results showed e (3359; 96.66%) to be most prevalent in the Iranian population, followed by D (3152; 90.71%), C (2677; 77.04%), c (2557; 73.58%), and E (1059; 30.47%). In the Kell blood group system, 3293 (94.76%) samples were typed as K−k+. For the Kidd and Duffy blood group systems, the following were the most common phenotypes: Jk(a+b+) (1703; 49%), Jk(a+b−) (1006; 28.95%), Fy(a+b+) (1495; 43.02%), and Fy(a+b−) (1005; 28.92%). In the MNS blood group system, the following were the most common phenotypes: M+N+ (1668; 48%), M+N− (1310; 37.70%), S+s+ (1564; 45%), and S−s+ (1392; 40.06%). In the Lutheran and P1PK blood group systems, Lu(a−b+) and P1+ phenotypes were observed in 3292 (94.73%) and 1966 (56.58%) samples, respectively. The Xg antigen was present in 1953 (56.20%) samples versus 1522 (43.80%) samples identified as Xg(a−). Knowledge of the prevalence of RBC antigen phenotypes in a population can be useful in databank creation for providing antigen-negative compatible blood to patients with multiple alloantibodies. ***Immunohematology* 2016;32: 135–139.**

Key Words: blood group systems, red blood cell antigens, phenotype prevalence, MNS, ABO, Rh, Kell, Kidd, Duffy

The nine major blood group systems include ABO, Rh, Kell, Kidd, Duffy, MNS, P1PK, Lewis, and Lutheran.[1,2] Identifying blood group antigens is very important in blood transfusion and organ transplantation to minimize major transfusion reactions.[3,4] Determining the prevalence of red blood cell (RBC) antigens of various blood groups in first-time voluntary blood donors would help us obtain insight into blood group distribution in a geographic population. This information can also help us lay the foundation for starting a databank of antigen-negative blood, which would aid in the prevention of transfusion reactions in alloimmunized patients.[5] There are some studies limited to ABO and Rh blood groups,[6–10] but other than one older study of blood group distribution,[11] no current data are available on RBC antigens and phenotype prevalence in Iran. The present study is the first comprehensive report of the prevalence of various RBC antigens and phenotypes of various blood groups in the Iranian general population.

Materials and Methods

This retrospective study was carried out using the data from 3475 individuals referred for paternity testing during a period of 10 years (from 1998 to 2008) to the Immunohematology Reference Laboratory (IRL) of the Iranian Blood Transfusion Organization (IBTO), Tehran, Iran, to derive information on the population prevalence of antigens and phenotypes in ABO, Rh, and seven other major blood group systems.

Ethical Considerations

This study was approved by the ethics committee of IBTO and its health services. Individuals were asked to sign an informed consent form before blood samples were obtained. All the terms of the Helsinki Declaration were met, and the personal information remained anonymous.

Sample Size

The sample size included all blood samples from individuals referred to the national forensic laboratory in Tehran, Iran, and the IRL of IBTO. During these 10 years, blood samples were collected and tested independently in two centers.

Blood Samples

Testing for ABO and Rh Antigens

Six milliliters of peripheral blood was drawn from each person into a vial containing ethylenediaminetetraacetic acid (EDTA) anticoagulant. Blood samples were collected under aseptic conditions from an anticubital vein for determination of blood group antigens. Initial ABO blood grouping was

determined by the tube method using commercially prepared antisera provided by Iranian Blood Research and Fractionation [IBRF], Tehran, Iran. The presence of D was determined serologically using reagents from IBRF. Weak-D testing was performed on samples initially tested as D–. Repeat ABO and D testing for blood group confirmation was performed by the conventional tube method as per our standard operating procedure using monoclonal reagents from different commercial companies: anti-A (Bio-Rad, Munich, Germany), anti-B (Bio-Rad), and anti-D (CE-Immumodiagnostika, Heidelberg, Germany). Testing for the presence of the weak-D phenotype was done on all samples typed as D– as per manufacturer's instructions. The tube method using a 2–5 percent RBC suspension and monoclonal Rh antisera (anti-C, anti-c, anti-E, and anti-e, Diamed AG, Cressier sur Morat, Switzerland) as per the manufacturer's instructions was performed for the common Rh antigens (C, E, c, e). A reaction range of 1+ to 4+ agglutination indicated the presence of the corresponding antigen. The absence of agglutination was confirmed macroscopically per the manufacturer's instructions indicating the antigen's absence.

Testing for Kell, Kidd, Duffy, MNSs, Lutheran, P1, and Xg Antigens

Cells with either the presence or absence of the antigens to be tested were selected from an in-house screening cell panel that was validated with commercial panel cells (Diacell, Diamed AG). These cells were used as positive and negative controls to ensure expected reactivity of antisera to be used in the testing.

Interpretation of Tube Testing Results:

Positive: Various-sized clumps of RBCs on the bottom of the tube, graded from 1+ to 4+, indicated the presence of the corresponding antigen.

Negative: A smooth RBC suspension after re-suspension of cells on the bottom of the tube indicated the absence of the corresponding antigen.

Statistical Analysis

RBC antigen and phenotype prevalence within the various blood group systems was calculated by totaling the number of individuals positive for a particular antigen or phenotype divided by the total number of individuals screened. Results are expressed as a percentage.

Results

The prevalence of different blood group antigens and phenotypes in a total of 3475 (1857 male and 1618 female) individual samples was compared.

ABO and Rh Blood Group Systems

The breakdown of results for ABO blood grouping was 1268 (36.49%) typed as group O followed by 1115 (32.09%) as group A, 823 (23.68%) as group B, and 269 (7.74%) as group AB. D phenotype prevalence analysis showed 3152 (90.71%) D+ individuals and 323 (9.27%) D– individuals. In total, among D+ individuals, group O (1148; 33.06%) was found to be most prevalent followed by groups A (1014; 29.19%), B (743; 21.41%), and AB (247; 7.11%). Among D– individuals, blood groups O (128; 3.7%) and A (107; 3.10%) were the most common, followed by groups B (75; 2.17%) and AB (13; 0.62%). Testing for Rh antigens found Rh5(e) (3359; 96.66%) to be most prevalent of the common Rh antigens in this Iranian population, followed by Rh1(D) (3152; 90.70%), Rh2(C) (2677; 77.04%), Rh4(c) (2557; 73.58%), and Rh3(E) (1059; 30.47%). Nine probable Rh phenotype combinations were found to be present in our D+ population; the most common phenotype was DCe/dce (R_1r; 873; 27.70%) (Tables 1 and 2).

Other Blood Group Systems

In the Kell blood group system, 3293 (94.76%) samples were typed as K–k+. The Kp(a+b–) phenotype was rarely found. In the Kidd and Duffy blood group systems, Jk(a+b+) (1670; 48.06%) and Fy(a+b+) (1466; 42.19%) were the most common phenotypes observed. Jk(a–b–) and Fy(a–b–) were found as rare phenotypes in the Kidd and Duffy blood group systems, respectively. M+N+ (1660; 47.77%) and S+s+ (1569; 45.15%) were the most common phenotypes observed in the

Table 1. Prevalence of D+ phenotypes in the Iranian study population (*N* = 3152)

	Phenotype		
Antigens present	Fisher-Race	Modified Weiner	Prevalence (%)
D Cc e	DCe/dce	R_1r	27.70
D C e	DCe/DCe	R_1R_1	22.38
D Cc Ee	DCe/DcE	R_1R_2	14.59
D c Ee	DcE/dce	R_2r	10.35
D c E	DcE/DcE	R_2R_2	2.30
D c e	Dce/dce	R_0r	1.78
D C Ee	DCE/DCe	R_zR_1	0.08
D Cc E	DCE/DcE	R_zR_2	0.01
D C E	DCE/DCE	R_zR_z	0.008

Table 2. Prevalence of D– phenotypes in the Iranian study population (*N* = 323)

Antigens present	Phenotype		Prevalence (%)
	Fisher-Race (assumed)	Modified Weiner	
c e	dce/dce	rr	9.59
Cc e	dCe/dce	r′r	1.95
c Ee	dcE/dce	r″r	0.45
Cc Ee	dCe/dcE	r′r″	0.05
C e	dCe/dCe	r′r′	0.04
c E	dcE/dcE	r″r″	0.001
C Ee	dCE/dCe	r^{y}r′	Rare
CE	dCE/dCE	$r^{y}r^{y}$	Rare
Cc E	dCE/dcE	r^{y}r″	Rare
Cc Ee	dCE/dce	r^{y}r	Rare

MNS blood group system. In the Lutheran and P1PK blood group systems, Lu(a–b+) and P1+ phenotypes were observed in 3292 (94.73%) and 1966 (56.58%) samples, respectively. The rare phenotype Lu(a–b–) was observed in none (0%) of the samples. The Xg antigen was identified in 1953 samples (56.20%), versus 1522 (43.80%) samples that typed as Xg(a–) (Table 3).

Discussion

In our study, blood group O was the most prevalent, followed by groups A, B, and AB. According to some studies, in the United States, group O is the most prevalent, followed by groups A, B, and AB.[12] Klein and Anstee[13] showed that the most common blood groups in Australians were O and A. These results are in line with our findings. According to a previous Iranian study, blood group O was the most common.[14] The prevalence is different between that study and ours (41% vs. 36%). In contrast, in some other studies of populations in other countries,[3,15–18] blood group B was most prevalent. According to Tomilin et al.,[19] blood group A was the most prevalent group in the Russian Federation.

In our study, the targeted population showed D– prevalence of 9 percent, as compared with 17 percent in Britain and 4.29 percent in India.[20,21] This result suggests that the expected rate of Rh isoimmunization would be lower in our population than that encountered in the British population. Studies have reported that in the United States, 85 percent of the population were found to be D+.[22] The prevalence of D– individuals varies from 20 percent to 40 percent in Basque populations to 0 percent to 1 percent in Japanese, Chinese, Burmese, Melanesian, Mauri, American Indian, and Eskimo populations.[3]

Table 3. Prevalence of phenotypes in various blood group systems in the Iranian study population (*N* = 3475)

System	Phenotype	Prevalence (%)
Kell	Kp(a–b+)	99
	K–k+	95
	K+k+	4.8
	Kp(a+b+)	0.8
	K+k–	0.2
	Kp(a+b–)	Rare
Kidd	Jk(a+b+)	49
	Jk(a+b–)	29
	Jk(a–b+)	22
	Jk(a–b–)	Rare
Duffy	Fy(a+b+)	43
	Fy(a+b–)	29
	Fy(a–b+)	28
	Fy(a–b–)	1
MNS	M+N+	48
	M+N–	38
	M–N+	14
	S+s+	45
	S–s+	40
	S+s–	15
	S–s–	0
Lutheran	Lu(a–b+)	91
	Lu(a+b+)	6
	Lu(a+b–)	3
	Lu(a–b–)	0
P1PK	P1+	67
	P1–	33
Xg	Xg(a+)	58
	Xg(a–)	42

The worldwide prevalence of D differs between ethnic groups—from 85 percent in white populations to 92 percent in black populations.[23,24] In the present study, we found the prevalence of D to be 91 percent.

We report for the first time the prevalence of other common antigens in the Rh system, including C, c, E, and e, in a general Iranian population. We found e to be the most prevalent Rh antigen in this population. DCe/dce (R_1r) was the most common phenotype in the D+ population versus dce/dce (rr) in that of the D–. In previous studies on Thai and Chinese individuals, CDe/CDe (R_1R_1) has been reported to be of the highest prevalence.[25] Nanu and Thapliyal also found DCe/DCe (R_1R_1) to be the most common phenotype in that population.[26] The prevalence of cde/cde (rr) varies among different ethnic groups. It was reported in 35 percent of white individuals, 26 percent of black individuals, and in only 3 percent of individuals of Asian descent.[27–30]

In the Kell system, the most common phenotype in our population was found to be K–k+ (95%), which is found in 100 percent of peoples from Southeast Asia.[27] The prevalence of K+k– in this study was 0.2 percent. None of the individuals of the Thakral et al. study[5] were found to be K+k–. The rarest phenotype in our study was Kp(a+b–), whereas Kp(a+b+) was found to be rare in the Thakral et al. study population.[5] This shows that a rare phenotype in a certain population does not necessarily imply rarity of that phenotype in another population.

In the Duffy system, Fy(a+b+) and Fy(a–b–) were the most common and the rarest phenotypes, respectively. According to Agarwal et al.,[15] Fy(a–b–) was rare in Indian and white populations, although it was common in a black population. In virtually all Indian studies, Fy(a+b+) was the most common phenotype in the white population.[15] *Plasmodium vivax* is endemic to India, and therefore we expect India to have a high prevalence of the Fy(a–b–) phenotype because the Duffy antigen has been proposed to be the receptor for entry of *P. vivax* into red blood cells.[31] Lack of this phenotype in studies from India could be attributable to fewer *P. vivax* infections. In Iran, thanks to preventive measures and treatment protocols, malaria has been eradicated, and this may account for the low prevalence of the Fy(a–b–) phenotype in our population.

Jk(a+b+) was the most common Kidd phenotype in our study. Nathalang et al.[27] reported similar findings in Asian and Thai populations. Moreover, Jk(a–b–) has rarely been found.

In our study, the M+N+ phenotype was the most common in the MNS blood group system. In the Nanu and Thapliyal study, the M+N– phenotype was reported to be the most common.[26]

S+s+ was the most common phenotype in our study. In other studies, S–s+ has been reported as the most common phenotype.[5,15] Further, the M+N+S+s+ phenotype was reported to be the most common by Nanu and Thapliyal, as well as in people of European descent and in African Americans.[26,32,33] In our population study, M+N+S+s+ was found to be the most common phenotype. It is possible that in areas where heterozygous phenotypes are most prominent, less alloimmunization occurs. In these areas, the value of a donor database might be in question.

In the Lutheran system, Lu(a–b+) was the most common phenotype found in our study, which is true for most of the populations around the world.[15] In our study, there also was no one with the Lu(a–b–) phenotype. Lu(a–b–) was reported as a very rare phenotype in the study by Thakral et al.[5]

In the P1PK system, P1+ was the most common phenotype in our study. Musa et al.[34] demonstrated that Malays and Chinese populations had high prevalence of the P1– phenotype, whereas the Indian population had higher prevalence of P1+. A lower prevalence of the P1– phenotype was reported among Thai individuals.[35]

In our study, the Xg(a+) phenotype was found as the most common phenotype in the Xg blood system. The distribution of the Xg antigen in our sample population is comparable with that reported in the Daniels study.[36]

8

Conclusions

We reported on the distribution of various blood group antigens and phenotypes among a general Iranian population. The study has a vital impact on the management of blood bank and transfusion services in this area. Knowledge of blood group antigen distribution is also important for clinical studies, for worldwide reliable geographical information, and for forensic research in various populations.

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Maternal red blood cell alloantibodies identified in blood samples obtained from Iranian pregnant women: the first population study in Iran

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BACKGROUND: The objective was to determine the frequency of occurrence of alloantibodies among pregnant women in Iran.

STUDY DESIGN AND METHODS: This was a prospective cross-sectional study, which was carried out in the immunohematology reference laboratory of the Iranian Blood Transfusion Organization in Tehran, Iran, in 2008 to 2015. Screening and identification of red blood cell (RBC) alloantibodies was done on the sera of 7340 pregnant females using the standard tube method and gel column agglutination technique.

RESULTS: Alloantibodies were identified in the serum of 332 of the 7340 (4.5%) pregnant women. A total of 410 antibodies were detected in 332 positive maternal serum samples with no previous history of blood transfusion. Anti-D was the most common antibody accounting for 70.5% of all the antibodies formed in D– women. The incidence of specific alloimmunization other than Rh group was 14.4%.

CONCLUSION: We concluded that the alloimmunization rate was high in comparison with wide pattern in previous studies. In Iran, like other developing countries, alloimmunization screening tests are performed only to detect anti-D in pregnant D– women. This high rate of alloimmunization, quite possibly, is due to the fact that the majority of blood samples came from pregnant women known to have previous obstetric problems. However, we suggest that RBC antibody screening tests should be extended to all D+ women.

Alloimmunization of red blood cells (RBCs) in pregnant women is still a challenge to clinicians. Maternal immunoglobulin G (IgG) antibodies are the main cause of fetal RBC hemolysis by targeting fetal RBC antigens. Although advances, including the implementation of RhIG to prevent anti-D hemolytic disease of the fetus and newborn (HDFN) in the 1960s, have been made, HDFN caused by anti-D as well as by non-D antibodies is still a serious concern. There are more than 50 RBC alloantibodies that cause HDFN, with anti-D followed by anti-c and anti-K having the highest probability of causing severe HDFN.[1] Despite use of D prophylaxis, anti-D is the most common and severe form of immunization.[2,3]

The prevalence of alloantibodies in pregnancy has been reported in various countries.[1,4-7] A compilation of similar data from Iran is limited. An evaluation of such data from a large and main referral laboratory to which blood samples from pregnant women and thalassemia patients are referred from all over the country would help to reiterate the importance of screening for and

ABBREVIATIONS: HDFN = hemolytic disease of the fetus and newborn; IBTO = Iranian Blood Transfusion Organization.

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monitoring those pregnancies that present with antibodies that may put the fetus at risk for HDFN. Therefore, we aimed to determine the frequency of alloimmunization in pregnant women who were referred to the immunohematology reference laboratory of the Iranian Blood Transfusion Organization (IBTO). Before 2015 there was no national protocol for testing of pregnant women for the detection and identification of RBC antibodies.

MATERIALS AND METHODS

This prospective cross-sectional study was carried out on 7340 pregnant women referred for ABO/D typing and antibody screening during a period of 8 years from 2008 to December 2015 in the immunohematology reference laboratory of the IBTO. The majority of samples were collected from the patients with positive history of multiple unsuccessful pregnancies (at least two times) to identify suspicious alloimmunization, prenatal and postnatal titration evaluation, and better management of possible need for intrauterine transfusion for alloimmunization in their pregnancy. All mothers with only anti-D as a result of RhIG were excluded from the study.

Sample size

Sample size included blood samples from individuals referred to the immunohematology reference laboratory of the IBTO.

Blood samples

ABO and D tests

Six milliliters of a peripheral blood sample was drawn from each individual into a vial containing EDTA. Blood samples were collected under aseptic condition from antecubital vein for determination of blood groups. Initial ABO blood grouping was determined by tube method using commercially prepared antisera, anti-A, anti-B, anti-AB (Iranian Blood Research and Fractionation). Presence of D antigen was determined by anti-D (Iranian Blood Research and Fractionation). For D– weak D test was performed. Repeat ABO and D blood grouping for confirmation of blood group was performed by conventional tube technique as per our standard operating procedure using monoclonal reagents from different commercial companies included Bio-Rad and CE-Immumodiagnostika: anti-A (Bio-Rad), anti-B (Bio-Rad), and anti-D (CE-Immumodiagnostika). Testing for the presence of weak D phenotype was done for all individuals typed as D as per the manufacturer's instructions. Tube technique using 2% to 5% RBC suspension and anti-C, anti-c, anti-E, and anti-e monoclonal antibodies (Diamed AG) as per the manufacturer's instruction was performed for Rh antigen (C, E, c, e) typing. A positive reaction of 3+ to 4+ strength of agglutination indicated the presence of corresponding antigen and absence of agglutination was confirmed macroscopically per the manufacturer's instruction indicating its absence.

Interpretation of results for the tube tests

Positive: RBCs formed a solid complete agglutinate on the bottom of the tube in several medium or small agglutinates. These were graded from 4+ to 1+ and indicated the presence of the corresponding antigens.

Negative: A compact button of cells on the bottom of the tube indicated the absence of the corresponding antigen.

Antibody screening and identification

A homemade available three-cell antigen panel (IBTO minipanel) was used for the antibody screening procedure in which the patient's serum was added to RBCs with and without papain enzyme using low-ionic-strength saline (LISS). IBTO minipanel and antibody screening kit and also selected cells were validated in a 2-year period using commercial CE-marked Diamed three-cell kits. Antibody screening test were performed twice in parallel using IBTO produced kits and Diamed kits. The results were compared and in case of positive results, the 11-cell antibody panel from Diamed was used simultaneously with IBTO 11-cell antibody panel. An IBTO-homemade antibody panel and selected cells were used to exclude and include alloantibodies.

A commercial antiglobulin gel card (Invitrogel AHG Coombs) and standard tube methods (Bio-Rad AHG) were used. The gel cards were incubated at 37°C for 15 minutes and then centrifuged for 10 minutes. On those samples found to be positive on the screening test, antibody identification was performed using a homemade 11-cell antibody identification panel expressing at least 18 US Food and Drug Administration–recommended RBC antigens, that is, D, C, c, E, e, M, N, S, s, P_1, Le^a, Le^b, K, k, Fy^a, Fy^b, Jk^a, and Jk^b in LISS (DiaLISS, Bio-Rad) with and without papain (Bio-Rad). Homozygous expression of Rh, Duffy, Kidd, and MNs antigens were included in the panel.

Clinically significant alloantibodies were defined as those antibodies that potentially could cause RBC destruction based on the reactivity at 37°C and/or antihuman globulin phase. Antibodies were categorized as passive anti-D due to RhIG injections based on three criteria, antibody reaction strength of less than 2+, anti-D titer of equal to or less than 4, and RhIG injection within past 2 to 3 months of testing. We also defined unspecified antibodies as those that are not defined in any of 31 blood group systems.

Statistical analysis

RBC antigen calculation and phenotype frequencies of the various blood group systems was done by totaling the number of individuals positive for a particular antigen phenotype divided by the total number of individuals screened. Results are expressed as percentages.

Ethical considerations

This study was approved by the ethics committee of IBTO and health services. Individuals were asked to sign an informed consent form before blood samples were obtained. All terms of the Helsinki Declaration were considered and the personal information remained anonymous.

RESULTS

During the study period, 7340 pregnant women were typed for ABO and D and screened for RBC alloantibodies. Of them, 332 women with an age range between 21 to 43 years old had positive antibody screen test results with one or more identified alloantibodies.

Blood group A was the most prevalent among patients with positive antibodies. In D+ patients (n = 6305), 68 (20.5%) were alloimmunized and 6237 (89%) were nonalloimmunized versus 264 (79.5%) alloimmunized and 771 (11%) nonalloimmunized in D– donor's (Table 1).

A total of 313 specific antibodies were detected in 332 positive maternal serums females with a total number of 1110 pregnancies. The number of unsuccessful pregnancies ranged between 1 and 14 times with a median of three pregnancies. Alloimmunizations occurred in 3% of women during their first pregnancy. An overall prevalence of alloimmunization including clinically significant and nonsignificant antibodies was 4.5%. A total of 23.6% were unspecified clinically significant, nonsignificant antibodies and antibodies reacted due to the RhIG injection (13.53% were unspecified clinically significant and nonsignificant antibodies and 10.07% were passive anti-D).

Of all 410 antibodies detected in this study, 55 were found in D+ women, giving an overall alloimmunization prevalence of 13.4% in the D+ patients. No anti-D alloantibody was identified in D+ women.

Among the 264 alloimmunized pregnant women in the D– patients, 193 cases developed specific antibodies versus 42 cases of 68 D+ alloimmunized pregnant women (prevalence of specific antibodies alloimmunization of 73.1% vs. 61.8%). Among D– women, despite receiving RhIG, anti-D was the most common antibody, accounting for 70.5% of all the antibodies formed alone in D– women. Within the D– group, the most common maternal dual and triple antibodies were anti-D+C (n = 37), investigation of anti-G was not performed among women with anti-D+C and anti-D+E+C (n = 4), respectively.

Within the whole study group, anti-D alone contributed for 39.9% of all specific antibodies, anti-D in combination with other antibodies was 64.3% of all specific clinically significant antibodies (n = 283), and specific alloimmunization other than Rh group was 14.4%. Table 2 shows all antibodies that were detected in maternal serum.

TABLE 1. Frequency of blood groups in our study population*

Blood group	Alloimmunized women	Nonalloimmunized women
A	123 (37)	2313 (33)
B	90 (27)	1682 (24)
O	85 (25.6)	2453 (35)
AB	34 (10.2)	560 (8)
D+	68 (20.5)	6237 (89)
D–	264 (79.5)	771 (11)

*Data are reported as number (%).

DISCUSSION

This is the first report of a study on RBC alloantibodies during pregnancy in Iranian pregnant women who were referred to the immunohematology reference laboratory at the IBTO. This study revealed that the alloimmunization rate was 4.5%, which was high in comparison with wide pattern in previous studies.[2,3,8] This high rate of alloimmunization, quite possibly, is due to the fact that the majority of blood samples came from pregnant women known to have previous obstetric problems. It should be mentioned that comparison of the results of different studies with each other is problematic, because of differences in population selection and heterogeneity, laboratory methods, and national blood transfusion practices.[3,6]

In the studies by Smith and colleagues[9] and Garratty and colleagues,[10] blood group O was the most common while in a recent study, blood group A was the most prevalent among patients with positive antibodies. In our study, the rate of antibody alloimmunization in the D– group was 25%, which is different to a study by Karim and coworkers.[3] This high rate is probably due to the nature of the services provided by our laboratory as a referral center and so data from nonreferral laboratories might be different from data provided by the recent study.

In the D+ group, the alloimmunization rate was 0.9%, but the total number of women with a positive antibody screen test was 17.6%. This significant allosensitization rate in D+ women shows that a routine screening program should be encouraged despite the negative opinion about the cost and benefit of this test.

However, we suggest that for a better prenatal immunohematologic care, antibody screening should be extended routinely to all D+ women at least once during their pregnancy. Koelewijn and colleagues[6] and De Vrijer and colleagues[11] discussed that antibody screening in the first trimester of pregnancy should be encouraged to

TABLE 2. Frequency of alloantibodies according to blood group

Antibody specificity	Total number (%) of women	D+ women	D– women
Anti-D	125 (37.6)	0	125
Anti-D + anti-C	37 (11.1)	0	37
Anti-D + anti-E	7 (2.1)	0	7
Anti-D + anti-E + anti-C	4 (1.2)	0	4
Anti-D + anti-K	2 (0.6)	0	2
Anti-D + anti-C + anti-S	2 (0.6)	0	2
Anti-D+ anti-M	1 (0.3)	0	1
Anti-D + anti-s	1 (0.3)	0	1
Anti-D + anti-C + anti- Jk^a	1 (0.3)	0	1
Anti-D + anti-C + anti- Jk^b	1 (0.3)	0	1
Anti-D + anti-C + anti- Le^a	1 (0.3)	0	1
Anti-E + anti-c	6 (1.8)	5	1
Anti-E	5 (1.5)	5	0
Anti-c	3 (0.9)	3	0
Anti-K	3 (0.9)	3	0
Anti-E + anti-c + anti- Jk^b	2 (0.6)	2	0
Anti-C	2 (0.6)	1	1
Anti-E + anti-Kp^a	1 (0.3)	1	0
Anti-E + anti-Le^b	1 (0.3)	1	0
Anti-e	1 (0.3)	1	0
Anti-M	10 (0.3)	7	3
Anti-P1	5 (1.5)	3	2
Anti-Jk^b	1 (0.3)	1	0
Anti-M + anti-c	1 (0.3)	1	0
Anti-S	1 (0.3)	1	0
Anti-Le^a	2 (0.6)	2	0
Anti-Le^b	8 (2.4)	4	4
Anti-c + anti-Jk^b	1 (0.3)	1	0
Unspecified antibodies	45 (13.55)	26	19

decrease fetal hemolysis and they recommended antibody screening in the first trimester of pregnancy.

Today, with the use of wider screening panels, it is possible to detect various other irregular antibodies that have been found to cause fetal hemolysis.[12] We used a minipanel antibody (IBTO3cells) to detect alloantibodies. The rate of antibody alloimmunization other than Rh was 14.4%, which is higher than studies performed by Karim and coworkers[3] and Pujol and coworkers.[13]

In Iran, like other developing countries, during pregnancy alloimmunization screening tests are performed just on D– women to detect anti-D. Despite the academic teaching offered in the text to the obstetrics and gynecology specialists, there is no national guideline to follow for detecting other unexpected RBC alloantibodies, but in developing countries there is a guideline for screening all pregnant women for irregular RBC antibodies. Also, in North America and several European countries, there are guidelines to recommend D prophylaxis for all D– pregnant women unless the father of the fetus is D–.[14] According to our national policy for RhIG to prevent alloimmunization to D antigen, antepartum and postpartum Rh immunoprophylaxis, which is available in all centers in Iran and is covered by universal health insurance at affordable prices, is administered at 28 weeks of pregnancy, but the patient's improper management during pregnancy by not screening for the possible risk of any alloimmunizations during multiparous pregnancy, the absence of the correct test to the determine exact number of RhIG doses needed for injection in a D– mother with a D+ child, and lack of follow-up for post–RhIG injection effectiveness are all important issues that we think need to be considered in further studies. It is not known if individuals with multiple antibodies were alloimmunized after first or subsequent pregnancies. It is recommended to study the clinical relevance of the non-D antibodies. It is also recommended to perform such a study in nonreferral laboratories.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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Case Report

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Hyperhemolysis Syndrome in a Patient With B-Thalassemia Due to an Anti-Jka Alloantibody

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Abstract

Hyperhemolysis syndrome (HS) is a delayed type of transfusion reactions (DTHRs). These may occur in patients with hemoglobinopathies due to destruction of red blood cells (RBCs). A 17-year-old boy with B-thalassemia and a history of multiple transfusions, after several RBCs transfusions developed hemolysis and hemoglobinuria with a decreased hemoglobin (Hb) level in the absence of bleeding that continued with splenomegaly. The antibody screen test (LISS AHG) and direct anti-globulin test (DAT) were performed. An 11-cell antibody identification panel (IBTO home-made) was used. Acid elution procedure was done using a commercial kit. The patient RBC phenotyping was attempted by treating the cells, following a chloroquine treatment procedure. Laboratory findings showed that his blood group was "A" Rh positive. His RBC phenotyping was negative for Jka antigen. The LISS antibody screening test result was negative with a weakly positive auto control result but DAT was positive for both C3d and IgG. The antibody identification test showed the presence of anti-Jka alloantibody and also anti-Jka was identified with eluate reacting stronger with homozygous cells (Jka+Jka+). The patient received steroid and IVIG as the main treatment and followed by receiving compatible blood units but hemolysis was not resolved and his Hb level was not increased, finally he was splenectomized. The patient's Hb level and clinical symptoms improved after the splenectomy, showing that the spleen may have a role in the destruction of the recipient and the donor blood cells.

Keywords: Thalassemia major; DAT; Anti-Jka; DHTR; Hyperhemolysis

Introduction

Hyperhemolysis is a delayed type of hemolytic blood transfusion reaction [1] reported in sickle cell anemia [2] applied when pre-transfusion hemoglobin (Hb) level is greater than post-transfusion level. This phenomenon is described in beta-thalassemia also and rarely in patients without hemoglobinopathies [3]. The exact mechanism is not well known but it involves destruction of both transfused donor cells and recipients' red blood cells (RBCs) [4, 5] via alloimmunization. Azarkeivan et al [6] reported that alloimmunization rate in 441 thalassemic patients was 11.3%.

They found that there was a significant association between antibody production and history of transfusion reactions.

Case Report

A 17-years-old male college student with thalassemia major from the North West province of Iran was referred to the Tehran Thalassemia Clinic with compliant of decreased transfusion interval and low Hb level. Since he was 6 months old he was diagnosed as B-thalassemia patient and was on regular blood transfusion every 30 days with the RBCs that were not typed. The transfusion reaction was worse since 1 year ago with decreased Hb level, dark urine and decreased transfusion intervals. In his native city, he was admitted in the hospital, was injected by IVIG and used prednisolone before every transfusion (three units every 15 days) but no improvement was observed. Finally, he was referred to the Tehran Thalassemia Clinic for further diagnosis and treatment. Clinical exams and laboratory tests in the clinic showed that his hematocrit and Hb level were 24.9% and 8.7 g/dL, respectively, in spite of blood transfusion in 2 days prior to the admission. Splenomegaly (5 cm below costal margin) was found in his clinical exam that was confirmed by abdominal sonography that showed a huge spleen (190 mm span) with multiple stones in his gall bladder.

ABO blood typing was done (Anti-A, Anti-B & Anti-D blend IBRF holding Co., Tehran, Iran) using automated method (Hemos II Bio-Rad, Cressier FR Switzerland). The antibody screening test with LISS (low ionic strength saline, DiaLISS; Bio-Rad, Cressier FR Switzerland) as enhancement media and AHG (AHG; CE-Immunodiagnostika, Am Seerain 13 Germany, Eschelbronn) manual technique and acid-eluate test with a commercial kit were performed (Red Cell-Eluate Kit/Lorne laboratories Ltd., Lower Earley, UK). The eluate was used to identify any suspected alloantibody to red cell antigens attached to the cells but not present in the patient's plasma. Direct antiglobulin test (DAT) was performed using automated technique DC-Lys

EM® (Diagast 251/AV.AVINEE-59120 Loos, France). An 11-cell antibody identification panel (IBTO home-made (IBTO-Homemade 11 Cells ID Kit, Registration No. 63882) was used. Chloroquine treatment procedure was used for the patients RBC phenotyping (JUDD's methods in Immunohematology 3rd ed AABB 2008).

Patient's ABO blood group was shown to be an "A" and Rh (D) positive. Panel cells for antibody identification showed specificity for anti-Jka. His direct Coombs test (DAT) was positive for both IgG and C3d.

The antibody screening test was negative with LISS as enhancement media and AHG manual technique, and the results are summarized in Table 1. The auto control showed auto antibody presence.

Tables

Table 1. Antibody Screening Test Results With LISS Enhancement

Incubation phases	IS	37 °C LISS	AHG LISS	CCC
IS: immediate spin; AHG: anti-human globulin; CCC: Coombs control cells; AC: auto control; NT: not tested. Cell followed by a Latin number indicates an antibody screening cell. √: positive reaction (1^+ - 2^+).				
Cell I	0	0	0	NT
Cell II	0	0	0	√
Cell III	0	0	0	√
AC	0	0	1+	NT

DAT, using automated Diagast kit, is constituted by a well of antiglobulin anti-IgG, a well of antiglobulin anti-C3d and a well of negative control. The differential DAT was positive as it is shown in Table 2

Table 2. DAT Result: Automated Technique

IgG	C3d	Negative control
4+	3+	0

Due to patient's history of frequent RBCs transfusion and positive DAT results, an acid elution procedure was performed using a commercial kit. The eluate was tested against the cells of a home-made identification panel that is shown in Table 3.

Table 3. Acid Elution Test Results

Cell No.	Rh-hr	Kell	Duffy	Kidd		Lewis	MNS	P1	Lutheran	Xga	AHG	CCC	Control (last wash)	CCC
				Jka	Jkb									
Cell 1				+	+						W+	NT	0	√
Cell 2				+	0						2+	NT	0	√
Cell 3				0	+						0	√	0	√
Cell 4				0	+						0	√	0	√
Cell 5				+	+						1+	NT	0	√
Cell 6				+	0						2+	NT	0	√
Cell 7				+	0						2+	NT	0	√
Cell 8				0	+						0	√	0	√
Cell 9				0	+						0	√	0	√
Cell 10				+	0						2+	NT	0	√
Cell 11				0	+						0	√	0	√

Cell followed by a Latin number indicates an antibody screening cell. AHG: anti-human globulin; CCC: Coombs control cells. √: positive reaction (1^+ - 2^+).

Anti-Jka was identified with eluate reacting stronger with homozygous cells (Jka+Jka+).

A complement reaction in DAT test results was probably indicative of an anti-Jka complement dependent alloantibody.

Despite the fact that the patient had recently received blood transfusion and the anti-IgG in DAT test was positive, patient's RBCs were phenotyped and found to be Jka negative (Table 4).

Table 4. Patient Phenotype Post-Chloroquine Treatment

C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s
+	+	mf	+	0	+	+	0	0	+	+	+	+	+

One week after the admission his Hb level was 7 g/dL, in the absence of bleeding, in addition to treatment with prednisolone and IVIG and receiving one phenotype-matched RBC unit. His clinical condition did not improve and his Hb level was not rising. Finally the splenectomy procedure was done after consulting with an experienced surgeon. The patient underwent emergency surgery for splenectomy and cholecystectomy; after surgery his condition was better and his Hb level was slowly increased to 8.3 g/dL during 14 days.

Discussion

Chronic multiple transfusion causes alloimmunization frequently [7]. Rate of alloimmunization in thalassemia is 4-37% in comparison to general population (1-4%). RBC antigen difference between recipient and donor is the main cause of alloimmunization [8] that may cause hyperhemolysis syndrome (HS). Alloantibody production occurs in DTHR that destroys transfused RBCs; delayed hyperhemolysis is suggested to be a subset of DTHR with destruction of both donor and host RBCs [3].

HS first was known in patients with sickle cell anemia [4, 5], and it is rarely reported in thalassemia. Several theories are suggested to explain the hemolytic destruction of RBCs like: macrophages hyperactivation, defects in complement regulation, HLA antigen-antibody reactions, a bystander hemolysis and suppression of erythropoiesis [2, 9, 10]. Anti-Jka causes over one-third of DHTRs [8] may be severe. In the absence of HLA antibodies and RBC alloantibodies, it is suggested that macrophages are involved in RBCs destruction [10]. Morawakage et al (2009) reported a case of HS in a child with thalassemia with 2+ DAT for C3b but they could not find any antibody in the patient's serum. They discuss that IgA may lyse RBCs while common serologic tests are negative. They explain that ADCC may occur with low level of antibodies that were not detectable by serologic tests [4].

Laboratory and clinical finding shows that our patient seems to be a case of delayed hemolysis, with post-transfusion Hb level lower than pre-transfusion and a DAT positive. Santos et al [5] described two acute (with negative DAT) and chronic (with positive DAT) forms of HS. Transfusions in a hyperhemolytic episode are able to accelerate hemolysis [3], which was seen in our case. Jka negative blood product (compatible blood) could not increase Hb level in our patient, and his positive auto control result directed that autologous RBC destruction may occur.

It is suggested that antibodies reacting with foreign antigens on transfused cells can cause activation of complement, leading to a phenomenon known as "bystander hemolysis" [3]. Our patient RBC was negative for Jka but had anti-Jka antibodies with a DAT 4+ result for IgG and 3+ result for C3b reflecting RBCs sensitization.

Eberly et al [3] recently reported a case with hyperhemolysis without hematologic disease, that her laboratory tests results were as the same as our patient results. IVIG and steroids [8], avoiding further blood transfusion [9], plasma-to-RBC replacement [11] are different treatment of the HS. Hemolysis was a sever condition in our patient. Additional transfusion was not avoided in this case in his history, but other therapeutic options were chosen, e.g. IV steroid and IVIG without any improvement. The patient was splenectomized finally to decrease RBC destruction by macrophages. His Hb level was increased to 8.3 g/dL during 14 days. Mechery et al suggested that splenectomy could resolve the crisis of hyperhemolysis in a child with beta-thalassemia also [9].

Conclusion

Alloimmunization is a big problem in patients with chronic and multiple transfusions that may be deceased by compatible blood transfusion, transfusion avoiding, using suitable medications and finally splenectomy, due to spleen macroghages role in RBC destruction.

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Conflict of Interest

There is no conflict of interest.

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Case Report OMICS International

A Fatal Case of Cefazolin-Induced Immune Hemolytic Anemia in Iran

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Abstract

Introduction: Drug-induced immune hemolytic anemia (DIIHA) is known as an unpredictable and in some cases serious complication occurring upon pharmacotherapy. Cefazolin, as a first generation cephalosporin used against gram-negative bacteria, has rarely been reported to cause hemolytic anemia. Here, we report the first fatal case of hemolytic anemia associated with cefazolin treatment.

Materials and methods: A 24-year-old woman undergoing operative hysteroscopy for resection of the uterine septum developed hemolytic anemia upon receiving cefazolin immediately before and after the operation. A few hours after surgery, clinical signs of disseminated intravascular coagulation (DIC) appeared. Despite all supportive medical measures that were taken, the patient died of multiple organ failure about 24 hours after admission to ICU. The patient had no history of transfusion or allergies to any types of drugs including antibiotics. Direct anti-globulin test (DAT) was conducted, and the presence of antibody and complement on patient's RBCs was investigated. In addition, the reaction between patient's RBC eluate and cefazolin-coated control RBCs was explored.

Results: DAT was strongly positive (4+); in addition, the presence of IgG4 and C3d on the patient's RBCs was shown. Moreover, the eluate from patient's RBCs reacted (3+) with RBCs pre-coated with cefazolin.

Discussion: The results confirmed the presence of cefazolin-dependent antibodies. This case reveals the importance of taking precaution before using cefazolin even in cases where there is no previous evidence of drug sensitization.

Keywords: Cefazolin; Direct anti-globulin test; Disseminated intravascular coagulation; Immune-mediated hemolytic anemia

Introduction

Drug-induced immune hemolytic anemia (DIIHA) is a rare and unpredictable complication of pharmacotherapy. Accurate estimates of the current incidence of DIIHA are not available because of the difficulty in capturing all cases and estimating the true number of individuals exposed to a drug. The pharmacopeia associated with DIIHA has changed over the recent decades, because the prototypical drugs (e.g., high-dose penicillin and α-methyldopa) have declined in use [1].

However, about 130 new agents have been documented to cause immune system-mediated hemolysis [2]. Approximately 3-4% of patients receiving high doses (e.g., millions of units per day) of intravenous penicillin or first or second-generation cephalosporins develop a positive direct antiglobulin test (DAT). In contrast, in only a small fraction of these patients hemolytic anemia begins to manifest itself. The second and third generation cephalosporins are responsible for most cases of DIIHA today [1,3]. The first useful clue to the presence of an antibody directed against a drug may be that an eluate from the DAT positive red blood cells (RBCs) fails to react with normal RBCs. If the patient is being treated with penicillin or cephalothin, the next step is to test the eluate against drug-coated RBCs [4].

In present case, a 24-year-old woman underwent operative hysteroscopy for resection of the uterine septum. For prophylaxis against bacterial infection, due to a history of cervicitis and pelvic infection, she received 1 gr intravenous cefazolin immediately before and after the operation. Prior to admission she had taken an oral course of doxycycline. Her presenting complaint was primary infertility for six years and her past medical history was ordinary, with no history of transfusion or drug allergies. A complete blood count, few days before admission documented hemoglobin of 12.5 g/dl. About three hours after the operation (at 2:30 pm), she complained of dizziness and urinary retention. Her vital signs indicated collapse with a PR above 130 per min and a BP of 80/50 mmHg. CBC revealed hemoglobin of 7 g/dl, WBCs 20,000, and Platelets: 180000. Multiple blood samples exhibited severe intravascular hemolysis with schistocytes in a peripheral blood smear. Two units of red blood cells were ordered. Severe hemoglobinuria was noticed after urethral catheterization. Primary supportive measures did not make significant improvement in vital conditions. Shortly after, clinical signs of disseminated intravascular coagulation (DIC) with persistent oozing of blood from every needle puncture site were added to the scenario. Elevated values of PT: 36.8, PTT: 69, and INR: 5.7, D-dimer>10000 µg/l (reference value<500), Fibrinogen<50 mg/dl (reference value:

200-400), and Fibrin Degradation Products>20 µg/mL (reference value<5) confirmed the clinical diagnosis of DIC. At 4:30, her O_2 saturation dropped to 85 and clinical signs of pulmonary edema started to manifest. Due to the progressive courses of respiratory failure and pulmonary edema (ARDS), the patient was transferred to ICU. At 18:00, she was incubated. From this time on, complete anuria developed. Despite receiving 6 units of RBCs, 12 units of FFP and 18 units of cryoprecipitates, the patient died about 24 hours after admission to ICU with multiple organ failure. Immunohematologic testing was performed at the blood center reference laboratory. Specific laboratory parameters for the patient are listed (Tables 1-5). Through reporting this case, we aim at drawing the attention of patients' clinical teams (e.g. physicians and nurses) to the importance of close monitoring of patient status and recognizing DIIHA upon utilizing cefazolin.

Patient and Methods

The Iranian Blood Transfusion Organization's Immunohematology Reference Laboratory (IBTO - IRL) performed immunohematologic work-up for possible drug-related antibody analyses. All serological tests were performed on the post cefazolin blood samples. ABO and Rh (D) typing was conducted manually using standard tube methods (Anti-A, Anti-B and Anti-D blend, Diagast/Loos, France). In addition, the patient's RBCs were treated with chloroquine diphosphate solution (Sigma-Aldrich, UK) and subsequently ABO and Rh (D) tests were repeated using these cells. The antibody screening and auto-control tests were performed under three conditions including immediate spin, 37°C incubation, and in the presence of anti-human globulin (AHG; Bio-Rad Dreieich, Germany) according to standard low ionic strength saline (LISS) method (DiaLISS; Bio-Rad ,Cressier FR Switzerland).

The conventional direct anti-globulin test (DAT) was conducted using two different sources of poly-specific anti-human globulin (clone 1; Bio-Rad Dreieich, Germany and clone 2; IBRF holding co. Tehran, Iran); also differential DAT was performed using anti-IgG (Bio-Rad Dreieich, Germany), and anti-C3d (Diagast Loos, France) antibodies. DATs were carried out as per standard internationally accepted serologic methods [4]. An acid elution was performed to prepare an eluate solution from the patient's RBCs using a commercial kit (Red Cell–Elute Kit CE, Lorne Laboratories LTD, Lower Early, RG6 4UT, UK) according to the manufacturer's recommendation. The eluate was then tested for reactivity with a panel of 11 antibody identification cells (IBTO-IRL homemade 11 cell ID panel Kit).

To evaluate the cause of DIIHA and also due to the patient's physician request, cefazolin (antibiotic) and provive MCT-LCT 1% (propofol 10 mg/ml; a short acting general anesthetic agent), which were suspected of causing the fatal immune hemolysis were included in drug investigations. Both drugs were provided for the reference laboratory by the patient's physician; the drugs used in performing the laboratory experiments were the same as those given to the patient. Three separate sets of normal O RBCs were prepared (IBTO homemade 3 cell mini-panel). The first set was RBCs washed but not coated with a drug. The second and third sets of RBCs were those pre-incubated with cefazolin (0.04 g/ml in PBS) and provive (undiluted), respectively according to the method previously described by JUDD [5]. Three test tubes were allocated to each RBC set (Table 5). Assays were performed in three conditions: RT, 37°C and in the presence of AHG. Normal serum was tested against the untreated and drug-treated RBCs as the negative control.

Results

As for the patient's blood group typing, the records showed that four days prior to the surgery ABO and Rh (D) tests had been performed by Baghyat-Allah hospital laboratory and reported as A Rh (D) positive with no indications of discrepancy between forward and reverse reactions (Table 1).

a. Pre-Cefazolin treatment								
ABO & Rh	**Forward type**		**Reverse type**		**ABO Inter pretation**	**Rh(D) type**		**Rh Interpretation**
	anti-A	anti-B	A1 cells	B cells	anti-D	Rh control		
	4+	0	0	4+	A	4+	0	Positive
b. Post-Cefazolin treatment								
ABO & Rh	**Forward type**		**Reverse type**		**ABO Interpretation**	**Rh(D) type**		**Rh Interpretation**
	anti-A	anti-B	A1 cells	B cells	anti-D	Rh control		
	4+	W +	0	4+	Unresolved†	4+	W+	Unresolved
ABO & Rh repeated with chloroquine treated cells	4+	0	0	4+	A	4+	0	Positive
Weak Inconclusive result Strength of positive reactions from (W+,1+,2+,3+,4+) weakest to strongest Negative								

Table 1: ABO and Rh results.

However, upon performing blood group typing tests on post-operative samples we found out unexpected reactions (weak+) to anti-B and Rh (D) control (Table 1); this made ABO and Rh (D) interpretations inconclusive.

Hence, we repeated ABO and Rh (D) tests using both warm saline method and chloroquine-treated cells to disassociate potentially existing IgM/IgG from RBCs membranes [4]. Warm saline technique failed to resolve the discrepancy; however, the chloroquine results were consistent with the pre-operative results so that they confirmed the patient's ABO and Rh (D) blood group to be A Rh (D) positive.

The results for antibody screening test were negative and showed no alloantibody presence in patient's serum. In contrast, the auto-control was strongly reactive (4+) at all conditions (Table 2).

Incubation Phases	IS	37ºC	AHG	CCC
Cell 1 §	0	0	0	
Cell 2	0	0	0	
Cell 3	0	0	0	
AC	4+	4+	4+	

* Immediate Spin
AHG: Anti-Human Globulin
CCC: Coombs Control Cells
Numbers indicate antibody Screening Cells Positive reaction (1+- 2+)

Table 2: Antibody screening test results post-cefazolin specimen (patient serum).

DAT was strongly positive (4+) showing agglutination of patient's RBCs with two different clones of polyspecific anti-human globulin. In addition, differential DAT revealed the presence of IgG (4+) and C3d (4+) on the surface of patient's RBCs (Table 3). As control, 6% albumin control was used.

PS1*	CCC	PS2	CCC	Anti IgG	CCC	Anti C3d	CCC
4+	NT	4+	NT	4+	NT	4+	NT

* Poly specific anti-human globulin
Coombs Control Cells
Not Tested

Table 3: Direct antiglobulin test results (Post-Cefazolin Specimen).

The eluate from the patient's RBCs did not react with any the panel RBCs (Table 4). The patient's serum and the eluate did not react with either the untreated or provive-treated RBCs (Table 5). Also, the patient serum did not react with cefazolin-treated RBCs, while the eluate from the patient's RBCs reacted strongly (3+) at AHG phase with cefazolin-coated RBCs. The normal serum control in all three sets was non-reactive. Accordingly, a suspected drug-induced warm autoimmune hemolytic antibody was indicated (Tables 4 and 5).

	AHG	CCC	Control (last wash)	CCC
Cell 1*	0		0	
Cell 2	0		0	
Cell 3	0		0	
Cell 4	0		0	
Cell 5	0		0	
Cell 6	0		0	
Cell 7	0		0	
Cell 8	0		0	
Cell 9	0		0	
Cell 10	0		0	
Cell 11	0		0	

* Numbers indicate antibody Screening Cells
Anti-Human Globulin
Coombs Control Cells
Positive reaction (1+ - 2+)

Table 4: Acid elution results post cefazolin specimen (patient eluate).

Discussion

Although the field of drug-induced hemolytic anemia abounds with cases of reactions to second and/or third generation cephalosporins, such complications have been less commonly observed upon utilization of first generation cephalosporins [1,2]. In particular, among the latter group, only a few reports of cefazolin–associated hemolysis could be found in the literature [6-8]. Here, we described the first fatal case of cefazolin-associated hemolytic anemia.

To diagnose a case of antibiotic-induced hemolytic anemia one needs to exclude all other possible causes [9]. Having suspected that a drug might be the cause of hemolytic reactions, we performed a set of laboratory experiments to find the underlying mechanisms. Due to the short interval between patient's surgery (drug intake) and her death (which was quite unpredictable) very limited amount of blood sample was available for complete blood bank serology testing. Furthermore, no pre-operative (pre-drug intake) blood sample was available. Nonetheless, the Immunohematology Reference laboratory was able to perform the most necessary laboratory experiments on post-operative samples. These tests included resolving patient's ABO and Rh (D) discrepancies by pre-treating the patient's RBCs with chloroquin in order to separate any unwanted antibodies attached to the RBCs membrane. It is important to rule out any misidentification of patient's blood sample received by the laboratory. Since the patient received multiple blood transfusions at the onset of DIC event, correct identification of patient blood sample by comparing the ABO and Rh (D) blood group results, at hand, with previous records of patient's blood group was very important. As it was seen, auto control test on patient's blood sample was strongly reactive on all phases including AHG phase (Table 2). DAT test followed by a special immunohematology technique (acid elution process) were two other important procedures needed to be considered. Therefore, utmost care was applied to perform these tests (Table 5).

a. **Untreated Cells**			
Incubation phase	**Untreated cells + Patient serum**	**Untreated cells + Normal sera complement source**	**Untreated cells + Patient eluate**
RT	0	0	0
37ºC	0	0	0
AHG	0	0	0
CCC			
b. Cefazolin-coated cells			
Incubation phase	**Cefazolin-coated RBCs + Patient serum**	**Cefazolin-coated RBCs + normal serum**	**Cefazolin-coated RBCs + Patient eluate**
RT	0	0	0
37ºC	0	0	0
AHG	0	0	3+
CCC			NT
c. Provive-coated cells			
Incubation phase	**Cells treated with provive + Patient serum**	**Cells treated with provive + normal serum**	**Cells treated with provive + Patient eluate**
RT	0	0	0
37ºC	0	0	0
AHG	0	0	0
CCC			

Table 5: Reaction of normal sera, patient's serum and patient's RBC eluate with untreated RBCs, cefazolin-coated RBCs and provive-coated RBCs under different experimental conditions.

The result of one DAT is an important and basic requirement for suggesting a case as being drug-induced hemolytic anemia. In the present case, strongly positive DAT results on post-drug treatment specimens pointed to immune-mediated hemolytic anemia following drug injection. In addition, according to differential DAT results, IgG and C3d were present on the patient's RBCs, proposing that antibody attachment together with complement pathway, rather than non-specific binding of serum proteins, was responsible for RBC lysis. Here, the duration between drug administration and antibody-dependent drug-induced hemolytic anemia was surprisingly short suggesting the possibility that high titers of pre-existing anti-drug antibodies might have been responsible for the observed drug-induced anemia. Also, the clinical signs of DIC, observed in this case, are in favor of intravascular rather than extracellular hemolysis.

To find the main drug responsible for the observed DIIHA, normal O erythrocytes were coated with either cefazolin (prophylactically used antibiotic) or provive (general anesthetic agent) and examined for reaction with either patient's RBC eluate or her serum. As control groups, uncoated RBCs were tested accordingly. The eluate showed positive results in presence of cefazolin-treated RBSs, while no provive-related reactions were observed; these finding introduce cefazolin as the main cause of RBC hemolysis in the current case. Nonetheless, the specificity of eluate reaction with the drug could have been further corroborated through addition of a cefazolin solution for inhibition of the reaction; however, as mentioned earlier in this section, we had very limited patient's blood sample. In line with previous eluate results, no reaction was detected when the eluate was mixed with uncoated RBCs suggesting that the antibodies existing in the eluate function in a drug-specific manner. Notably, the patient's serum did not react with neither of the two groups of drug-coated RBCs. This raises the possibility that most of serum IgG specific for cefazolin-coated RBCs had been adsorbed to patient's erythrocytes leading to this fatal case of drug-associated hemolytic anemia.

Commonly, patients with drug-induced hemolytic anemia have a history of previous drug intake with no major problem; this could be the reason for generation anti-drug antibodies causing DIIHA following next treatments. The first case of cefazolin-induced DHIIA goes back to 1977 when a patient admitted for renal artery bypass surgery was prophylactically treated with cefazolin and showed signs of hemolytic anemia [8]. In that case, the patient had a history of penicillin sensitivity although had never received cephalosporins; hence, the authors assumed the structural similarities between penicillin and cefazolin as the reason for development of cross-reactive antibodies. In addition, it has been demonstrated that anti-penicillin antibodies cross-reactively bind to cephalothin-coated RBCs and also that such erythrocytes have shorter survivals in patients with

penicillin-induced hemolytic anemia. In our case, a noticeable drop in hemoglobin level only a few hours after drug injection suggests that preexisting antibodies, rather than newly developed ones, have been involved in erythrocyte lysis. However, our patient had no history of being treated with cefazolin, other cephalosporins, or penicillin. Similar to our case, a patient with no history of drug sensitivity developed hemolytic anemia after a total knee arthroplastic surgery [7]; multiple prior blood transfusions were assumed as the potential cause for the presence of preexisting in that case. However, our case had never experienced any kind of transplantation or blood transfusion according to her medical history making the clinical picture complicated. In fact, this phenomenon has always been difficult to explain; in this regard, it has been shown that a single dose of cefotetan can cause severe hemolytic anemia in patients with no history of cefotetan intake. It is of note that, it commonly happens that people have drugs not prescribed by their physicians and do not mention or recall it at the time of admission. In addition, a wide range of antibiotics are prophylactically given to cattle and chicken. Although meat and dairy products are used by a large portion of Iranian population, sensitivity to cefazolin is not commonly observed; nonetheless, it is still plausible that there have been epitopic similarities between the antibiotics given to cattle and those taken by our patient without a doctor's prescription; this could have led to the development of cross-reactive antibodies responsible for the observed clinical complications following cefazolin intake in the present case. However, whether or not our patient had developed cross-reactive B- or T- cell clones activated against a type of antibiotic is currently unclear to us.

Taken together, it seems that the attachment of preexisting antibodies to the surface of cefazolin-coated RBCs in patient's blood has caused severe hemolysis of erythrocytes leading to the patient's death despite all the standard supportive measures that were taken. Post-mortem pathological examination revealed the uterus and cervix within normal limits without significant pathological findings. Local hematoma identified in right pelvic and femur region, possibly due to multiple blood sampling from femoral route. There have been a few previous reports regarding sensitivity to cefazolin in the past and here we describe the first case of death following cefazolin-related hemolytic anemia. We wish to alert the physicians to the probability of facing fatal cases of cefazolin even when there is no previous history of drug sensitivity.

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National rare donor program in Iran

M. Moghaddam

Iranian Blood Transfusion Organization (IBTO) is the sole organization providing blood services to hospitals and other medical centers throughout Iran, with a network of over 220 blood centers in 31 provinces.

As a reference center, the immunohematology laboratory of the IBTO has been responsible for identifying and providing compatible blood for patients with rare blood types and for multiply transfused patients such as those with thalassemia who have developed several clinically important antibodies.

Prior to 2009, IBTO used traditional non-systematic services when managing rare blood for these patients. However, to provide better service to donors and patients, IBTO decided to expand the operation of identifying rare blood donors and storing units of blood by developing the Iranian Rare Donor Program. This program expanded the management of rare blood to a systematic approach whereby the organization was prepared for any critical time when the request for rare blood was received for a patient with a difficult blood type.

History

The first initiative toward developing the Iranian Rare Donor Program was triggered through a collaborative program called the Joint Program Review and Planning Mission (JPRM 2008–2009) between the World Health Organization (WHO) and IBTO with a goal to increase blood safety in Iran. Available resources were studied and the absence of processes and deficiencies in provided services were identified and analyzed.

We also contacted international organizations that were already experienced in rare donor activities. In this endeavor, contact was initiated between IBTO and the International Blood Group Reference Laboratory (IBGRL-WHO) in Bristol, UK, to learn from their experience and to receive their support. IBTO was interested in a longstanding collaboration with IBGRL-WHO.

Dr. G. Woodfield (past chair of the International Society of Blood Transfusion [ISBT] Working Party on Rare Donors) was kind enough to accept IBTO's invitation to travel to Tehran in 2010 to share his scientific knowledge and technical expertise through a series of lectures in a 2-week workshop.

Responsible heads of the IBTO's special serology laboratories attended the workshop. They were selected to be the future technical specialists who would participate in the national rare donor program. The goal was to review the approaches that would help in the establishment of a systematic and successful national rare donor program.

Other issues that needed close attention were designing the complete structure of the rare donor program, finding the correct approach to screening blood donors to identify rare red blood cell (RBC) phenotypes for the program's data registry, and deciding on the number of donors to be screened in a specific period of time.

National Rare Donor Database

To meet the requests of patients with rare blood types, IBTO decided to regularly screen blood donor samples received randomly from selected blood centers in 31 provinces at the Immunohematology Reference Laboratory in Tehran for rare blood types. Populations from these provinces are ethnically and geographically diverse. Today, more than 35,000 blood samples have been phenotyped serologically. The initial antigens screened are in the Rh (C, e, E, c), Kell (K, k), Duffy (Fy^a, Fy^b), Kidd (Jk^a, Jk^b), and MNS (S, s) systems. All blood samples are selected from group O blood donors.

The National Rare Donor Database currently consists of information from approximately 1000 active rare donors divided into two groups; this process was adopted from a review article published in *Immunohematology*.[1] Group I (rare donors) are defined as those negative for multiple common antigens (428 new donors added in 2012–2014) and Group II (very rare donors) are defined as those negative for a high-prevalence antigen (72 new donors added in 2012–2014). The inventory of very rare units currently consists of about 170 frozen RBC units stored at −80°C (Table 1). The national database also includes two Rh_{null} donors and two K_0 donors. Their molecular backgrounds have yet to be identified.

Where Are We Now?

In 2015, the Iranian Rare Donor Program is a well-established service in IBTO. Between 2012 and 2014, a total of 93 rare units were shipped domestically to medical centers in the country, with 2 requests not filled. One request was for a

Table 1. Identified donors and current inventory of very rare blood in Iran as of 2014

	Number of donors		
Phenotype	**2012**	**2014**	**Frozen RBC units stored at −80°C***
O_h (Bombay)	32	10	26
Fy(a−b−)	14	22	12
Lu(a−b−)	3	5	10
r′r′	1	9	10
k−	4	5	9
D− −	3	3	8
R_zR_1	7	10	5
r″r″	2	0	3
Kp(b−)	1	0	2
R_zR_2	1	2	2
R_zR_z	0	2	1
K_0	0	2	1
Rh_{null}	0	2	0

*RBC = red blood cell.

26-year-old labor and delivery patient from Ilam province with an antibody against an unknown high-prevalence antigen. The second request was for a 28-year-old male thalassemia intermediate patient with antibody of unknown specificity.

There were also three incompatible transfusion cases. All three patients were suffering from beta thalassemia intermediate disorder. One patient was identified as having warm autoimmune hemolytic anemia. Because blood transfusion was not helpful and due to extramedullary hematopoiesis, intravenous steroids and radiotherapy were started with good outcome. The other two patients were identified with multiple antibodies including anti-E, -c, -M, -Jk^a, and an antibody of unknown specificity. The patient with anti-M and -Jk^a experienced a severe hemolytic transfusion reaction and died due to the transfusion of multiple incompatible RBC units.

In general, the practice in Iran has been to use oral promethazine, diphenhydramine, and hydrocortisone as prophylaxis prior to RBC transfusion to a patient with a history of transfusion reactions rather than referring the patient's sample to an immunohematology reference laboratory to identify the antibody and provide antigen-negative blood. With the establishment of the Iranian Rare Donor Program, we hope to see this practice eliminated.

Encouraging Rare Donors

Personal appreciation, educational materials, and occasionally gift cards of no more than a $20 value, to compensate for donor time off from work or transportation expenses, are offered as incentives to encourage awareness and willingness among donors to collaborate with the program.

Every year, January 11th is celebrated as National Rare Donor Day in Iran for rare donor recognition and is attended by officials from the Ministry of Health and IBTO's higher management; the activities are covered by the national media.[2]

After joining the ISBT Working Party on Rare Donors in 2010, our goal has been for Iran to be one of the active members of the committee. It is currently the only country from the Eastern Mediterranean Region in the Working Party. For the first time in September 2014, IBTO organized an international meeting on rare donors supported by the ISBT Academy. International speakers and participants from Europe, Asia, New Zealand, Africa, and the Middle East attended the meeting.

IBTO is potentially ready to fill orders and ship rare units for international requests, particularly to meet requests from neighboring countries.

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Challenges of Establishing a National Rare Donor Program in Iran

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ABSTRACT

Background: Over the past decades, interest in establishing a National Rare Donor Program has increased significantly worldwide. The experience of developing countries, however, is still limited. Rare blood is defined as a blood group found in a 1000- 5000 population and donor has an absence of a high-prevalence antigen, or the absence of multiple common antigens. Iranian national rare donor program was established in 2009. This paper reports the experiences and challenges of establishing a national rare donor program in Iran.
Materials and Methods: This program provides services to all medical centers that need rare units. The main role of rare donor program is to maintain information of rare donors that are identified at the immunohematology reference laboratory located in Tehran. Good manufacturing practices and standard operating procedures are utilized to all activity. The IRL secures frozen blood to make them available when rare blood is required.
Results: As many as 1000 different types of rare donors have been identified in Iran, including several individuals whose blood group had developed clinically significant allo-antibodies. In addition to routine donors' personally identifiable information such as addresses and telephone numbers, we also access to the contact information of their close relatives or friends for emergency situation. Contact data are kept up to date at least twice annually. IRL staff are ready to provide services to patients with rare blood types, 24 hours per day, 7 days per week. To date, more than 80 donors with very rare blood group are listed on the IRL rare donor database in 31 centers. Current practice at IRL is to screen the first and second-degree relatives of any patient found to have a rare blood type for a matching blood donor. Iranian blood services need to establish special departments to provide rare blood RBCs and technical assistance for a quicker and more efficient responses to patients and request of their medical staff for blood transfusion. To achieve this aim, there were several challenges, including situation analysis and justification of the program, allocation of financial support by top managers, engineering and technical maintenance, facility and environmental services, employee awareness and communication between blood centers, technologist training in advanced immunohematology.
Conclusion: The results of this survey are encouraging and indicate that the information and database for rare donors will provide services to patients with very difficult and complex serology test results requiring rare blood transfusion. The experience of IRL may be helpful for other transfusion centers in developing countries.

Keywords: Rare blood program, Rare blood type, Rare donor

INTRODUCTION

For many years, one of the challenges in transfusion medicine was to ensure the availability of compatible units of rare blood for patients with either single or multiple blood group antibodies present[1]. These rare blood types may represent a significant impediment to both urgent and planned transfusion of red blood cells (RBCs) and also carry out important administrative and clinical challenges for the practitioner. Clinical guidelines address little about the management of patients with unique blood group. Patients who require blood from donors known as "rare blood donors" present unique challenges both at the preoperative health care delivery system at large and for the individual clinician who desires to maintain safe, efficient, and cost-effective care. It is most challenging to supply blood for a patient with an antibody to an antigen of high prevalence, with an occurrence rate of less than one in 1000[2] Often, there are other antibodies against common blood group antigens present with antibodies to high prevalence antigens, which provide a higher challenge[3]. Blood banks should answer the following questions:

How frequently one is expected to encounter these patients? How frequently does a non-ABO hemolytic transfusion reaction occur if an alloimmunized patient is transfused on an emergent basis? What effect will be the advent of red cell molecular genotyping played in providing blood for patients with rare blood types? Are regional and national rare donor programs often able to cope with the majority of requests for rare blood RBC units? Unfortunately, few medical centers are aware of the existence of the program that organizes information about rare blood donors and seeks to assist providing rare blood. This article introduces activity of the IRL and challenges of establishing a national rare donor program in Iran with task to answer the above questions.

History of pioneering rare blood activities

The need for a rare donor registry was noted at an early stage of blood transfusion development. The UK blood group reference laboratory was established in 1946 for the national blood transfusion service[3]. By 1959, the AABB had improved the rare donor file, and the American Red Cross created the rare donor registry. Before merging their programs in 1998, the ARC and AABB maintained separate rare donor databases and separate programs. About the same time, a similar panel was established in France to meet local needs[4].

The International Panel of Rare Blood Donors or International Donor Panel (IDP) was formed in 1965 under the directive of the ISBT and the day to day running of the IDP has been carried out by the red cell reference department of the International Blood Group Reference Laboratory (IBGRL) since that time[5]. The ISBT rare blood donor working party (ISBT WP) was appointed in 1985 by the ISBT to provide oversight of any matters related to the supply of rare blood donations on an international basis[1,4,5].

Chain of activities at Iranian National Rare Donor Program

Iranian blood transfusion organization (IBTO) was established in 1974. Thirty-five years later, Iranian national rare donor program was established in 2009. The central Iranian immunohaematology reference laboratory (IRL) has the responsibility of identification of donors with rare types and maintaining the database of rare blood groups in Tehran. Moreover, it is working to provide services for centers that are involved in the transfusion chain. The IRL role through Iranian national rare donor program is also to improve services and update the information on rare donors that have been identified at other centers. IRL provides services to 31 IBTO provincial blood donor centers, hospital blood bank laboratories and other medical centers, thalassemia centers, bone marrow & other transplantation centers. Annually, about 7000 to 10,000 samples from mentioned centers are referred to IRL. Department of Frozen blood processing and storage facility are part of the IRL.

When a patient with difficult blood type is identified in a hospital blood bank, the special serology laboratory is informed at the provincial blood centers. The physician's written request and 10 ml EDTA blood samples are forwarded to the laboratory. Preliminary work-up is performed at the provincial special serology laboratory. The central IRL in Tehran is contacted for consultation if necessary. If the serological test results are completed, the search to find matched RBC units is

initiated at the provincial level. If matched RBC units cannot be found or if the serological identification needs more extensive work-up, the samples are sent to central IRL in Tehran for complete antibody identifications. The rare blood database is searched and if there is any frozen blood available, this blood after processing, thawing and deglycerolization is transported to the hospital via air or land transport supervised by a trained technologist. Matching donors also from data registry are contacted for donation if they are eligible to donate.

A summary of the activities of the central Iranian reference immunohaematology laboratory and frozen blood department pertaining to rare donor program is as follows:

• Retention rare donor and effective continuous relation (education, public relation, recalling & updating personal information)

• Examining and resolving discrepancies in blood grouping complex, identifying alloantibodies and autoantibodies.

• Determining the principal blood group and other blood groups with different ways (Manual method, Automation & Molecular typing)

• Maintaining information on national bank of rare blood, keeping in touch with network of database of rare blood at the International Society of Blood Transfusion

• Participation in the evaluation and quality control of blood typing antisera according to international standards of WHO (NIBSC).

• Participation in External Quality Assessment Scheme (EQAS) with unknown samples from international centers

• Monthly production of RBC kits for determining antibody-screening strategy and detecting unexpected antibodies using frozen rare RBC aliquots.

• Training students and staff in specific serology.

Facts and figures

A total of 1300 rare donors were registered in this study. Fifty six individuals with rare Bombay blood group were identified in this survey. There were about 175 rare units in frozen state for transfusion. The most difficult types to find were Kpb negative, Rh null, K null, Jk(a-b-), S-s-U- . The average number of rare donor units used per year was close to 50 unites. Approximately 20 units of rare blood are delivered to the hospital via air or land transport annually. There are procedures in place to collect information when rare blood is forwarded and used. Special delivering form has been designed for this purpose.

Challenges and important step to ensure successful establishment of a National rare donor program

Packaging and reliable transportation

In order to transport the rare blood in liquid phase, a monitored temperature at 1-10 C should be maintained and standard commercially insulated and leak-proof boxes should be used. Several days may be required to keep a frozen supply of units of rare blood. As transport of such units over long distances presents a number of difficulties, special containers should be designed[1]. It is easier to ship frozen cells by glycerol and these can be stored in dry ice but deglycerolizing equipment should be available at the receiving facility. First of all, containers should be validated for their ability to maintain low-temperature storage for the required time period. Also, it is necessary to use data logger to record temperature information during transportation.

Labeling of boxes should always be adequate. The labels should also have the full shipper address, full details of properly classified biological or infectious material, and the information on the container should indicate the contact party in case of delay in blood transport. For long-distance transport, the contract with specific airlines should be signed in order to transport the blood in a short period of time. The name and phone number of receiving center should be displayed on the outside of the container.

Conformation of serological antibody testing and rare blood group identification are done at central IRL. It performs a crossmatch of any rare RBC units before shipping them in order to prevent misidentification or any discrepancies between IRL and medical center testing results. This might cause unnecessary delay or result in the loss of units. Therefore, the collection of a properly labeled pre-transfusion blood specimen from the intended

recipient is critical for safe blood transfusion. The majority of hemolytic transfusion reactions arise from misidentification of patients or pre-transfusion specimen labeling errors[6].

Standard Operating Procedures and use of a comprehensive Information Technology

All centers have specific standard operating procedures designed to support the activities related to the handling of Immunohematology procedures and rare blood units. Because of sudden and unexpected requests for rare blood types, all centers are equipped with computers. Some of the data items which need to be collected and placed in a computer database are as follows:

- Donor personal information
- Rare donor database of donors and their antigen type
- Database for frozen unit inventory

Requests must contain sufficient information for accurate recipient identification. Each center is required to develop and apply policies and procedures for accurate patient identification and specimen collection. Problems might exist in the preparation, storage and transportation of rare blood. Survey shows few known failures in the systems have resulted in: wasting rare blood, receiving the rare blood in an unsatisfactory condition, or a delay in the delivery of rare blood.

Receiving centers are validated through acceptable performance of the procedure as well as proficiency of staff members.

Engineering and technical maintenance

Engineering staff are trained to follow proper storage requirements upon receipt of the blood from the remote blood centers during holidays and non-working hours. Temperature requirements during blood transport, monitoring of storage temperatures in refrigerators and freezers of blood component storage are available with continuous temperature monitoring devices that would be able to detect a temperature deviation before blood components might be affected. Daily checks of the temperature should be recorded by engineering staff in order to ensure proper operation of equipment and recorder.

Deviations from acceptable temperature ranges are annotated, dated and initialed on the temperature recording chart by the engineering staff who note the deviation, and appropriate correcting action is taken either in emergency or routine situations. The equipment should be so constructed as to sound an audible alarm when an abnormal situation is sensed.

Facility and freezing condition

Glycerol is most commonly used in RBC cryoprotectant and RBC units, in frozen state, are stored at 80°C (40% wt/vol glycerol, high-glycerol method). Frozen RBC expires after 10 years, but AABB standards permit storage of frozen units for 10 years [7].

Frozen units must be thawed, and prior to transfusion, the glycerol must be removed. The use of thawed RBCs is limited to 24-hour expiration when stored at 1°C to 6°C because of concerns related to potential bacterial contamination when a functionally open system is used to add and remove the glycerol. Thus, the critical care specialist should understand the transfusion planning purposes and notice that preservation of post-thaw rare blood unit is limited to hours, whereas frozen rare blood units may be preserved and stored for many years. The recently developed closed systems have an extended expiration date to 14 days for thawed, non– leukocyte-reduced, and deglycerolized RBCs when stored at 1°C to 6°C [8]. For extended storage of units in the case of antigen negative rare donor that is used in autologous or allogeneic transfusion, cellular components of RBCs are frozen, and for this reason, it is considered to provide appropriate equipment and various devices such as refrigerator and freezer. However, loss of absolute red cell mass which is resulted from red cell damage, will occur.

High-glycerol freezing method is utilized, the 57.1% wt/vol high-glycerol, slow-freeze technique.

During, thawing and deglycerolizing RBCs the freezing canister in which the RBC unit has been stored is placed in a 37 °C dry heaters, or, after overwrapping, is placed in a 37 °C water bath. When the red cells have been frozen in the primary blood container, then the container is thawed at 42 °C. Thawing is completed within 40 minutes [9,10].

Different situation requires different numbers of staff and different skill levels. Type of patient surgery, either routine or emergency, and number of rare RBC units will determine the type of staff and resources. Moreover, performing procedures require knowledge and skills.

Employee awareness and communication between regional blood centers

One of the key components of quality management and quality improvement is the employee knowledge and awareness, then a key component of employee performance is training. To do the job well, staff must be properly trained to fulfill responsibilities. Clear communication is an important issue in different blood centers that are involved in requesting blood for a patient with rare blood types or receiving a rare blood shipment. An assigned person in each blood center is responsible for communicating the relevant information, including physician request, the patient's blood sample, packaging for shipping and receiving samples.

Technologist Education & Training in Advanced Immunohematology

Different donor centers may require different numbers of staff and skills in rare blood collection, processing, compatibility testing, storage, or distribution of rare blood. All personnel are required to have educational background, job training and experience to perform their duties.

The type of staff resources will be determined by the donor center's immunohematology activity level, number of difficult samples received and number of medical centers in the region which need rare blood services. Specific licensing requirements need to be provided by technologists who are practicing as professionals in Immunohematology reference serologic testing need to be provided. Job descriptions is enhanced and reflect employment requirements by job title.

At management level, staff members supervise the daily operations of the unit, write procedures; train the serology staff, and enforce compliance with applicable procedures, standards and equipment. Education and training of the employees are applied in the preparation of rare blood and performance of Immunohematology standards.

Employees with a college degree in one of the sciences such as laboratory science can be trained successfully to perform specific serologic tests. Staff should be promoted and provided opportunities to attend continuing education seminars on topics in serologic and related fields. In numerous states, continuing education is required for the renewal of licenses.

Financial & Administrative Support by Higher Management

Due to the cost of equipment and consumables used in rare blood banks, especially freezing and storage devices, budgeting in rare blood services need special attention and dedication by higher management and top managers of the blood-service systems. So, the government is required to provide financial support to blood banks. According to the study of Geralyn et al., the cost of collecting, testing, storing and transporting blood to hospital transfusion service in the United States is approximately $925 to $1150 per RBC unit[11]. According to The American Rare Donor Program (ARDP), the cost of special antigen typing will rise to $500 - $1200 per each unit. Meanwhile, nearly $1940 to $2800 may be added to units imported to the United States [12]. Thus, the cost of a rare unit of blood in the United States ranges from approximately $1148 to $1373.

Cost of preparing and handling a rare blood unit may differ between countries according to the policies of blood centers. To date, the rare blood services for patients are provided free of charge in Iran and all is paid by the government, including transportation charges.

DISCUSSION

During the past decades, interest in national rare donor program has increased in a significant manner worldwide. This is especially true in developed countries. In developing countries, however, the experience is not a lot. We report the results and challenges of the rare donor program, which was initiated in 2009.

All activities used at IRL was based on those reported previously by different international

organizations. In the current year, database consisted of 1300 personal information of rare donors and 308 RBC units in frozen state. As the proportion is similar to those reported by different countries, it is important to notice that this is the initial phase of our program, and we have evidence that this number will increase in the following years, as our collection program of donor and rare units improves.

According to the study of Woodfield, many areas of the world have problems in the storage, transportation and financing of rare blood[1]. Between January 2005 and June 2006, there were approximately 51 000 active donors in the rare donor registry. Rare units were not always available to fill a transfusion request. Flickinger examined the effect of ARDP in filling requests for high incidence antigen-negative RBC units for patients with sickle cell disease. She reported that 88% of 141 requests were completely or partially filled. The top 3 requested phenotypes for this patient population were U−D+, Js(b−), and U−D− [13]. The study of Goodell et al. focused on patients with routinely encountered red cell antibodies. Seltsam et al. studied blood banks in Germany, Switzerland, and Austria to identify transfusion support[14, 15].

During the 20-month survey period, 52 patients with antibodies to high-frequency antigens were hospitalized on 56 occasions (49 routines, 3 urgent, and 4 unknown). The top 5 antibody specificities identified included anti-Kp^b, anti-Vel, anti-Lu^b, anti-Yt^a, and anti- Co^a. Insufficient blood was offered to 40% (23/56) of transfusion cases. Procedures continued without transfusion in 10 cases. Antigen-positive RBCs were transfused in 8 cases and led to 5 cases of delayed hemolytic transfusion. The authors note that the additional time is required to achieve these rare units and recommend that additional group O donors be typed for blood for Kp^b, Vel, Lu^b, and Yt^a. Thus, for a patient with previously identified multiple alloantibodies, the critical care specialist may consider transfusing alternative units such as D-positive units to a D-negative patient or antigen-positive units for those antibodies that are no longer serologically detectable until compatible units are provided by the ARDP. One can then evaluate the patient for the presence of intravascular and extra vascular hemolysis[16]. Nonetheless, one should not withhold transfusion from a patient whose life depends on urgent transfusion.

If an autoantibody appears to be present and one is uncertain whether underlying alloantibodies are present, transfusing blood matched for ABO, Rh (D, C, and E), and K (Kell) should be considered. Additional antigens may be matched if time permits. Selecting “least incompatible” blood for transfusion is unreliable [17].

CONCLUSION

According to the results and reports of different countries in ISBT working party 2012 for rare donors, the majority of member countries have nationalized rare donor program. The number of donors in database and number of frozen rare cells are different in various countries. The definition of a rare blood 1/1000 is the same in all member states, except 1/250 in South Africa. Also, the most difficult rare blood types differ in *various regions*: in China (Rhnull; D - -), Finland (Vel neg; Oh; hr S −), France (U-; Fy(a−b−); Vel− ; Rhnull; D - -; Hr−; HrB –) and in Iran (D - -; E− c− K- Jk(b−); E−c− K− Jk(b−) Fy(b-); C− E− Jk(b−) S− M−; E− C− c− e−). Rhnull has been reported in 9 countries: Spain, Switzerland, Taiwan, Netherlands, Israel, India, France, Germany and China. D --, U- and Ko have been reported in 6 countries. Moreover, the availability of especially effective reagent is controversial in many countries[11].

Finally, in order to improve rare donor program, we have to overcome economic and other issues. So, the government should allocate sufficient budget for initial establishment and continuous improvement program of rare donor services of Iranian National Rare Donor Program. It is necessary to put a heavy emphasis on continuous education, training and improving knowledge of technologists with special reference to serology in various provincial blood donor centers. The importance level of services offered to patients with difficult samples by rare donor program should be promoted to prevent transfusion-related adverse effect. To overcome the lack of experience in using special immunohematology procedures as well as technical and human errors in reporting test results, effective contact and collaboration with

international immunohematology reference laboratory for technical help and testing confirmation is required. Easy access to expensive internationally accepted anti-sera availability of automated machines, supplies and consumables for glycerolization and deglycerolization of rare RBCs should be provided. Moreover, there is a need for access to sufficient storage area and good environmental condition, sufficient number of -80°C standard freezers for longer blood storage, empty back up freezer, controlled access area temperature, controlled environment (-18 °C -22°C) by central heating and cooling system, central 24-hour temperature control, and alert system for any electrical failure. Finally, serious difficulties that occur in delivery of blood by Air and Land (bureaucratic procedures /ticket purchase / cancellation of flights/ flight delays), due to lack of experienced private companies, should also be considered.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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Review

Proceedings from the International Society of Blood Transfusion Working Party on Immunohaematology, Workshop on the Clinical Significance of Red Blood Cell Alloantibodies, September 2, 2016, Dubai

Clinical significance of antibodies to antigens in the Raph, John Milton Hagen, I, Globoside, Gill, Rh-associated glycoprotein, FORS, JR, LAN, Vel, CD59, and Augustine blood group systems

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This article reviews information on the clinical significance of antibodies to antigens in the Raph, John Milton Hagen, I, Globoside, Gill, Rh-associated glycoprotein, FORS, JR, LAN, Vel, CD59, and Augustine blood group systems. Antibodies to many of the antigens in these groups are rarely encountered because of the high prevalence of the associated antigens in most populations. For many of these antibodies, the clinical significance—that is, the potential to cause reduced survival of transfused antigen-positive red blood cells or a transfusion reaction (e.g., anti-P, anti-Jr^a, and anti-Lan), and/or hemolytic disease of the fetus and newborn (e.g., anti-RHAG4 and anti-Vel)—has been documented. For other antibodies, their prevalence is so rare that information on the clinical significance of their antibodies is not available (e.g., anti-FORS1). ***Immunohematology* 2018;34:85–90.**

Key Words: clinical significance, antibodies to red blood cell antigens, Raph, John Milton Hagen, I, Globoside, Gill, Rh-associated glycoprotein, FORS, JR, LAN, Vel, CD59, Augustine

Raph Blood Group System

The Raph blood group system contains just one antigen, MER2 (RAPH1), located on the tetraspanin CD151 glycoprotein (TM4SF).[1,2] The true MER2– phenotype, associated with the presence of anti-MER2, is very rare and results from mutations in *CD151,* but there is a quantitative red blood cell (RBC) polymorphism in which RBCs of about 8 percent of white individuals are serologically MER2–.[2]

Clinical Significance

There have been six reports of human alloantibodies to MER2. Three of the subjects were found to have a stop codon in the *CD151* gene, which encodes a member of the tetraspanin family of proteins. These three individuals had nephropathy and deafness, and two of the three, who were siblings, also had skin lesions, deafness, and β-thalassemia minor. The fourth subject had missense mutation c.533G>A (p.Argl78His). Subjects 5 and 6 shared missense mutation c.511C>T (p.Argl71Cys) as well as a synonymous single-nucleotide mutation (c.579A>G) and had no clinical features. Although the CD151 protein is critical to cell adhesion and signaling and is implicated in cancer progression, its significance in transfusion medicine is limited to only one report of a hemolytic transfusion reaction (HTR).[3] Least-incompatible RBC units should be selected for transfusion to patients with anti-MER2.[2] No information on anti-MER2 causing hemolytic disease of the fetus and newborn (HDFN) is available.[4]

John Milton Hagen Blood Group System

The John Milton Hagen (JMH) blood group system consists of six high-prevalence antigens that are recognized by the International Society of Blood Transfusion (ISBT) and are numbered sequentially from JMH1 through JMH6. Confirmed JMH variants are named with the first letter from the antibody maker's first name following JMH (JMH2 named JMHK, JMH3 named JMHL, JMH4 named JMHG, JMH5 named JMHM, JMH6 named JMHQ).[5] These antigens are located on the Sema7A protein.[5,6] The chromosomal location of *SEMA7A* is 15q22.3-q23. JMH1 is the primary antigen in the system and is present in greater than 99 percent of all individuals. The JMH1– phenotype is more commonly acquired by depression of the antigen. This finding might explain the serologic observation of a positive direct antiglobulin test (DAT) seen in many individuals with anti-JMH.[5]

Clinical Significance

JMH1, commonly known as JMH, is most notable because transient depression of the antigen occurs, and (auto)anti-JMH may develop.[5] JMH– patients with anti-JMH often have no history of transfusion or pregnancy. Of seven JMH antibodies,

five were IgG4 and two were IgG1, although IgG3 anti-JMH has been described. There are numerous cases where patients with anti-JMH have been transfused with JMH+ blood with no adverse effects. One such patient received 20 units of JMH+ blood in 10 months, with the expected hemoglobin rise. There are no reports of JMH antibodies causing HDFN, which is unsurprising considering that JMH antigens are expressed very weakly on cord cells. One patient with anti-JMH is reported to have experienced an acute intravascular HTR, but evidence that the JMH antibody was responsible is limited. Antibodies developed in the rare JMH variant types may cause reduced RBC survival.[2] Today, rapid detection of JMH antibodies with recombinant SEMA7A protein and the particle gel immunoassay has been developed.[7]

I Blood Group System

The I antigen, together with i, used to be part of the Ii blood group collection. The gene encoding the I β-1, 6-*N*-acetylglucosamine transferase (IGNT/GCNT2) responsible for converting i active straight chains of carbohydrates to I active branched chains has been cloned,[8] and some mutations responsible for adult i phenotype have been identified.[9,10] Hence, I has been promoted to the I blood group system comprising only a single antigen, the I antigen, and i remains in the Ii collection. RBCs from adults predominantly express I antigens and only low levels of i antigens, higher levels of the latter predominate in fetal and neonatal RBCs. In a small number of individuals, only very low levels of I can be detected, and their RBCs show high levels of i (adult i phenotype). This phenotype is believed to result from lack of activity of the I branching transferase, a product of the *GCNT2* gene.[2]

Clinical Significance

Potent cold reactive antibodies responsible for cold agglutinin disease are usually of I specificity. These antibodies are generally monoclonal and are usually IgM, but IgG autoanti-I can also occur. They directly agglutinate I+ RBCs at 4°C with varying thermal amplitude but are generally inactive above 30°C. One autoanti-I active at 30°C caused an acute HTR in a small child when 2 units of blood were transfused immediately after removal from the refrigerator. Transient, polyclonal, or oligoclonal autoanti-I may arise from infection, most typically by *Mycoplasma pneumoniae*.

Alloanti-I of high titer is rare and usually presents in the sera of i adults. These antibodies are almost invariably IgM and are active only at low temperatures. Rare examples may be hemolytic and have a thermal range up to 37°C, and some anti-I with a thermal range below 37°C can cause shortened survival of transfused I+ RBCs. One anti-I became potentially clinically significant after transfusion of 6 units of I+ blood.

Globoside Blood Group System

The P blood group antigen of the Globoside system is a glycolipid structure, also known as globoside, on the RBCs of almost all individuals worldwide. The P antigen (Gb4) is intimately related to the P^k and NOR (P1PK4) antigens.

The molecular genetic basis of globoside deficiency is the absence of functional P synthase caused by mutations at the *B3GALNT1* locus. Other related glycolipid structures, the LKE and PX2 antigens, remain in the Globoside blood group collection pending further evidence concerning the genes and gene products responsible for their synthesis.[11]

Clinical Significance

Anti-P is found in the serum of all P^k individuals and can be separated from serum of p individuals by adsorption with P_1^k or P_2^k cells or by inhibition with hydatid cyst fluid.[2] When complement is present, anti-P will hemolyze P_1 or P_2 phenotype RBCs.[2] P antibodies are IgM and often also IgG, are usually reactive at 37°C, and can cause severe intravascular HTRs. Autoanti-P is associated with paroxysmal cold hemoglobinuria. P antigen is also a receptor of parvovirus B19.

Cytotoxic IgM and IgG3 antibodies directed against P or P^k antigens are associated with a higher-than-normal rate of spontaneous abortion in women with the rare p, P_1^k, and P_2^k phenotypes.[4]

Gill Blood Group System

The Gill blood group system was added to the list of systems already recognized by the ISBT in 2002. GIL, the only antigen of the Gill system, is an antigen of high prevalence located on the water and glycerol channel aquaporin-3 (AQP3).[2,12] The GIL– phenotype results from homozygosity for a splice mutation in *AQP3*. Anti-GIL has been identified in five Gil– white women who had been pregnant at least twice.[2]

Clinical Significance

Five examples of anti-GIL have been identified, all in white women who had been pregnant at least twice. No GIL– individual was found by screening 23,251 white American and 2841 African American women with anti-GIL. RBCs from two

of the babies of mothers with anti-GIL gave a positive DAT, but there were no clinical symptoms of HDFN. Anti-GIL may have been responsible for an HTR, and results of monocyte monolayer assay (MMA) with two GIL antibodies suggested a potential to cause accelerated destruction of transfused GIL+ RBCs.[2]

The Rh-Associated Glycoprotein Blood Group System

In 2010, the recognition that three RBC surface antigens were located on RhAG encoded by *RHAG* led to the establishment of a new blood group system. Two high-prevalence antigens, Duclos (RHAG1) and DSLK (RHAG3), two low-prevalence antigens, OI(a) and (RHAG2), and RHAG4, have serologic characteristics suggestive of expression on RhAG, but RHAG4 has been shown to not exist and is under investigation by ISBT to be retracted.[13]

Clinical Significance

No data are available.[4]

FORS Blood Group System

This blood group system has been named FORS after its original finder, Lund professor John Forssman. The FORS antigen (originally recognized as the A_{pae} phenotype) was discovered by weak reactivity of RBCs against polyclonal anti-A reagents, reactivity against the lectin *Helix pomatia* (snail anti-A), and no reactivity with the plant anti-A_1 lectin, *Dolichos biflorus,* in two different families. Genomic analysis of the ABO locus in both samples revealed that they were genetically group O, and the reactivity described must be due to other phenomena.[14,15]

Clinical Significance

The clinical significance of anti-FORS1 is not known.[4]

JR Blood Group System

The JR blood group system (ISBT 032) consists of one antigen, Jr^a, which is highly prevalent in all populations (>99%). Jr^a is located on the ABCG2 transporter, a multipass membrane glycoprotein (also known as the breast cancer resistance protein [BCRP]), which is encoded by the *ABCG2* gene on chromosome 4q22.1.[16–19] The rare Jr(a–) phenotype has been found mostly in Japanese and other Asian populations, but also in people of northern European ancestry, in Bedouin Arabs, and in one Mexican individual. The rare Jr(a–) phenotype mostly results from recessive inheritance of *ABCG2* null alleles caused by frameshift or nonsense changes.[17,20–22] To date, more than 25 different mutations responsible for the absence of ABCG2 as well as mutations giving rise to weakened Jr^a expression have been identified.[16]

Clinical Significance

ABCG2 expression levels in cord RBCs are higher than those in adult RBCs, and the change of ABCG2 expression in erythroid lineage cells may influence the clinical course of fetal anemia with anti-Jr^a.[23] Anti-Jr^a may be stimulated by transfusion or pregnancy and has been detected in untransfused Jr(a–) women during their first pregnancy.[24] Most anti-Jr^a are IgG1 and sometimes IgG3. Anti-Jr^a may fix complement, can be a dangerous antibody in pregnancy, and has been implicated in severe and fatal HDFN; in other pregnancies with anti-Jr^a, however, indications of HDFN have been no more than a positive DAT on cord cells or mild neonatal jaundice. Many transfusions of Jr(a+) RBCs to patients have resulted in no signs of hemolysis, although incompatible transfusion may cause a sharp rise in the titer of anti-Jr^a, resulting in signs of an acute HTR in subsequent transfusions. A patient with anti-Jr^a developed rigors after transfusion of 150 mL crossmatch-incompatible blood. Least-incompatible RBC units may be suitable for transfusion to most patients with anti-Jr^a, but Jr(a–) RBCs should be selected in cases where the anti-Jr^a is of high titer.[2]

LAN Blood Group System

LAN (Langereis) was officially recognized by the ISBT in 2012 as the 33rd human blood group system. It consists of one high-prevalence antigen, Lan (LAN1).[25] The ABCB6 protein is the carrier of the Lan blood group antigen.[16,25] The *ABCB6* gene (chromosome 2q36, 19 exons) encodes the ABCB6 polypeptide, known as a porphyrin transporter. The exceptional Lan– individuals do not express ABCB6 (Lan_{null} phenotype) owing to several different frameshift and missense mutations.[25] To date, more than 40 *ABCB6* alleles that encode Lan– or $Lan+^w$ phenotypes have been described,[26] and quantitation of Lan antigen in Lan+, $Lan+^w$, and Lan– phenotypes have been performed.[27] Despite the Lan antigen role in erythropoiesis and detoxification of cells, Lan– individuals do not appear to demonstrate susceptibility to any disease.

Clinical Significance

Anti-Lan has been reported in two African American individuals as well as in other populations including Caucasians and Asians and may be stimulated by transfusion or pregnancy. The original anti-Lan was responsible for an immediate HTR characterized by fever and chills. There is no report of naturally occurring alloanti-Lan; none of the Lan– siblings of the Lan– propositi have anti-Lan. Anti-Lan has been described as having variable clinical significance, either for HTRs (none to severe) or HDFN (none to mild).[25] Lan alloantibodies are mostly IgG1 and IgG3, although IgG2 and IgG4 may also be present. Some anti-Lan fix complement; others do not.[2] Despite challenging conditions caused by the scarcity of Lan– donors worldwide, ideally Lan– RBCs should be selected for transfusion to patients with anti-Lan, especially individuals with a high-titer antibody,[2,25] although least incompatible RBCs may be suitable for patients with weak examples of the antibody. The only autoanti-Lan was reported in a patient with mild autoimmune hemolytic anemia (AIHA) with depressed Lan antigen expression.[2]

Vel Blood Group System

Vel is an RBC antigen that is expressed by more than 99.9 percent of the population.[28] The recognition of Vel dates to 1952, when a patient, named Mrs. Vel, suffered a transfusion reaction due to the presence of an antibody that was found to agglutinate sera of over 10,000 individuals. The antibody was named anti-Vel.[29]

Recently, the SMIM1 protein was shown to carry the Vel blood group antigen. Using a high-density single nucleotide polymorphism array, Storry et al.[30] identified the *SMIM1* gene residing in a 97-kb region of homozygosity on chromosome 1p36 in the vicinity of the *RH* locus. A frameshift deletion of 17 nucleotides in exon 3 of *SMIM1* is responsible for the Vel– phenotype.[16,31,32] Genotype screening estimated that ~1 in 17 Swedish blood donors is a heterozygous deletion carrier and ~1 in 1200 is a homozygous deletion knockout.[31]

Clinical Significance

The high clinical significance of Vel is related to what happens to individuals with the Vel– phenotype upon transfusion or pregnancy. Vel alloantibodies are never naturally occurring, and most producers of anti-Vel have been transfused, yet Vel antibodies are predominantly IgM and fix complement. Of the two IgG anti-Vel, one was IgG1, and the other contained IgG1 and IgG3. Anti-Vel is a dangerous antibody, and patients with anti-Vel should be transfused with Vel– RBCs. The first anti-Vel and other examples since have caused severe immediate HTRs. Anti-Vel may be missed in compatibility testing if inappropriate methods are used.[33] Although many examples of anti-Vel have been found in pregnant women, anti-Vel does not usually cause HDFN, probably because most anti-Vel are predominantly IgM, and the Vel antigen is usually expressed weakly on neonatal RBCs.

Two examples of autoanti-Vel were responsible for (AIHA), although in one case, a nine-week-old infant, the RBCs gave a negative DAT.[2]

CD59

CD59, also known as the membrane inhibitor of reactive lysis (MIRL), homologous restriction factor (HRF), and membrane attack complex inhibitory factor (MACIF), is a cell surface glycoprotein of approximately 20 kDa that limits the activity of the terminal complement complex C5b-9 and is more effective than decay accelerating factor (DAF) or CD55 in this respect.[28,34] The first demonstration of anti-CD59 was in a patient homozygous for a CD59 deficiency, which led to the discovery of a new blood group system, CD59, and a null allele (c.146delA).

CD59 is attached by a glycosylphosphatidylinositol (GPI) anchor not only to erythrocytes but also to various other cellular membranes. Seven cases of an isolated CD59 deficiency due to three distinct null alleles of the *CD59* gene have been published so far.[34] As well as being cell-bound through its GPI anchor, soluble CD59 is also found in plasma, urine, and cerebrospinal fluid.

Absence of CD59 is associated with hemolytic anemia and with thrombosis as well as with other autoimmune diseases such as systemic lupus erythematosus.[2]

Clinical Significance

No data are available.

Augustine Blood Group System

At^a is a high-prevalence antigen found on the RBCs of over 99 percent of individuals.[35] The first literature referring to the At^a antibody, which can be responsible for severe HTRs and mild HDFN, dates to the 1960s. Applewhaite et al.[36] identified an antibody with a novel specificity in the serum of Mrs. Augustine when the RBCs of her third child gave a positive DAT at birth. Her alloantibody, abbreviated as anti-At^a after

her name, reacted with more than 6600 blood donors tested at that time, indicating that At^a was a high-prevalence antigen. All anti-At^a producers reported thus far have been of African ancestry, like Mrs. Augustine.

Recently, it has been shown that *SLC29A1* encoding the equilibrative nucleoside transporter 1 (ENT1) specifies a new candidate gene for a novel blood group system that includes the At^a antigen. Daniels et al.[37] reported that a nonsynonymous SNP in *SLC29A1* (rs45458701) is responsible for the At(a–) phenotype. Although all At(a–)-reported propositi are of African ancestry with functional ENT1, they identified three siblings of European ancestry who were homozygous for a null mutation in *SLC29A1* (c.589+1G>C) and thus have the Augustine$_{null}$ phenotype. These individuals lacking ENT1 exhibit periarticular and ectopic mineralization, which confirms an important role for ENT1/SLC29A1 in human bone homeostasis,[34] cardioprotection, and drug transport in erythrocytes.[38]

Clinical Significance

At^a antibodies are mostly IgG, but IgM could also be present and can directly agglutinate At(a+) RBCs. Of two IgG anti-At^a, one was IgG1, and the other consisted of IgG1, IgG3, and IgG4. At^a antibodies facilitate rapid destruction of ^{51}Cr-labeled At(a+) RBCs in vivo and give positive results in the in vitro functional assay, the MMA. Ideally, At(a–) RBCs should be selected for transfusion to patients with anti-At^a, although least incompatible RBC units may be suitable for patients with weak examples of the antibody.[2]

One anti-At^a caused an immediate HTR with chills and nausea during an RBC survival study, and another caused a severe delayed HTR after transfusion of multiple units of At(a+) RBCs. Despite numerous pregnancies involving anti-At^a, only one of the infants had moderately severe HDFN requiring phototherapy.[2] In three At(a–)-reported patients from the southern United States, the anti-At^a was concomitant with autoimmune disease.[5]

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The First Comprehensive Study of H-Deficient Phenotypes in Iran

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Anti-H antibody · H-deficient (Bombay) · H antigen · Iran

Summary
Background: The lack of correct blood grouping practices can lead to missing of the rare Bombay Oh phenotype and subjecting patients to the risk of severe hemolytic transfusion reaction. In the absence of blood donor registry, transfusion management of patients is a challenge. We performed this study in order to estimate the prevalence of the Bombay blood group (Oh) in Iran and to determine whether consanguinity plays a role in the prevalence of Oh group. **Methods:** This is a descriptive study in the Immunohematology Reference Laboratory of the Iranian Blood Transfusion Organization (IBTO) Tehran, Iran, over a period of 7 years. All donor blood samples showing blood group O and a strong initial reaction with blood group O RBC control cells were tested with anti-H lectin. Also blood samples from blood group O patients were tested with anti-H lectin if all cells on both antibody screening tests and antibody identification panels were reactive with negative auto control test. Specialized tests like adsorption/elution technique and inhibition assay for determination of secretor status were performed on Oh cases. Any history of consanguineous marriages were recorded. All variables were categorical variables, and percentage and proportions were calculated manually. **Results:** Analysis of the results of over 7 million first-time blood donors in Iran showed that the most common ABO blood group was O, with 2,520,000 (36%) subjects. 56 Oh individuals' (donors and patients) phenotypes (0.0008%) were detected. Consanguinity was observed in 50 cases (89%). **Conclusions:** This study shows that the prevalence of Bombay blood group in the general population of Iran is relatively high (0.0008%) and associated with consanguineous marriage. Thus, consanguinity is still an important risk factor present.
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Introduction

The serum of individuals with Oh (Bombay) blood type contain strong anti-H in addition to anti-A and anti-B [1]. If patients with anti-H in their circulation receive transfusions of blood that contains the H antigen (e.g., blood group O), they are at risk of acute hemolytic transfusion reaction. Since their red blood cells (RBCs) do not react with anti-A, anti-B and anti-AB antisera, they mimic O blood group in cell typing. The Oh individuals can only be transfused with autologous RBCs or blood from individuals of Bombay Oh phenotype which is very rare. We present our experience of detection of H-deficient rare blood group Oh phenotype donors/patients in Iranian Blood Transfusion Organization (IBTO). The prevalence of Bombay blood group is rare in the Iranian general population. As this rare blood type is believed to be associated with incidence of consanguineous marriages, it is expected to be more prevalent in rural than in urban populations. We, thus expected the prevalence of Oh (Bombay) blood group to be high in consanguineous marriages in Iran.

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Material and Methods

This is a descriptive study that was done in the Immunohematology Reference Laboratory of the IBTO, Tehran, Iran. The general population was covered and investigated through the Iranian National Rare Donor Program. Both the recipients and donor were included in the recent study over a period of 7 years (2009–2016). Donors were selected according to the blood banking norms. Initial ABO blood grouping was determined by tube method using commercially prepared antisera, anti-A, anti-B, anti-AB (Iranian Blood Research and Fractionation, Tehran, Iran). The presence of RhD antigen was determined by anti-D (Iranian Blood Research and Fractionation-IBRF and DIAGAST, Loos, France). Cell grouping and serum grouping were done using commercial blood grouping reagents. Although, antibody screening test is not part of routine serology donor testing in Iran but part of pre-transfusion testing, antibody screening test is performed on recipients' blood samples. All suspicious patients' blood samples showing 'blood group O with strong positive results (anti-H reacting at 37 °*C* and AHG phase) in the antibody screening test, antibody identification panel (IBTO in-house 3-cell and 11-cell ID panel), and negative auto control results were tested for confirmation of Bombay blood group by tube method. In tube method, one drop of anti-H lectin and one drop of 5% RBC suspension, washed in isotonic saline, solution were mixed, shaken to homogenize, and then centrifuged and checked for agglutination.

Adsorption/elution technique was used to confirm absence of A and B antigens and to rule out para-Bombay phenotype which carry weak antigens of A or B. RBCs of H-deficient individuals were washed, reagent antibody added (anti-A or anti-B), mixed, incubated at 4 °C for at least 2 h, and packed. After washing, it was eluted at –30 °C, and elute was tested with A or B cells.

Inhibition test was performed to check the secretory status of 45% of the subjects. These are subjects that we could reach for saliva collection, and their adsorption/elution test showed negative reactivity. Fresh saliva of these individuals was collected, boiled, centrifuged, and the supernatant was used for testing. In two tubes marked one and two, two drops each of anti-H lectin was added. Saliva and normal saline (each 100 µl) were added in each tube, mixed, and incubated. To this, 50 µl of 5% cell suspension of known blood group O RBCs were added, mixed, incubated, and centrifuged. All the procedures were done according to AABB Technical Manual [2].

History of any consanguineous marriages among subjects' genetic parents was recorded in the index cases. All individual parents who volunteered to participate in the study were checked for the presence or absence of A, B, and H antigens

Ethical Considerations

This study was approved by the ethics committee of IBTO and health services. Individuals were asked to sign an informed consent form before blood samples were obtained. All terms of the Helsinki declaration were considered, and the personal information remained anonymous.

Results

Analysis of the results of over 7 million first-time blood donors and recipients in Iran during 2009–2016 showed that the most common ABO blood group was blood group O, with 2,520,000 (36%) subjects. 56 (44.6% female vs 55.4% male) Oh phenotypes (0.0008%), including 42 (75%) donor and 14 (25%) patients were detected in the entire study group, consisting of blood donors and individuals identified through other incidental means. 50 (89.3%) individuals were born out of consanguineous marriage.

19 (33.9%) Oh phenotype individuals were identified during 2015 and 2016; this was the highest rate of detection within the study period. In 2013–2015, 15 (26.7%), in 2011–2013, 18 (32.1%) and in 2009 to 2010, 4 (7.1%) of Oh individuals were detected.

Among blood group O donors detected on routine blood grouping from 2009 to 2016, as many as 0.001% had Oh blood group. Among all 56 subjects in this study, 46 (82.1%) were Rh+ versus 10 Rh– (17.9%). When applying the adsorption/elution technique, there was no agglutination with A or B cells confirming these donors to be Bombay, and not para-Bombay phenotype. Saliva testing of 45% of the cases by inhibition assay showed agglutination and proved them to be non-secretor of H antigen (see table 1 for more serological test results).

The population distribution of rare H-deficient blood group in Iran is shown in table 2. The Oh (Bombay) phenotypes were from 12 provinces. The maximum number of Oh individuals (10 donors and 4 patients) were identified in Tehran province that includes the metropolitan city of Tehran, capital of Iran, a city with close to 8.6 million inhabitants as well as adjacent small cities and neighboring rural areas which in total inhibits a population of above 14.5 million (table 2).

Discussion

The discovery of the rare 'Bombay phenotype' blood group, by Bhende et al. [3] was a vital event in the field of immunohematology. This phenotype was characterized by the absence of A, B, and H antigens on RBCs, and the serum of these persons had anti-A, anti-B, and anti-H; thus these individuals had antibodies in plasma reacting with all RBC ABO phenotypes. These individuals were termed as homozygous recessive 'hh' or Bombay phenotype. They were typed as blood group O on normal ABO grouping but on cross-matching showed incompatibility to blood group O. They were Lewis antigen-positive (Le (a+)) [3, 4]. In our recent study about 82% of the individuals were Le (a+).

More than 130 Bombay phenotypes have been reported in various parts of the world. Bombay phenotype is rare, since it occurs in about 1 in 10,000 individuals in India while it occurs in about 1/1,000,000 individuals in Europe [5, 6].

In a study from South India, 13 Oh phenotypes (0.048%) were detected of which 7 were males and 6 females. Among these 13 Oh phenotypes, only 3 were RhD–, and consanguinity among parents was observed in 10 cases (77%) in a study amongst Bombay phenotypes [7].

The surprisingly high proportion of the Bombay phenotype caused by homozygosis is explained by the average inbreeding occurring even in well-mixed populations. This finding was supported by previous studies that the Bombay phenotype is more prevalent in populations with high rate of consanguineous marriage [8].

Balgir [9] concluded that the practice of endogamy and consanguinity were the main cause of the high prevalence of Bombay blood group. Consanguinity leads to increased homozygous expression of rare recessive genetic characters like the Bombay phenotype. A consanguinity among parents was observed in 50 cases (89%) in the recent study.

Table 1. Laboratory data of H-deficient individuals*

Laboratory results by saline-tube tests		
DAT	auto control test	Lewis phenotype
Positive 1 (1.7%) (IgG–/C3d+^{weak}) Negative 55 (98.3%) (IgG–/C3d–)	positive 0 (0%) negative 56 (100%)	Le (a+b–) 46 (82.2%) Le (a–b–) 10 (17.8%)

DAT = direct antiglobulin test.
*Number of suspicious cases considered for Oh confirmation: 64. Number of suspicious cases that were not confirmed as Oh phenotype: 8.

Table 2. Population distribution of rare H-deficient individuals in Iran

Province	Number of donors (%)	Number of patients (%)	Total number of donors + patients	Subjects from urban population	Subjects from rural population	Subjects from consanguineous marriages
Tehran	10 (17.9%*)	4 (7.1%)	14 (25%)	9	5	10
Fars	7 (12.5%)	0 (0%)	7 (12.5%)	3	4	7
Khorasan Razavi	2 (3.6%)	1 (1.8%)	3 (5.3%)	1	2	3
Semnan	1 (1.8%)	0 (0%)	1 (1.8%)	0	1	1
Bushehr	3 (5.4%)	0 (0%)	3 (5.4%)	0	3	3
Zanjan	1 (1.8%)	0 (0%)	1 (1.8%)	0	1	1
Sistan and Baluchestan	1 (1.8%)	0 (0%)	1 (1.8%)	0	1	1
Mazandaran	5 (8.9%)	1 (1.8%)	6 (10.7%)	0	6	6
East Azerbaijan	3 (5.4%)	4 (7.1%)	7 (12.5%)	5	2	5
Hormozgan	1 (1.8%)	1 (1.8%)	2 (3.6%)	0	2	2
Isfahan	5 (8.9%)	2 (3.6%)	7 (12.5%)	1	6	7
Yazd	3 (5.4%)	1 (1.8%)	4 (7.1%)	1	3	4
Total	42 (75%)	14 (25%)	56 (100%)	20 (36%)	36 (64%)	50 (89%)

* Percentages were rounded to one decimal place.

The Bombay phenotype is also reported from other countries in Asia like Japan [10], Malaysia [11], Sri Lanka [12], and Thailand [13]. In a small study, Ravanparvar et al. [14] found one Bombay blood type among three different population in Tehran. Furthermore Zanjani et.al. [15] reported the first FUT1 deletion in Iranian individuals. FUT2 deletion has been reported previously. The finding of two FUT1 novel alleles in Iranian people is indicative of mutation diversity in this gene.

Moreover, 7 individuals with Bombay phenotype in the US in an Indian family were reported in Yunis et al. [16]. In previous studies, cases of Bombay blood group in South Africa [11] and a large series of H-deficient individuals (around 1:1,000) were reported in Reunion Island near Madagascar, indicating that Bombay blood group is mostly confined to South East Asian countries [17].

An acute hemolytic transfusion reaction will occur in patients with anti-H in their circulation if they receive transfusions of blood with the H antigen (e.g., blood group O) [3].

Physicians should be aware of the management of such patients. In a report from Iran, a transfusion reaction occurred in a case of Bombay blood group patient because only forward grouping was performed in routine testing using a crude slide method and inappropriate documentation of cross-matching resulting in not detecting the Bombay group [18]. It is very important that a simple test like the antibody screening test be implemented as standard method of pre-transfusion testing, including both forward and reverse grouping so that no patient is missed or receives wrong blood, which could lead to serious hemolysis due to transfusion.

Implementing a complete standard pre-transfusion testing consisting of ABO/RhD antibody screening test and avoiding reliance solely on a complete cross match test in the laboratory minimizes errors and makes certain that the right test is performed, the right results are obtained, and the right blood product is provided to the right patient at the right time.

It has been suggested that in developing countries where the antibody screening test is not part of a routine pre-transfusion test to reduce the risk of fatal hemolytic transfusion reaction, transfusion medicine departments or blood banks or blood donor centers should merge 'routine serum typing or reverse grouping confirmation' along with 'blood group O cell control in the reverse grouping procedure [19]. It is important to obtain accurate results in blood group serology tests of the donor and patient compatibility testing, and it is equally essential that the results are transcribed, collated, and interpreted correctly so that compatible blood products are issued. Errors in the blood bank laboratory can be due to technical failure in serological testing, insufficient procedures leading to misidentification of the samples, or misinterpretation of results [5].

In Iran, the immunohematology reference laboratory (IRL) of

the IBTO attempts to confirm the blood group discrepancies and can also provide confirmation on blood donors with Bombay Oh phenotype as they maintained the national rare donor registry. It should be emphasized that due to the blood group rarity individuals with Bombay blood group (Oh) can either receive autologous blood or blood from an individual of Bombay phenotype only. If transfusion is required in such patients, IRL services are easily available nationwide with no cost option and with the potential advantage of a reduction in blood transfusion complication.

Conclusion

The present study shows the prevalence of H-deficient blood groups in the Iranian general population to be 0.0008%. Overall, the rare prevalence is possibly related to more homogenous and currently rare rate of consanguineous marriages in modern society of Iran. High prevalence is found wherever consanguineous mating is still highly followed in more traditional society, which remains a dominant risk factor. Even though feasibility and economics are still critical factors in a large population like Iran, in addition to donor education and awareness and the long distances the donors may have to travel, there is a need for establishing regional or main provincial rare donor registries. Advances in molecular research and technology in blood services may play a very important role in a developing country like Iran since it is still the matter of concern that individuals with rare blood groups like Bombay phenotype are missed and not truly identified and are at risk of being transfused with wrong blood group leading to hemolytic transfusion reactions and possible patient mortality. All blood banks in developing countries need to adopt up to date quality standards for performing basic blood grouping tests and stay away from antiquated methods in order to avoid life-threatening situations in recipients and donors of blood products. Programs for implementation of the standard pre-transfusion methods (voluntary antibody screening test) in hospital blood banking have been started through training on a national level in Iran, but it seems that they still need the due attention. The establishment of a Iranian hemovigilance system in 2010 has raised questions with respect to transfusion-related complications contributed to understanding the gaps in this area.

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Disclosure Statement

The authors report no conflicts of interest.

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First report of the rare RhCE-depleted D--phenotype in sixteen people of Iranian origin

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Vox Sanguinis

Background and Objectives In transfusion medicine, it may be a challenge to acquire compatible blood for patients who have clinically important alloantibodies to high-prevalence antigens. The aim of this study was to study prevalence of rare D-- phenotype in samples from patients and their relatives referred to the Immunohematology reference laboratory of the Iranian Blood Transfusion Organization and the detection and identification of the phenotype and associated antibodies, particularly in an antenatal setting. This is the first report of the cases evaluated by the IBTO and family studies of the D-- proposita in Iran and possibly the first attempted comprehensive study in the current transfusion-related literatures.

Materials and Methods This retrospective cross-sectional study was carried out on 6720 pregnant women and individuals with difficult positive pretransfusion testing referred for ABO/Rh(D) typing and antibody screening during a period of 8 years from 2008 to December 2016 in the Immunohematology Reference Laboratory of the Iranian Blood Transfusion Organization, Tehran, Iran.

Results During 2008 to December 2016, 16 persons from ten families were detected to have rare D-- phenotype. Anti-Rh17 and anti-c were identified in plasma of the 11 persons, including 10 females with a history of multiple unsuccessful pregnancy and the total number of 24 abortions and one male with history of blood transfusion vs. 5 individuals, including an unmarried single woman, 1 person with a history of first-time pregnancy and 3 persons with a history of multiple pregnancy, who showed no alloimmunization. Based on these collective findings, we interpreted these results as being confirmed as D-- phenotype (0.23%).

Conclusion Irrespective of Rh (D) group a serological antibody screening test is recommended to be required in a National prenatal testing guideline.

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Key words: Anti-Rh17 (anti-Hro), D-- phenotype, haemolytic disease of the fetus and newborn.

Introduction

The Rh blood group is one of the more polymorphic blood group systems in humans. Due to its immunogenicity in transfusion and in pregnancy receives special attention in transfusion medicine [1]. More than 100 variants have been identified and the frequency of some alleles is extremely low [2].

The rare *Rh* gene complex D-- ISBT number Rh17 (004017), first was described by Race *et al.* in 1950, is characterized by the complete absence of C, c and E, e antigens and the elevated expression of D on the surface of red blood cells (RBCs) [3–8]. D-- phenotype people have been found in many populations with a high consanguinity rate of the parents. RhCE-depleted D-- phenotype individuals, homozygous for D--, are very rare and may become sensitized to high-frequency

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antigens of the Rh system by transfusion or pregnancy. Sensitized pregnant women can have different antibodies, including anti-Rh17 (Hro), which cause mild to severe hemolytic disease of the fetus and newborn (HDFN) in their fetuses and newborns [9–13].

Based on our knowledge, this is the first population study in Iran and also possibly the first of its kind comprehensive study in the current transfusion-related literatures. To date, there is no evidence-based and unified international protocol for testing and management of D-- phenotype individuals, particularly D-- phenotype pregnant women for the detection and identification of anti-Rh17 antibodies.

Materials and Methods

This retrospective cross-sectional study was carried out on 6720 pregnant women and individuals referred for ABO/Rh (D) typing and antibody screening tests during a period of 8 years from 2008 and December 2016 in the Immunohematology Reference Laboratory of the Iranian Blood Transfusion Organization (IBTO), Tehran, Iran. The samples were collected both from the individuals for routine pregnancy testing and with positive history of multiple unsuccessful pregnancy (at least two times) and also from individuals with incompatible cross-match test to identify suspicious alloimmunization, prenatal and postnatal titration evaluation and better management of possible need for intrauterine transfusion for haemolytic disease of the fetus and newborn.

Blood samples

ABO and Rh(D) tests

Six millilitre of peripheral blood sample was drawn from each individual into a vial containing Ethylenediamine Tetraacetic ethylenediaminetetraacetic acid (EDTA) anticoagulant. Blood samples were collected under aseptic condition from an antecubital vein for determination of blood groups. Initial ABO blood grouping was determined by tube method using commercially prepared anti-sera, anti-A, anti-B, anti-AB (Iranian Blood Research and Fractionation – IBRF). The presence of Rh D antigen was determined by anti-D (Iranian Blood Research and Fractionation – IBRF). Repeat ABO and Rh D blood grouping for confirmation of blood group was performed by the conventional tube technique as per IBTO's standard operating procedure using monoclonal reagents from different commercial companies included Bio-Rad and CE- Immunodiagnostika. Testing for the presence of weak D phenotype was done for all individuals typed as D negative as per manufacturer's instructions. Tube technique using 2%–5% red cell suspension and anti-C, anti-c, anti-E and anti-e monoclonal antibodies (CE-Immunodiagnostika, Germany) as per the manufacturer's instruction was performed for Rh antigen (C, E, c, e) typing. A positive reaction of 3+ to 4+ strength of agglutination indicated the presence of corresponding antigen, and the absence of agglutination was confirmed microscopically per manufacturer's instruction indicating its absence.

Antibody screening and identification

A home-made available three-cell antigen panel (IBTO mini-panel) was used for the antibody screening procedure in which the patient's plasma was added to RBCs with and without papain enzyme using the Low Ionic Strength Solution (LISS). IBTO mini-panel and antibody screening kit and also selected cells were validated in 2-year period using commercial CE marked Diamed 3 cells kits.

Clinically significant alloantibodies were defined as those antibodies that potentially could cause RBC destruction based on the reactivity at 37°C and/or anti-human globulin (AHG) phase.

Rh phenotyping with DiaMed-ID (Bio-Rad, Philadelphia, PA, USA) was done. If the D antigen was present, but C, c, E, and e antigens were not expressed on the RBCs of the patients; further, Rh antibody identification tests were performed using an in-house panel of 11 donor cells with ID-IAT at the Immunohematology reference laboratory of the Iranian Blood Transfusion Organization (IBTO), Tehran, Iran.

Adsorption and Elution technique was used to confirm the absence or presence of Rh phenotypes C, c, E and e antigens. History of any consanguineous marriage and a previous history of RBC transfusion were recorded in the index cases.

Ethical considerations

This study was approved by the ethics committee of IBTO and health services. Individuals were asked to sign an informed consent form before obtaining blood samples. All the terms of the Helsinki declaration were considered, and the personal information remained anonymous.

Results

In analysis of the results of over 6720 individuals, who were referred to Immunohematology reference laboratory of the Iranian Blood Transfusion Organization (IBTO), Tehran, Iran during 2008–2016, 16 persons from ten families, including 9 females with history of multiple abortion and one individual candidate of kidney transplantation and 6 persons from 23 siblings, with the mean age of 35.06 ± 10.23 years consisting of individuals identified through incidental means, were detected to have rare D-- phenotype (Table 1). Anti-Rh17 was identified in plasma of 10 patients, including 9 females with a history

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of multiple unsuccessful pregnancies (i.e. intrauterine fetal death, missed abortion and gross fetal abnormalities) with the total number of 24 abortions, one male patient with Rh17 antibody had history of blood transfusion. Among six siblings, five individuals, including an unmarried single woman, one person with a history of first-time pregnancy and three persons with a history of multiple pregnancies, showed no alloimmunization except for one sibling that was alloimmunized with anti-Rh 17 and anti-c antibodies.

Based on these collective findings, we interpreted these results as being confirmed for the D-- phenotype (0.23%). In 7 (43.75%) patients, a consanguineous was detected. Among individuals with D-- phenotypes, the total of ABO blood group was group A 7 (43.75%) followed by group O 4 (25%), group AB 3 (18.75%) and group B 2 (12.5%). In Adsorption and Elution technique, there was no agglutination with C, E, c and e red blood cells confirming it to be D-- phenotype. Previously known anti-Rh17 was not available to perform further testing and confirmation.

The results of anti-Rh17 titration showed that there was only one person with anti-Rh17 titration under 256. Four, three, one and one persons showed titration range of 256, 512, 1024 and 2048, respectively (Table 1). According to AABB recommendation, any anti-human globulin (AHG) phase titre above 16 would be clinically significant and would be a reason for concern to cause HDFN [14].

The majority of patients had multiple histories of miscarriages. Few numbers of them, who had delivered, their infants experienced mild to severe HDFN, which required blood transfusion.

Discussion

Rh-deletion RBCs are presented by a lack of expression of RhCE antigens and an over-expression of the D antigen. The frequency of the Hro D-- phenotype is very rare, with only few unrelated and consanguineous families documented worldwide [1, 15].

In Western populations, D-- phenotype is an extremely rare Rh phenotype, while the frequency of the D-- phenotype in Japan is much higher (approximately 1 in 100 000) and 50 cases of haemolytic disease of the fetus and newborn in Hro (Rh17) women have been reported in Japan [15]. Our recent study showed a rare frequency of D-- phenotype in our population study (0.23%).

Hro (Rh17)-negative individuals can become immunized by pregnancy or through transfusion and individuals are occasionally identified when detailed blood grouping of families is carried out, such as in disputed paternity cases. In this report, one alloimmunized individual during her first pregnancy and one unmarried woman were reported, while an anamnestic immune response occurred during pregnancy in a majority of the cases. Three cases did not show any alloimmunization. We speculate that they have a nonresponsive immune system (nonresponders) or they are individuals with a very weak expression of Rh phenotypes with unusual Rh haplotypes, which further molecular studies should be considered. Partners and children of these selected patients also were phenotyped for Rh antigens, and none of them was negative for all antigens.

As a result, we think that alloimmunization due to anti-Rh17 should be known as a critical state causing haemolytic disease of the fetus and newborn (HDFN). Further, its rarity and high tendency of the maternal immune response may lead to higher risk than alloimmunization in Rh (D) negative women.

Offspring from an immunized D-- phenotype woman are often obligate heterozygotes for the wild-type *RHCE* gene unless there is parental consanguinity. It shows that it is necessary to test the siblings of D-- individuals because they also may be negative for Rh17 [11]. The genetic changes leading to the D-- phenotype are not completely known. But it is well known that its molecular characterization would greatly benefit newborn identification. So, according to the recent study and previous studies, it seems that parental consanguinity plays an important role that increases the likelihood of an Rh17 infant [1].

Cherif-Zahar *et al*. assessed the *RH* gene structure in D-- individuals using Southern analysis and reported no major deletion or structural alteration of the gene [16].

Currently, there are total of 5 allogenic and 3 autologous frozen red blood cell units from D-- donors are in stock at Immunohematology Reference Laboratory of the Iranian Blood Transfusion Organization (IBTO), Tehran, Iran and easily obtainable, but several days are needed to collect, process and prepare fresh packed red cells from registered donors of D-- phenotype, thus heightening the risk. Better quality and longer life make fresh packed red cells preferable for transfusion in fetal or neonatal, though access to donor blood may be difficult.

All pregnant women (with a history of multiple abortions and also primigravida women), with a positive anti-Rh17 (anti-Hro) titre, should be evaluated via serial fetal ultrasonography and measurements of the antibody titre need to be conducted for management until the middle of the second trimester.

The aim of management of maternal red cell alloimmunization is to allow the pregnancy to continue to a safe gestational age without fetal risk. An invasive interventions such as amniocentesis or cordocentesis should be introduced timely in selected cases, even if it may provoke an anamnestic immune response that is deleterious for the fetus [17], which occurred in case of Hirose *et al*. where alloimmunization was probably induced following a cordocentesis procedure [15]. Although an intrauterine

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Table 1 Data on Patients with D– rare blood type

No	Diagnosis	Age	Sex	N.ch	ABO	ABS	Antibody ID	Frozen autologous	Hx of Transfusion	No of abortion	G. Area
1	Multiple abortion	34	F	1	O	Positive	Anti-Rh17 titre 256	2	No	4	Borujerd
2	Kidney transplant	52	M	NA	O	Positive	Anti-Rh17, titre nt	1	Yes (anaemia Hg 6.8 g/dl) NA	Mashhad	
3	Multiple abortion	37	F	1	O	Positive	Anti-Rh17 titre 512	1	No	3	Tehran
4	Multiple abortion	35	F	1	O	Positive	Anti-Rh17 titre 512	0	No	4	Ahvaz
5	Multiple abortion	44	F	1	A	Positive	Anti-Rh17 titre, titre nt	0	Yes (anaemia Hg 6 g/dl)	3	Tehran
6	Multiple abortion	29	F	2	A	Positive	Anti-Rh17 titre 256	0	Yes (infant transfusion)	1	Tehran
7	Sibling	36	F	3	A	Negative	None, titre nt	0	No	1	Shiraz
8	Sibling	57	F	5	A	Negative	None, titre nt	0	No	2	Shiraz
9	Multiple abortion	35	F	1	A	Positive	Anti-Rh17 titre 512	0	No	3	Urmia
10	Multiple abortion	24	F	1	A	Positive	Anti-Rh17 titre 2048	0	Yes (infant transfusion)	2	Tehran
11	Multiple abortion	30	F	2	B	Positive	Anti-Rh17 titre 256	0	Yes (infant transfusion)	0	Tehran
12	Sibling	19	F	None	B	Negative	None, titre nt	1	No	0	Mashhad
13	Multiple abortion	41	F	1	AB	Positive	Anti-Rh17 titre 1024	2	Yes (IUT)	6	Zahedan
14	Sibling	36	F	None	AB	Negative	None, titre nt	0	No	0	Gorgan
15	Sibling	32	F	1	AB	Negative	None, titre nt	0	No	0	Gonabad
16	Sibling	20	F	1	A	Positive	Anti-Rh17, anti-c, titre 64,16	0	No	0	Tehran

F, Female; M, Male; N.ch, number of children; G.Area, geographic Area; nt, not tested; NA, not applicable.

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transfusion at 28 weeks of gestation might have been useful to mitigate the subsequent development of fetal anaemia, but only one previous study reported successful management with an intrauterine transfusion for fetal haemolytic anaemia in a sensitized D-- phenotype woman, though fetal activity and an unfavourable position prevented of cordocentesis at 33 weeks of gestation in that patient [12].

As Mari *et al.* [18] reported previously, elevations of antibody titre and blood flow velocity in the fetal middle cerebral artery in Hro (Rh17) women strongly suggest the occurrence of fetal anaemia, which needs monitoring using of an invasive intervention, including cordocentesis until the early third trimester and immediate delivery in the middle to late third trimester.

Due to the scarcity of antigen-negative blood for fetal and neonatal transfusion, the management of D-- phenotype pregnant women is difficult. However, washed maternal RBCs are a potential source of blood with little risk. Care should be taken so that these donations would not be harmful to the mother or neonate. Of common practice among major regional referral centres is now serial maternal blood donation for intrauterine transfusion (IUT), that treat these high-risk pregnancies [19].

We think that a suitable assessment of the interval between sensitization and pregnancy, and also antibody titre, IgG subclass determination, not performed in our recent study due to unavailability of reagents, and fetal Doppler ultrasonography findings, might help to avoid some of the invasive interventions and prevent the second immune response, resulting in pregnancy prolongation.

Iran has been a member of the International Society for Blood Transfusion (ISBT) working party on rare donors since 2010 [20]. Recognition of blood donors with D-- phenotype are reported to the working party in order to be added in the list of countries with D-- phenotype donors.

Conclusion

Sixteen individuals from ten families of Iranian ancestries were reported. Specificity of antibodies against high-frequency antigen Hro (Rh17) were identified and counselling for obstetrical outcome, neonatal management and one case of transfusion support for kidney transplantation was arranged. The IRL of the IBTO contributed technical help and counselling to the physicians and medical team. Transfusion support was provided through the National rare donor database and frozen blood bank. Rare D-- phenotype red blood cells, compatible with maternal plasma were provided for infant and intrauterine transfusion. Patient's siblings were phenotyped to find compatible donors. It is necessary, to do an assessment and phenotype siblings of such patients.

In places, where antibody screening test is not a required prenatal test, it is recommended that irrespective of Rh (D) group, serological screening tests to be done as a National prenatal testing guideline.

Though one individual appeared to have anti-Rh17 and anti-c specificity and was compatible when cross-matched with RBCs from D-- propends, due to lack of access to different selected anti-Rh17, we did not confirm Rh $_{mod}$ phenotype.

Further studies will be needed using RHCE molecular genotyping of individuals to identify possible mutations in the families.

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Conflict of interest

There is no conflict of interest.

Author contributions

All authors contributed equally in study design; in the collection, analysis, interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

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FIRST REPORT OF THE RARE LITTLE P BLOOD TYPE IN PEOPLE OF IRANIAN DESCENT

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Background

Anti-PP1P^k (previously known as anti-Tj^a) is a naturally occurring antibody made by the rare little p individuals. This antibody to a high-frequency antigen is considered highly clinically significant, either in transfusion (risk of severe and acute hemolytic transfusion reaction) or obstetrics (frequent and recurrent early miscarriages). In case of blood transfusion, p negative rare blood must systematically be used, but finding compatible donors represents a real challenge for blood bankers in many countries due to extreme scarcity of this phenotype.

Summary of Antibody characteristics*

ANTIBODY	REACTIVITY			ENZYMES	BIND COMPLEMENT	IN VITRO HEMOLYSIS	HTR	HDFN	PREVALENCE	
	≤RT	37	AHG						Whites	Blacks
P1^a	Most	Some	Rare	Enhance	Rare	Rare	Rare	No	21%	6%
PP1P$^{k[1]}$	Most	Some	Some	Enhance	Most	Most	Yes	Mild	Rare	Rare
p^a	Most	Some	Some	Enhance	Most	Some	Yes	Mild-server	Very Rare	Very Rare

[1]Usually clinically insignificant
[2]Potent hemolysins may be associated with early abortions
[3]Associated with severe delayed HTR
*Reference: Modern Blood Banking and Transfusion Practices, 6th Edition 2012 (Table 8-24)

Aims

To investigate antibodies to a high-frequency antigen of undetermined specificity in 9 patients originating from Iran, through an international collaboration.

Methods

EDTA blood samples were collected from 9 patients with pan-agglutinating antibodies of undetermined specificity. The blood samples were referred to the French National Immunohematology Reference Laboratory (IRL) for serological investigations, together with the initial test results and clinical information obtained by the IRL of the Iranian Blood Transfusion Organization.

Results

Five out of the 9 patients were identified as carrying a rare p phenotype, with anti-PP1P^k in their plasma. No underlying common alloantibodies were identified in the 5 p patients; autocontrols and DAT were negative. Three out of those 5 p patients were initially referred to the Tehran IRL by obstetricians/gynecologists specialists, because of spontaneous and multiple miscarriages. The close relatives of the 3 p female patients were also studied and 2 of their brothers were identified as presenting a p rare type as well.

Summery Of The Test Results

Case number	Sex	Age	ABO/Rh(D)	Indications (Diagnosis)	History of Transfusion	No. of		No. of Frozen units	
						Gravida	Para	Red Blood cells	Plasma
Case#1	F	33	O$^+$	history of recurrent miscarriages	None	4	0	2 units	29 aliquots
	M	29	O$^+$	Case#1 sibling	None	0	0	0	0
	M	31	O$^+$	Case#1 sibling	None	0	0	0	0
	F	36	O$^+$	Case#1 sibling	None	0	0	1 unit	10 aliquots
Case#2	F	31	O$^+$	Placenta Accreta Surgery	None	2	2	0	0
	M	22	O$^+$	Case#2 sibling	None	0	0	1 unit	0
Case#3	F	38	O$^+$	history of recurrent miscarriages	None	7	1+currently pregnant at (16 weeks)	0	0
	M	36	O$^+$	Case#3 sibling	None	0	0	0	0

Summary/Conclusions

The plasma from 9 patients from Iran, with antibodies to a high-frequency antigen of unknown specificity, were studied and 5 of them were found to show an exceptional p phenotype, also known as Tj(a-), with a potent anti-PP1P^k (anti-Tj^a). As the rare p phenotype was never detected or reported in Iran before, the antibodies of those 5 patients were considered in the initial investigation performed in Tehran as antibodies against a high-frequency antigen with an undetermined specificity.
The patients and their siblings with a p phenotype were informed about their unique blood group and the precautions they should take in case of hospitalization or pregnancy. The results of the 3 female patients with a history of recurrent miscarriages were sent to their respective obstetrician/gynecologist, with information about a specific clinical follow up in case of further pregnancy.
The rare p phenotype was previously described to be more frequently encountered in Scandinavia (Finland, Sweden), Japan and Northern Africa, with several different molecular backgrounds. We plan to further investigate the molecular basis of our p cases, which might be similar to the one reported in Northern Africa.
Iran has implemented with great enthusiasm a 'National Rare Donor Program' since 2010.This interesting simultaneous finding of 5 individuals with an exceptional p type allows for the opportunity to store their own blood (autologous donation) at -80°C for a possible future use, the better management of possible future pregnancies in the three p female patients, and the possibility to cryopreserve reagent red blood cells with a p type as well as anti-PP1P^k antisera, in order to identify new similar cases in an autonomous way in the IRL of Tehran.

Design: rastarpou.ir