

## Red Blood Cell Alloantibodies in Healthy Egyptian Blood Donors

Azza Elsayed Abdelfattah Hashem, Gehan Mostafa Hamed Mostafa, Mariam Samy Mohamed El-Faramawy

Department of Clinical Pathology, Faculty of Medicine – Ain Shams University

Corresponding author: Mariam Samy Mohamed El-Faramawy, Mobile: 01004050341; Email: mariam.s.faramawy@gmail.com

### ABSTRACT

**Background:** Alloantibodies are antibodies that are produced in response to foreign antigens, the main stimuli responsible for erythrocyte alloantibodies in healthy adult donors are previous pregnancies and transfusions. Red blood cell (RBC) alloantibodies, especially IgG class, are clinically significant because of the association with hemolytic disease of newborns (HDN), hemolytic transfusion reaction (HTR) and a significant reduction in lifespan of transfused red blood cells. It has been stated that hemolytic reactions due to erythrocyte alloantibodies in donor blood is a relatively rare occurrence; however, they can occasionally cause severe transfusion reaction, particularly if given to infants and in the setting of massive transfusion.

**Objective:** We aimed in this study to screen healthy Egyptian blood donors for the presence of red blood cells alloantibodies in a trial to prevent complications of blood transfusion, other secondary possible outcomes are to reveal the differential frequency of these alloantibodies among donors with previous blood transfusion, multiple pregnancies, different age groups and gender.

**Subjects and Methods:** This study was carried out on 200 healthy Egyptian blood donors coming to Ain Shams University Hospitals Blood Banks. Data was collected on: gender, age, frequent donor or first time to donate, history of previous blood transfusion, number of pregnancies (in female donors), history of hepatitis or other viral infection, history of diabetes mellitus, hypertension, and history of recent surgery.

**Results:** Sera from donors was subjected to alloantibody screening by manufacturer's antibody screening cells (Grifols I+II+III), when positive screening test, alloantibodies identification was done by manufacturer's antibody identification panel (Grifols), which consists of 11 panels. Out of the 200 donors, alloantibodies were detected in 8 donors (4%), a percent which is higher than other studies, may be due to the smaller sample size, alloantibodies were: anti-Kell in 1 case (12.5%), anti-M in 1 case (12.5%), and anti-C in 1 case (12.5%), anti-c in 1 case (12.5%), and anti P in 1 case (12.5%), and 3 (37.5%) cases were non-specific antibodies, 130(65%) of donors were males and 70 (35%) were females with a male to female ratio 1.8:1, their ages ranged from 19 years to 65 years with median of 32 years.

**Conclusion:** Relation between alloantibodies and gender, multipara, repeated blood transfusion and previous surgeries was done showing statistically significance of previous surgeries and presence of allo-antibodies.

**Keywords:** Red Blood Cell, alloantibodies, blood transfusion, multipara

### INTRODUCTION

Alloantibodies are antibodies that are produced in response to foreign antigens <sup>(1)</sup>. The main stimuli responsible for erythrocyte alloantibodies in healthy adult donors are previous pregnancies and transfusions <sup>(2)</sup>.

Red blood cell (RBC) alloantibodies, especially IgG class, are clinically significant because of the association with hemolytic disease of newborns (HDN), hemolytic transfusion reaction (HTR) and a significant reduction in lifespan of transfused red blood cells <sup>(3)</sup>.

It has been stated that hemolytic reactions due to erythrocyte alloantibodies in donor blood is a relatively rare occurrence; however, they can occasionally cause severe transfusion reaction, particularly if given to infants and in the setting of massive transfusion <sup>(2)</sup>.

Screening of donated blood for the presence of RBCs alloantibodies is significant. The American Association of Blood Banks (AABB) Standards

stipulate that blood from donors with a history of prior transfusion or pregnancy be tested for RBC alloantibodies. Identification of donors' antibodies helps with the selection of suitable products for the transfusion, so the risk of complications from incompatible blood transfusions are greatly reduced <sup>(4)</sup>.

There is a paucity of literature on the prevalence of RBC alloantibody in the general population probably due to difference in selected population and laboratory test sensitivity <sup>(5)</sup>.

Reported incidence of RBC alloantibodies in these literatures varied significantly from 0.5% to up to 60% <sup>(6)</sup>.

Antibody screening test is a simple and inexpensive method for identification of large number of important antibodies; which are generally against minor blood group antigens <sup>(3)</sup>. Because of the importance of these alloantibodies, paucity of literature and variability in their reported prevalence in normal population, we decided to examine the frequency of occurrence of alloantibodies in blood donors.

**AIM OF THE WORK**

The primary outcome of this study is to screen healthy Egyptian blood donors for the presence of red blood cells alloantibodies in a trial to prevent complications of blood transfusion. Other secondary possible outcomes are to reveal the differential frequency of these alloantibodies among donors with previous blood transfusion, multiple pregnancies, different age groups and gender.

**SUBJECTS AND METHODS**

**SUBJECTS**

The study was carried on two hundred healthy Egyptian blood donors, both sexes are included, from 19 to 65 years, who are attending to Ain Shams University Hospitals Blood Banks. **The study was approved by the Ethics Board of Ain Shams University.**

**All donors were subjected to:**

A structured questionnaire about: Gender. Age. Smoking. Frequent donor or first time to donate. History of previous blood transfusion. Marital status (in female donors). Number of pregnancies and abortions (in female donors).

**Laboratory investigations using standard blood bank techniques in the Central Blood Bank of Ain Shams University including: Antibody Screening** to detect alloantibodies against foreign RBCs antigens [IAT using antibody screening panel (Grifols I+II +III) (Diagnostic Grifols, S.A. (Barcelona), Spain)]. **Antibody Identification**, in cases of positive screening test to identify the antibody type [IAT using Grifols Antibody identification panel (1-11) (Diagnostic Grifols, S.A. (Barcelona), Spain)], (e.g. anti-Kell and anti-C)]. **Auto-control (AC) column**, in cases of pan positivity in antibody screening test to detect autoantibodies. **N.B: Immediate spin** was done to all cases showed positive auto-control to differentiate between cold and warm antibodies.

**METHODS**

**Sample**

The samples used in the laboratory tests were venous blood samples (3ml) on EDTA, which routinely collected for the pre-donation tests. Then samples were centrifuged at 3,500 g for 3 minutes to obtain plasma and packed RBCs (PRBCs). **The plasma was used in:** Antibody (Ab) screening & identification. Auto control

(AC). Immediate spin (IS). **The PRBCs were used to prepare suspensions used in:** AC. Immediate spin.

**RESULTS**

**Table (1):** Descriptive data of the study population is shown in the following.

		Number	Percent %
Sex	Male	130	65
	Female	70	35
ABO Blood group	A	71	35.5
	B	44	22.5
	O	65	32.5
	AB	20	10
	Rh	Positive	194
	Negative	6	3
Previous blood transfusion	Yes	7	3.5
	No	193	96.5
Previous surgery	Yes	54	27
	No	146	73
multipara	Yes	54	77.1
	No	16	22.9
Alloantibodies	Positive	8	4
	Negative	192	96

**Table (2):** Descriptive data for the 8 positive cases.

		Number	Percent %	P value
Sex	Male	3	37.5	.096
	Female	5	62.5	
ABO Blood group	A	3	37.5	.926
	B	1	12.5	
	O	3	37.5	
	AB	1	12.5	
Rh	Positive	8	100	.612
	Negative	0	0	
Previous blood transfusion	Yes	1	12.5	.157
	no	7	87.5	
Previous surgery	Yes	8	100	.000
	no	0	0	
multipara	Yes	3	60	.343
	no	2	40	
Alloantibodies	Anti-Kell	1	4	
	Anti-M	1	12.5	
	Anti-C	1	12.5	
	Anti-c	1	12.5	
	Anti-P	1	12.5	
	Nonspecific	3	37.5	

**Table (3):** Demonstrates the distribution of males and females between the study population and between the positive cases, there is a predominance in males in the whole population, but females are predominant in the positive cases. The p value is < 0.1 which is clinically significant but not statistically.

			Alloantibodies		Total
			Neg.	Pos.	
Sex	Female	Count	65	5	70
		%	33.9%	62.5%	35.0%
	Male	Count	127	3	130
		%	66.1%	37.5%	65.0%
Total	Count	192	8	200	
	%	100.0%	100.0%	100.0%	
Chi-Square Tests					
			Value	P	
Pearson Chi-Square			2.770*	.096	

\*: clinically significant but not statistically significant

**Table (4):** Demonstrates the distribution of blood groups among the whole study population and the positive cases, blood group A is the most common then blood group O, as regard positive cases, blood group A and O are equal to each other.

		Alloantibodies		Total	
		Neg.	Pos.		
ABO.type	A	Count	68	3	71
		%	35.4%	37.5%	35.5%
	AB	Count	19	1	20
		%	9.9%	12.5%	10.0%
	B	Count	43	1	44
		%	22.4%	12.5%	22.0%
	O	Count	62	3	65
		%	32.3%	37.5%	32.5%
Total		Count	192	8	200
		%	100.0%	100.0%	100.0%
<b>Chi-Square Tests</b>					
		Value	P		
Pearson Chi-Square		.467 <sup>a</sup>	.926		

a: non significant

**Table (5):** Demonstrates the percentage of blood transfusion between the study population and between the positive cases which was only 12.5 % with a non-significant p value (> 0.05).

		Alloantibodies		Total	
		Neg.	Pos.		
Prev. Bl. Tx	No	Count	186	7	193
		%	96.9%	87.5%	96.5%
	YES	Count	6	1	7
		%	3.1%	12.5%	3.5%
Total		Count	192	8	200
		%	100.0%	100.0%	100.0%
<b>Chi-Square Tests</b>					
		Value	P		
Pearson Chi-Square		1.999 <sup>a</sup>	.157		

a: non significant

**Table (6):** Shows the relation between previous surgery and presence of alloantibodies, with a highly significant p value of (< 0.05).

		Alloantib		Total	
		Neg.	Pos.		
Prev. Surg	No	Count	146	0	146
		%	76.0%	0.0%	73.0%
	YES	Count	46	8	54
		%	24.0%	100.0%	27.0%
Total		Count	192	8	200
		%	100.0%	100.0%	100.0%
<b>Chi-Square Tests</b>					
		Value	P		
Pearson Chi-Square		22.531 <sup>A</sup>	.000		

A: significant

**Table (7):** Shows the percentage of multipara females among the study population and positive cases with a non-significant p value (>0.05).

		Alloantib		Total	
		Neg.	Pos.		
Multipara	No	Count	14	2	16
		%	21.5%	40.0%	22.9%
	Yes	Count	51	3	54
		%	78.5%	60.0%	77.1%
Total		Count	65	5	70
		%	100.0%	100.0%	100.0%
<b>Chi-Square Tests</b>					
		Value	P		
Pearson Chi-Square		.897 <sup>a</sup>	.343		

a: non significant

## DISCUSSION

Red cell antibody anti-A and anti-B are the naturally occurring antibodies that are found in the human serum. All other antibodies are called “irregular red cell antibodies.” There are two types of irregular red cell antibodies: alloantibodies and autoantibodies. Alloantibody is produced against the antigen that is lacking, whereas autoantibody is produced to an antigen that is present. Such irregular alloantibodies/autoantibodies can be encountered in healthy blood donors who are either transfused previously or in multiparous females <sup>(7)</sup>.

Screening of donated blood for the presence of RBCs alloantibodies is significant. The American Association of Blood Banks (AABB) Standards stipulate that blood from donors with a history of prior transfusion or pregnancy be tested for RBC alloantibodies <sup>(8)</sup>.

The National Blood Policy, India, 2007 (National Aids Control Organization, Ministry of Health and Family Welfare) has laid down the guidelines for the screening of donated blood for the presence of irregular red cell antibodies. The incidence of transfusion reactions due to irregular red cell antibodies in donor blood is rarely seen <sup>(9)</sup>. However, the presence of such antibodies can occasionally cause severe transfusion reactions if a large amount of plasma or whole blood is transfused as in the cases of massive transfusions or in pediatric population. Only packed red blood cells (PRBCs) should be preferably transfused when irregular red cell antibodies are found <sup>(10)</sup>.

For safe blood transfusion blood donors testing for infectious markers, but also for irregular antibodies should be performed for safe and compatible blood transfusion, especially for previously alloimmunized individuals <sup>(11)</sup>.

Red blood cell (RBC) alloantibodies, especially IgG class, are clinically significant because of the association with hemolytic disease of newborns (HDN), hemolytic transfusion reaction (HTR) and a significant reduction in lifespan of transfused red blood cells<sup>(3)</sup>.

Identification of donors' antibodies helps with the selection of suitable products for the transfusion, so the risk of complications of blood transfusions are greatly reduced<sup>(4)</sup>.

There is a paucity of literature on the prevalence of RBC alloantibody in the general population. Reported incidence of RBC alloantibodies in these literatures varied significantly<sup>(6)</sup>. Probably due to difference in selected population and laboratory test sensitivity<sup>(5)</sup>.

This study was carried out on 200 healthy Egyptian blood donors coming to Ain Shams University Hospitals Blood Banks. Blood samples of those donors were examined for the presence of RBC alloantibodies and autoantibodies.

Of the 200 donors (130 (65%) males and 70 (35%) Females), eight (4%) were found to have alloantibodies to RBCs, in a study conducted in Iran on 75 donors of Zanjan population by **Babaei et al.**<sup>(3)</sup> alloantibodies were detected in serum of 6 donors (8%).

On the contrary, **Giblett**<sup>(12)</sup> had reported 0.32% incidence of RBC alloantibodies in blood donors, another study carried by **Winters et al.**<sup>(13)</sup> reported 0.89% prevalence among blood donors of Olmsted county, Minnesota that consisted of donors who were previously transfused and pregnant women in their study, which probably explains the higher percentage of alloantibodies, Similarly, **Shafini et al.**<sup>(14)</sup> reported 0.8% (four out of 500 prevalence of RBC alloantibody among the blood donors in a tertiary hospital in the east coast of Malaysia, in another study, in Kuwait carried out by **Ameen et al.**<sup>(15)</sup> among 179,045 Kuwaiti pregnant women, and allogeneic blood donors and patients the prevalence of alloantibodies was 0.49 %.

Lower alloantibodies incidence was detected in a study done by **Pahuja et al.**<sup>(2)</sup> which was 0.05%, in their donor population counted 7756, 7648 donors were males (98.6%) and 108 were females (1.4%), this very low incidence of alloantibodies may be due to the very low percentage of females in the study, who are known to be of higher probability for alloantibody formation, other studies were in accordance with this one as the done by **Garg et al.**<sup>(16)</sup> among 47,450 whole blood donors who reported

incidence of 0.09% for red cell alloantibody which is the same found by **Makroo et al.**<sup>(11)</sup> in his study in India among 82,153 donors, 93.40% were males and only 6.60% were females.

Our high incidence (4%) and the higher (8%) in the study of **Babaei et al.**<sup>(3)</sup> may be due to the very little sample size, 200 and 75 respectively, in comparison to other studies, which could not clearly exclude the ethnic difference within the studied sample. Other causes of this large variation may be due to the different screening method used, and characteristics of the population studied.

Other studies showed higher incidence like the study conducted in southwestern Iran by **Keikhaei et al.**<sup>(17)</sup> in which the prevalence of alloantibodies was 18.7%, a near result to the study done in southeastern Iran by **Mirzaeian et al.**<sup>(18)</sup> in which the prevalence of alloantibody was %17.9, other Egyptian study by **Ahmed et al.**<sup>(19)</sup> including 501 subjects was done and revealed an alloimmunization rate of 11.3%, these high incidences are explained by the different type of population in these studies, as the subjects were thalassemic patients who are exposed to repeated blood transfusion in contrast to the healthy donors in our study.

In the present study, we found a predominance of male donors compared to female donors 65% males and 35% Females, which is comparable with a study done by **Makroo et al.**<sup>(11)</sup> in which 93.40% were males and only 6.60% were females, in **Babaei et al.**<sup>(3)</sup> study 97.33% were male and 2.66% were females, in **Pahuja et al.**<sup>(2)</sup> study we found 98.6% of donors were males and only 1.4% were females, in our study there is predominance in male donors as other studies, but still the percentage of female donors is high in comparison to other studies, which may be due to the unintended bias to female donors especially who were multipara.

The frequency of blood groups in our population were A (35.5%), O (32.5%), B (22%), AB (10%), and as regard Rh (97%) had Rh positive and (3%) were Rh negative, a result which is some how similar to that of **Babaei et al.**<sup>(3)</sup> study which showed frequency of blood group A (52%), O (29.33%), B (14.66%) and AB (4%), Rh+(97.33%) and Rh-(2.67%).

In the current study in the 8 positive cases the encountered alloantibodies were: anti-Kell (12.5%), anti-C (12.5%), anti-M (12.5%), anti-c (12.5%), and anti P (12.5%), and (37.5%) of positive cases were non-specific antibodies, in **Babaei et al.**<sup>(3)</sup> the most

prevalent alloantibody was against K (66.66%), c (16.66%) and e (16.66%) antigens, also in a study conducted in Ardabil by **Reyhaneh *et al.***<sup>(20)</sup> the most prevalent of alloantibody was against K (30%), E (15%) and c (15%) antigens, a similar results in a study conducted in Tehran by **Reyhaneh *et al.*** in which alloantibodies were Anti-Kell (23.53%), Anti-E (20.59%), and Anti-c (17.56%), in all these studies it is apparent that anti-Kell, anti-E and Anti-c are the most prevalent alloantibodies, also other studies was done among patients not donors reveal the same prevalence as a study conducted by **Vaziri *et al.***<sup>(21)</sup> in Yazd (Iran) in patients with beta thalassemia, the rate of prevalence of alloantibody was 4% that these antibodies were against Kell, C and D antigens. In another study from northwest of Iran by **Davari *et al.***<sup>(22)</sup> in patient with beta thalassemia major, the rate of prevalence of alloantibody was 16.32% that these antibodies were against Kell, E and c antigens.

In our study some of the detected antibodies were also anti- k, anti C and anti c like the mentioned studies, but each of them has the same percent of (12.5%), this may be explained by the small sample size of our study and the different techniques used for antibody screening and identification, in our study, antibody screening and identification was performed by the indirect Coombs test (also known as the indirect antiglobulin test or IAT) whereas, they used column agglutination technique.

In contrary **Makroo *et al.***<sup>(11)</sup> study showed Anti-M was the most common antibody identified (56.57%) followed by anti-D (27.63%), then the frequency of anti-E and anti-C was found to be (2.63%) which is lower than our and other studies mentioned above, other study done by **Kaur *et al.***<sup>(23)</sup> also detect Anti-M as the most common antibody identified (42.8%), anti-lea (14.2) and (14.2%) were inconclusive, in our study anti-M was also detected by frequency of (12.5%), Anti-M is naturally occurring alloantibody which does not react at 37°C and is not clinically significant for transfusion but can cause a problem in pretransfusion testing. It is clinically significant when detected at 37°C, wherein, cross-match compatible antigen negative blood should be given to prevent any hemolytic transfusion reaction<sup>(24)</sup>. This difference could be due to the different techniques used for antibody screening and identification. In **Makroo *et al.***<sup>(11)</sup>, antibody screening and identification was performed by Solid Phase Red Cell Adherence Assay (SPRCA)

technique, whereas, other studies used column agglutination technique. The sensitivity of antibody detection by (SPRCA) is higher as compared to column agglutination<sup>(25)</sup>.

In our study 3(37.5%) positive cases were found to be non-specific, a result going with **Kaur *et al.***<sup>(23)</sup> study at which 1 case (14.2 %) was inconclusive.

Although the percentage of multipara, previously blood transfused donors and percentage of male and female in positive cases were not mentioned in other studies, their values were calculated in our study, out of the 8 positive cases 5 (62.5%) were females and 3 (37.5%) were male. only one (12.5%) of them received previous blood transfusion, of the 5 positive female cases 3 (60%) of them were multipara and 2 (40%) were single.

## CONCLUSION

Relation between alloantibodies and gender, multipara, repeated blood transfusion and previous surgeries was done showing statistically significance of previous surgeries and presence of allo-antibodies. Screening of donated blood for the presence of RBCs alloantibodies is of clinical importance.

## REFERENCES

1. **Lerner NB, Razaai MA, Blumberg N (2010):** Red cell Transfusion. In: Williams hematology. Kenneth Kaushansky, Marshall Lichtman, E. Beutler, Thomas Kipps, Josef Prchal, Uri Seligsohn (eds.) 8th ed. New York: McGraw-Hill, 2287-2298.
2. **Pahuja S, Kushwaha S, Sethi N, Pujani M, Jain M (2012):** Screening of blood donors for erythrocyte alloantibodies. *Hematology*, 17:302-305.
3. **Babaei K, Esmaeilzadeh A, Asadi S, Sohrabi R (2016):** Prevalence of Red Blood Cell Alloantibodies in Blood Donors of Zanjan Province; the Preliminary Report of the North West of Iran. *Biosci Biotech Res Asia.*, 13: 2207-2210.
4. **Eder AF, Chambers LA (2007):** Non-infectious complications of blood transfusion. *Arch Pathol Lab Med.*, 131:708-7018.
5. **Tormey CA, Fisk J, Stack G (2008):** RBC alloantibody frequency, specificity and properties in a population of male military veterans. *Transfusion*, 48:2069–2076.

6. **Nicholls MD (2003):** Transfusion: morbidity and mortality. *Anaesth Intensive Care*, 21:15-29.
7. **Harmening DM (2012):** Modern Blood Banking and Transfusion Practices. 6th ed. United States of America: FA Davis Company Publications; available at: <https://www.fadavis.com/product/modern-blood-banking-transfusion-practices-harmening>.
8. **Brecher M (2006):** AABB technical manual, ed 15, Baltimore, MD, American Association of Blood Banks. Available at: <https://books.google.com.eg/books>.
9. **Mohn JF, Lambert RM, Bowman HS, Brason FW (1961):** Experimental transfusion of donor plasma containing blood group antibodies into incompatible normal human recipients. I. Absence of destruction of red cell mass with anti Rh, anti-Kell and anti-M. *Br J Haematol.*, 7:112.
10. **Myhre BA, Greenwalt TJ, Gajewski M (1965):** Incidence of irregular antibodies occurring in healthy donor sera. *Transfusion*, 5:350-4.
11. **Makroo RN, Rajput S, Agarwal S, Chowdhry M, Prakash B, Karna P (2018):** Prevalence of irregular red cell antibody in healthy blood donors attending a tertiary care hospital in North India. *Asian journal of transfusion science*, 12(1): 17-20.
12. **Giblett ER (1977):** Blood group alloantibodies: An assessment of some laboratory practices. *Transfusion*, 17:299-308.
13. **Winters JL, Pineda AA, Gorden LD, Bryant SC, Melton LJ, Vamvakas EC et al. (2001):** RBC alloantibody specificity and antigen potency in Olmsted County, Minnesota. *Transfusion*, 41:1413-20.
14. **Shafini MY, Haslina N, Rosnah B, Salamah S, Marini R (2017):** Prevalence and Specificity of Red Cell Alloantibodies in Regular Blood Donors in a Tertiary Hospital in the East Coast of Malaysia. *International Medical Journal*, 24(1): 10-11.
15. **Ameen R, Al-Eyaadi O, Al-Shemmari S, Chowdhury R, Al-Bashir A (2005):** Frequency of red blood cell alloantibody in Kuwaiti population. *Medical Principles and Practice*, 14(4), 230-234.
16. **Garg N, Sharma T, Singh B (2014):** Prevalence of irregular red blood cell antibodies among healthy blood donors in Delhi population. *Transfus Apher Sci.*, 50:415-417.
17. **Keikhaei B, Hirad Far A, Abolghasemi H, Mousakhani H, Ghanavat M, Moghadam M et al. (2013):** Red blood cell alloimmunization in patients with thalassemia major and intermediate in southwest Iran. *IJBC.*, 6:41-46.
18. **Mirzaeian A, Tamaddon G, Naderi M, Hosseinpour M, Sargolzaie N (2013):** Prevalence of alloimmunization against RBC antigens in thalassemia major patients. *Zahedan J Res Med Sci.*, 15:55-58.
19. **Ahmed AM, Hasan NS, Ragab SH, Habib SA, Emara NA, Aly AA (2010):** Red cell alloimmunization and autoantibodies in Egyptian transfusion-dependent thalassaemia patients. *Archives of Medical Science*, 6(4): 592-598.
20. **Reyhaneh K, Ahmad G, Gharib K, Vida V, Raheleh K, Mehdi TN (2013):** Frequency & specificity of RBC alloantibodies in patients due for surgery in Iran. *The Indian journal of medical research*, 138(2): 252-256.
21. **Vaziri M, JavadzadehShahshahani H, Moghaddam M, Taghvaei N (2015):** Prevalence and specificities of red cell alloantibodies in transfusion-dependent beta thalassemia patients in Yazd. *Iranian journal of pediatric hematology and oncology*, 5(2): 93-99.
22. **Davari K and Soltanpour MS (2016):** Study of alloimmunization and autoimmunization in Iranian  $\beta$ -thalassemia major patients. *Asian journal of transfusion science*, 10(1): 88-92.
23. **Kaur D, Bains L, Kandwal M, Parmar I (2017):** Erythrocyte alloimmunization and autoimmunization among blood donors and recipients visiting a tertiary care hospital. *Journal of clinical and diagnostic research: JCDR.*, 11(3): 12.
24. **Fung MK, Grossman BJ, Hillyer CD, Westhoff CM (2014):** Technical Manual of American association of Blood Banks. 18th ed. Bethesda, Maryland: ABO, H, and Lewis Blood Groups and Structurally Related Antigens, Rh system and other blood group system. <https://archive.org/.../AABBTechnicalManual18thEd2014/AABB+Technical+Manual+18>.
25. **Bajpai M, Kaur R, Gupta E (2012):** Automation in immunohematology. *Asian J Transfus Sci.*, 6:140-144.