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Red Blood Cells Alloimmunization and Autoimmunization in Multi-transfused Thalassemia Patients in South of Iran

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Abstract

Background: Recurrent blood transfusion is a common treatment in patients with thalassemia. The development of antibodies against red blood cell (RBC) antigens complicates RBC cross-matching, enhances the in vivo destruction of transfused cells, accelerates tissue iron overloading, delays the provision of safe transfusion, and reduces health-related quality of life.

Materials and Methods: In total, 516 thalassemia patients with a mean age of 18.5 years were included in this cross-sectional study in Mashhad University of Medical Sciences, Razavi Khorasan Province, Iran, in cooperation with the Abu Rayhan Special Medical Center and Hormozgan Blood Transfusion Organization between June 2015 and May 2016. The detection and identification of alloantibodies were done using 3 screen cells and 11 panel cells, respectively. To detect autoantibodies, auto-control was performed using polyspecific Coombs (IgG + C3d) standard method.

Results: Alloantibodies and autoantibodies were observed in 16 (3.1%) and 21 (4.1%) patients, respectively. Among patients with alloantibodies, 2 patients (12.5%) developed 3 antibodies (Anti-c,E,P1; Anti-c,E,K), 1 patient (6.25%) developed 2 antibodies (Anti-D,C), and 13 patients developed 1 antibody (4 patients Anti-D (25%); 3 Anti-K (18.75%); 2 Anti-E (12.5%); 2 Anti-C (12.5%); 1 Anti-Jka (6.25%); and 1 Anti-Jkb (6.25%)). A statistically significant correlation between patient age (P = 0.031), age of splenectomy (P = 0.006), Rh(D) (P = 0.001), leukoreduction of RBCs (P = 0.043), and type of disease (P = 0.006) with RBC alloimmunization was seen.

Conclusions: This study emphasized the need for the determination of RBC minor antigens, especially for Rh, Kell, and Kidd blood group systems, before the first transfusion and transfusion of antigen-matched blood. In addition, transfusion of prestorage leukoreduced packed cells is recommended for these patients.

Keywords: Alloimmunization, Autoimmunization, Blood Transfusion, Thalassemia

Introduction

Thalassemia is a common genetic disorder and a global problem (1). Hormozgan Province, with a population of 1.5 million, is located in the south of Iran. Due to some reasons, especially consanguineous marriage, the frequency of thalassemia is high in this province (about 25%). It is estimated that 12% of the Hormozgan population have the thalassemia trait. About 1000 thalassemia patients have been registered in the Abu Rayhan Special Medical Center. Blood transfusion is the main method of treatment for these patients. However, regular blood transfusion leads to organ damage due to iron overload and immunization to RBC antigens (2). Iranian routine blood group typing of thalassemia patients identifies ABO and Rh(D) antigens only before blood transfusion for thalassemia patients. Other incompatibilities between minor RBC antigens of the blood donor and the recipient may cause alloimmunization (3-5). In this research, we evaluated the frequencies of alloimmunization and

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autoimmunization and demographic and clinical risk factors for RBC immunization in thalassemia patients.

Materials and Methods

Patients and data collection

This cross-sectional study was performed between June 2015 and May 2016 in Mashhad University of Medical Sciences in cooperation with the Abu Rayhan Special Medical Center and Hormozgan Blood Transfusion Organization. We initially included 528 multi-transfused thalassemia patients in the study; however, 12 patients were excluded because of incomplete information records. Patients were diagnosed based on the clinical and laboratory examinations performed in the Abu Rayhan Special Medical Center. The study was approved by the local ethics committee (ethics code: IR.MUMS.fm.REC.1394.640).

At first, written consent forms were obtained from patients and parents of children participated in this study. Then, patients' demographic and clinical information were extracted from previous records. Number of transfused pack cells calculated based on the transfusion duration and age of patients.

Antibody screening and identification

Five milliliters blood samples were taken from all patients before blood transfusion. Serum samples were separated one hour after sampling and aliquoted in two tubes for antibody detection and identification. Antibody screening was performed using three screening cells (Iranian Blood Research and Fractionation Co) two hours after sampling based on the standard blood banking methods. Antibody identification was done for positive screening results using 11 cell panels with standard tube method (Iranian Blood Research and Fractionation Co, 15IP11C96). For detecting auto antibodies, auto control test was carried out using poly specific anti human globulin (IgG+C3d) (Iranian Blood Research and Fractionation Co).

Statistical analysis

Results were analyzed with SPSS (version 11.5). Descriptive analyses of the results were performed first, followed by comparative analyses. The mean of parametric variables with the Gaussian distribution was compared with the independent sample t test and those with non-Gaussian distribution, the Mann-WHITNEY test. The chi-square test was used for the analysis of categorical variables. P value less than 0.05 was considered as statistically significant.

Results

The mean age ± SD of the studied population was 18.5 ± 9.2 years, with the age range of 1 to 45 years and male-to-female ratio of 0.9. The phenotypes of 516 studied patients were 392 (76.0%) thalassemia major, 101 (19.6%) thalassemia intermedia, and 21 (4.1%) sickle beta thalassemia. The demographic information of these groups is shown in Table I. A total of 89 (17.2%) patients were splenectomized. The mean (±SD) age of splenectomized patients was 28.4 ± 5.7 years and that of nonsplenectomized patients was 16.5 ± 8.4 years. There was a significant difference between these 2 groups in this regard ($P < 0.001$). A total of 40 patients received K antigen–negative RBCs and 24 patients Rh antigen–matched component but not from the beginning of transfusion. Clinically significant alloantibodies, including 21 alloantibodies against different RBC antigens as a single or multiple, were observed in 16 (3.1%) patients. Two patients (12.5%) developed 3 antibodies (Anti-c,E,P1; Anti-c,E,K), 1 patient (6.25%) developed 2 antibodies (Anti-D,C), and 13 patients developed 1 antibody. Among patients with 1 alloantibody, 4 patients had Anti-D (25%), 3 had Anti-K (18.75%), 2 had Anti-E

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(12.5%), 2 had Anti-c (12.5%), 1 had Anti-Jka (6.25%), and 1 had Anti-Jkb (6.25%). Two patients were from 1 family (brother and sister). In 85.6% of cases, alloantibodies were against Rh and Kell blood group systems. Five patients (1%) had cold nonclinical significant alloantibodies; 2 of them were brothers. Among patients with alloantibodies formation, 9 cases (2.3%) had thalassemia major, 4 (4.0%) had thalassemia intermedia, and 3 (14.3%) had sickle beta thalassemia. There was a clinically significant difference considering alloimmunization between thalassemia major and sickle beta thalassemia patients. Tables II and III show a comparison between alloimmunized and non-alloimmunized with regard to demographic and clinical factors. We also detected autoantibodies in 21 (4.1%) patients.

Table I: Comparison between patients based on disease type

Variables	Thalassemia major	Thalassemia intermedia	Sickle- beta thalassemia	p-value
	Mean±SD (Range)	Mean±SD (Range)	Mean±SD (Range)	
Age (years)	17.6±8.7 (1-45)	20.8±9.9 (3-43)	23.1±11.0 (9-45)	$p<0.001$
Age at diagnosis (Months)	12.9±18.2 (1-168)	57.6±59.0 (4-408)	47.9±56.7 (3-216)	$p<0.001$
Age at first transfusion (Months)	14.5±20.9 (2-168)	62.2±61.2 (6-408)	76.2±133.4 (2-576)	$p<0.001$
Transfusion rate (Unit)	318.8±212.1 (8-934)	221.3±143.5 (12-552)	200.4±149.5 (23-507)	$p<0.001$

Table II: Comparison between alloimmunized and non-alloimmunized patients

	Age (Years)		Age at diagnosis (Months)		Age at first transfusion (Months)		Age at Splenectomy (Years)		Transfusion rate (Unit)	
Allo-antibody	P	N	P	N	P	N	P	N	P	N
Mean± SD	22.9± 7.5	18.4± 9.2	26.2± 34.8	22.8± 37.1	32.6± 41.8	25.9± 46.4	21.5± 8.5	12.4± 6.2	358± 138	296± 205
Range	7-41	1-45	4-120	-1-408	10-30	10-100	11-31	1-27	95-639	8-934
***p*-value**	0.031*		0.717		0.567		0.006**		0.09	

P: Positive, N: negative

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Table III: Demographical and clinical data of alloimmunized and non-alloimmunized patients

Variables	No.	Alloimmunized n (%)	Non-alloimmunized n (%)	*p*-value
Gender				0.187
Male	245	5 (2.0)	240 (98.0)	
Female	271	11 (4.0)	259 (96.0)	
Age at diagnosis (Months)				
≥24	411	12 (2.9)		
<24	94	4 (4.3)	399 (97.1)	0.505
			90 (95.7)	
Age at firs transfusion				
(Month)	402	9 (2.2)		0.144
≥24	102	5 (5.0)	393 (97.8)	
<24			97 (95.0)	
Splenectomy (No)				0.409
Yes	89	4 (4.5)	85 (95.5)	
No	425	12 (2.8)	413 (97.2)	
Leukoreduction (No)				0.043*
Yes	188	2 (1.1)	186 (98.9)	
No	328	14 (4.3)	314 (95.7)	
Rh D (No)				
				0.001**
Positive	474	11 (2.3)	462 (97.7)	
Negative	42	5 (11.9)	37 (88.1)	

Discussion

Thalassemia was first described by Cooley and Lee in 1925, and alloimmunization to minor RBC antigens was first reported by Levine and Stetson in 1939(6, 7). Thalassemia patients are prone to RBC antigen immunization because of a high rate of blood transfusion as a common treatment. Immunization against RBC antigens can generally reduce health-related quality of life in affected patients(2). The frequency of alloimmunization in our study was 3.1% (16 cases with 21 antibodies). This finding is in accordance with other Iranian studies (8-10); however, Ameen et al., reported alloimmunization in 30%, Obeid et al., in 42%, and Singer et al., in 44% (11-13). Heterogeneity between the donors and recipients is the main reason for this discrepancy. Our patients had received packed RBCs from local donors living in the Hormozgan Province with a high rate of consanguineous marriages. This led to low heterogeneity and genetic diversities in blood donors. Indeed, some patients had received blood from their families because of the fear of transfusion-transmitted infections. Genetic heterogeneity between the blood donors and recipients affects the type of alloantibodies. Most of the identified alloantibodies (86%) were against Rh and Kell blood group systems. The most common alloantibody in this study was Anti-D (23.8%). In a study by Azarkeivan et al, 10.9%, was reported ,15.8% was reported by Karimi et al., and 88.88% was reported in Iranian alloimmunized thalassemia patients in a study by Sadeghian et al (10, 14, 15). A total of 80% of Rh(D)-negative patients can produce Anti-D if they receive Rh(D)-positive blood. Moreover, the Rh(D) antigen is the most immunogenic antigen after ABO antigens (16). Because Rh(D)-negative patients receive Rh(D)-negative-packed RBCs, we must not observe anti-D in transfused patients. We believe this alloantibody is because of false negative identification of weak Rh(D) (Du) antigen in donors and, subsequently, transfusion of this blood to Rh(D)-negative patients. In line with previous studies by Sadeghian et al, Ismahanisa Ismail et al, and Koçyiğit et al, our Rh D negative patients showed high

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significant amount of alloimmunization (15, 17, 18). We did not observe Anti e antibody in our patients because of low immunogenicity and high frequency (97.9%) of this antigen in Iranian blood donors (19). The frequencies of Kell blood group system phenotypes in Iranian blood donors are as follows: (K+k−) 2.3%;, (K+k+) 5.7%, and (K−k+) 92%. K antigen is the most immunogenic antigen after the Rh(D) antigen in minor blood group antigens. We also detected anti-K antibodies in 19% of patients. Since the frequency of the K antigen in the blood donor population of Iran is low (8%) (19), it is possible to prevent alloimmunization against the K antigen with the transfusion of antigen-negative RBCs to the patients.

Two patients developed anti-Kidd antibodies (Jka and Jkb). These antibodies are transient antibodies that reduce to the undetectable level after immune sensitization and are a reason for about one-third of delayed hemolytic transfusion reactions(20) (21). Thus, informing the patients about these antibodies and the presence of the accessible history of alloantibodies is imperative. In our study, the chi-square test showed a significant difference in terms of alloimmunization between thalassemia major, intermedia, and sickle beta thalassemia (P = .04). However, logistic regression analysis showed only a significant difference considering alloimmunization between thalassemia major and sickle beta thalassemia (odds ratio = 7.09, P =0.006). Significantly higher rate of transfusion in thalassemia major patients than sickle beta thalassemia patients can be a reason for this difference (Table I). The genetic background must be considered as an important factor for immune response to foreign antigens. Among 16 patients (3.1%) who developed warm alloantibodies, 2 patients were brother and sister, and among 5 patients (1%) who developed cold alloantibodies, 2 were brothers. Findings on autoimmunized patients in a study by Guirat-Dhouib were in accordance with those in our study (9). Growing up of patients increases the need for blood and consequently increases the rate of transfusion, leading to an increase in antigenic immune contacts, thereby stimulating the patient immune system. We also observed a significant correlation between age and frequency of alloimmunization. This result was consistent with some studies (22, 23), and in contrast to some others (8, 12, 14). It seems that gender is not a risk factor for minor red blood cell antigens alloimmunization. Amin et al., (8) reported a highly significant rate of alloimmunization in males (male-to-female ratio = 1.6:1); whereas, Sadeghian et al., reported a highly significant rate of alloimmunization in females (male-to-female ratio = 1:2) (15). On the other hand, Patel et al., and Koçyiğit et al demonstrated no relation between gender and alloimmunization (18, 24). We did not observe a relation between gender and alloimmunization either. Splenectomized thalassemia patients have higher levels of interleukin-6, C-reactive protein, and higher counts of circulating B cells (25, 26). Singer et al, Thompson et al, and Hussein et al (13, 23, 27) showed a correlation between splenectomy and alloimmunization; however, this difference was not seen in our study and some other investigations (12, 18). Donor white blood cells have immunomodulatory effect on the recipient immune system. The immune system shifts to Th2, and the stimulatory and costimulatory agents are expressed in the recipient (28, 29). In this study, patients receiving leukoreduced packed RBCs developed a significantly lower rate of alloimmunization. In addition, the mean age of patients who received leukoreduced packed RBCs throughout life (8.6 ± 3.4 years) was significantly low compared with non-leukoreduced recipients (24.2 ± 6.1 years; P < .001). Ameen et al., also expressed that the transfusion of non-leukoreduced packed RBCs is a reason for a higher rate of alloimmunization (11). On

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the other hand, Karimi et al and Tampson et al did not report any relation between them (10, 23). Immune tolerance to minor RBC antigens can be induced in the first years of life because of humoral immune system immaturity. Patients who start receiving transfusion during the first 2 years of life develop fewer alloantibodies (14, 18, 24). Although in our patients who transfused before two years old, the difference in alloimmunization rate was not significant than patients who initiate transfusion after two years. A total of 21 patients (4.1%) developed autoantibodies in our study. Autoimmunization to RBC antigens was described by Dameshek and Levine in 1943(30). Researchers reported a wide range of autoimmunization in thalassemia patients from 1.7% in the study by Karimi et al to 45% in the study by Ameen et al (10, 11). The persistent presence of common antigens secondary to recurrent blood transfusion can activate the anergic B and T cells (31). Patients' genetic background and long-term exposure to toxic substances (free iron) have been suggested for autoantibody formation (32). Although not always positive direct antiglobulin test is clinically significant, the presence of autoantibodies can accelerate hemolysis.

Conclusion

Since alloimmunization against Rh(D) antigens was frequent in this study, it is important to revise the method of determination of the Rh(D) antigen especially for weak D determination. Indeed, it is better to use K− antigen RBCs for transfusion. The use of prestorage leukoreduced packed RBCs is recommended to poststorage leukoreduced RBCs.

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Conflict of interest

Authors declared no conflict of interest.

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