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Frequency of Red Cell Alloimmunization in Patients with Thalassemia Major: A Report from the Southwest of Iran

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Abstract
Background: The mainstay of managing severe β-thalassemia remains lifelong blood transfusion. Mismatched red blood cell phenotypes between donors and recipients in multiple blood transfusions can result in the development of alloimmunization in recipients. The aim of this study was to determine the frequency of major and subgroup antigens and their phenotypes in thalassemia major patients.
Materials and Methods: This cross-sectional descriptive study was performed on 105 patients with thalassemia major who referred to Baghaei Hospital in Ahvaz in 2021. Their alloimmunization to erythrocyte antigens was determined with standard tubular antibody search kits.
Results: Among the thalassemia major patients participating in the study, 51 were female (48.45%). The mean age of the participants was 21.10 ± 5.8 years. Out of the 105 patients studied, 26 had detectable alloantibodies in the serum (24.7%). The two groups of patients with positive and negative alloantibodies were significantly different in terms of Rh and C blood groups (P-values of 0.03 and 0.05, respectively). There was no significant association between the existence alloantibody and age, gender, spleen condition and the time of first transfusion (P > 0.05).
Conclusion: It was concluded that red blood cell matching, at least for Rh and C groups, is necessary to prevent alloimmunization in thalassemia major patients.
Keywords: Alloimmunization, Major thalassemia, Transfusion

Introduction

Thalassemia is one of the most common congenital hemolytic disorders characterized by the decreased synthesis of one or more hemoglobin polypeptide chains (1-3). A constant characteristic of β-thalassemia major patients is severe anemia caused by ineffective erythropoiesis and hemolysis, both of which are due to the partial or complete suppression of β-globin chain synthesis (2, 4). The only way of survival in β-thalassemia major is regular blood transfusion (5). The purpose of treatment is to sustain the pre-transfusion hemoglobin level above 9.5 g/dl (6). Blood transfusion, despite being a life-saving process, is associated with inherent risks of alloimmunization against red cells antigens (7). Alloimmunization is a major concern in the treatment of thalassemia as it can be life-threatening (8). Since only ABO and RH groups are controlled when a blood type is selected for transfusion, most alloantibodies are developed against other blood groups and cause delayed hemolytic reactions, which are considered as cross-matching (9, 10). Early diagnosis and identification of alloantibodies are very critical because blood transfusion is crucial for patients' survival (10, 11). Antibody screening and recognition can help to detect antigens (11). It is to be noted that, in developed countries, antibody screening is included in the compatibility testing protocol, but it is not carried out for all thalassemia patients in Iran (12). Previous

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studies have shown that the rate of alloimmunization in thalassemia patients varies from 2.5% to 37% in different regions of the world (13). The risk of alloantibody development still remains a major challenge, especially in developing countries where the high costs of pre-transfusion phenotyping do not allow it to be part of the routine blood transfusion practice (14). In Iran, pre-transfusion testing includes ABO grouping and Rh (D) typing, and groups other than ABO and Rh "D" (such as Kell, Duffy, Kidd, MNS, and Lewis) are not routinely dealt with (15). So, the extent of erythrocyte antigen profiling among the donor population, especially groups other than ABO and Rh "D", is not clear. This is because there are very limited data in the literature for these subgroups, and statistical differences have been found among different racial groups (13). Considering the annual birth of more than 600,000 babies with thalassemia worldwide who need treatment and regular blood transfusions, it seems necessary to investigate the involved factors in different ethnicities and provinces of Iran (16). Due to the high number of the patients with thalassemia major in Khuzestan Province, southwest of Iran, and the importance of alloimmunization as a factor for treatment failure, the current study aimed to determine the frequency of major and subgroup antigens and their phenotypes in thalassemia major patients.

Materials and Methods

This cross-sectional and descriptive study was conducted on 105 Iranian β-thalassemia major patients referring to Baghaei Hospital in Ahvaz and receiving blood in 2021. There were 51 (48.45%) females with the mean age of 21.10 ± 5.8 years. The inclusion criteria were dependence on transfusion and a history of blood transfusion at least once a month. The patients with sickle cell anemia, positive history of intravenous gamma-globulin, and thalassemia intermedia were excluded from the study. The clinical and transfusion records of the enrolled patients were collected with a questionnaire on age, gender, blood groups, age at the first transfusion, the total number of transfused RBC units, and splenectomy status. After informed consent was received from the patients or their elderlies, blood samples were collected in ethylenediamine tetraacetic acid (EDTA) tubes on the purpose of ABO/RhD blood grouping. The antibody screening (through an indirect Coombs test) was carried out by the gel process. This test was conducted in compliance with the standard procedure. The samples were tested for ABO group and Rh (D) type, and an antibody screen test (using an IBTO home-made kit) was conducted through the standard tube procedure. To identify the alloantibodies, that standard tube procedure was implemented in three phases. Antibody screening was performed using a commercial 11-cell identification panel (Dia panel, Bio-Rad, Switzerland).

Finally, the rate of the identified alloantibodies was calculated, and the relationship between the occurrence of alloantibodies and the variables of age in the first blood transfusion, gender, frequency of blood injection, and spleen status was investigated.

Ethical Consideration

Written consent was obtained from the patients or their parents. This study was approved by the ethics committee of Jundishapur University of Medical Sciences, Ahvaz, Iran (IR.AJUMS.REC.1399.251).

Statistical analysis

The data analysis was performed using SPSS (version 22; SPSS Inc., Chicago, IL, USA). The quantitative and qualitative variables were reported with mean ± SD and frequencies (percentages), respectively. Chi-square tests or Fisher's

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extract tests were used to examinethe associations. Independent t-tests were also used for the quantitative comparisons of the two groups. A P-value less than 0.05 was considered statistically significant.

Results

Of the total number of 105 patients with thalassemia major, 51 (48.45%) were females with the mean age of 21.10 ± 5.8 years. The mean age in the first blood transfusion was 4.9 ± 1.3 years. As reported in Table I, the patients were divided into alloantibodies positive (n = 26) and negative groups (n = 79). These two groups were not significantly different in terms of the patients' current age and their age in the first transfusion (P = 0.31, P = 0.89, respectively). Also, no statistical difference was observed between the two groups in terms of gender (P = 0.28). The frequency of blood transfusion per year did not differ between the two groups either (P = 0.67). Eighteen patients (17.1%) underwent splenectomy. There was no statistically significant relationship between alloimmunization and splenectomy (P = 0.37). Table II shows the frequency of main and minor blood groups in the patients with thalassemia major. The highest and lowest frequencies belong to O and AB groups (40.84% and 4.76%, respectively). Blood group A was seen in 33.3 % of the patients. Also, 85.5% of the patients were Rh-positive. Among the minor blood groups, D and KidD were the most and the least frequent (82.1% and 1.0%, respectively). According to the Table III, the patients in the positive alloantibody group had higher frequencies of Rh- and C- than those the negative alloantibody group (P = 0.03 and P = 0.05, respectively). As for the main blood groups, cc and E did not differ between the positive and negative alloantibody groups (P > 0.05). Table IV shows the relationship between alloantibody and the different blood groups of patients. As it can be seen, the positive and negative alloantibody groups had no significant difference in the frequency of sub-blood groups (P > 0.05). Based on Table V, there was a significant relationship between Rh and alloantibody (P = 0.03), while age, gender, transfusion start time, splenectomy, and number of transfusions had no significant relationship with alloantibody (P > 0.05).

Table I: The comparison of the patients in positive and negative alloantibody groups in terms of baseline characteristics (n = 105)

Variable		*Alloantibody*		*P-value*
		Positive (n = 26)	Negative (n =79)	
Age: mean (SD)(Year)		23.4 ± 10.6	20.9 ± 11.0	0.31
Age of diagnosis: mean (SD)(Year)		17.2 ± 4.5	15.8 ± 3.6	0.2262
Age in first transfusion (Year): mean (SD)		5 ± 1.4	4.9 ± 1.3	0.89
Gender: n (%)	Female	15 (57.7)	36 (45.6)	0.28
	Male	11 (42.3)	43 (54.4)	
Transfusion frequency in year: n (%)	≤ 12	20 (76.9)	63 (80.8)	0.67
	> 12	6 (23.1)	15 (19.2)	
Splenectomy: n (%)	Positive	3 (11.5)	15 (19.2)	0.37
	Negative	23 (88.5)	63 (80.8)	
Independent t-tests were used for the quantitative comparison of the two groups.				

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Table II: Frequency of the main and minor blood groups in patients with thalassemia major

Main blood types	*N (%)*
A	35 (33.3)
B	22 (20.9)
AB	5 (4.76)
O	43 (40.84)
RH (Positive)	90 (85.5)
Additional blood types	**N (%)**
C (Positive)	86 (81.7)
cc (Positive)	86 (81.7)
D (Positive)	87 (82.1)
E (Positive)	39 (36.8)
ee (Positive)	69 (65.1)
Kell (Positive)	5 (4.7)
Fya (Positive)	82 (77.4)
Fyb (Positive)	82 (77.4)
jka (Positive)	82 (77.4)
Jkb (Positive)	67 (63.2)
duffy (posittive)	3 (2.8)
Kidd (Positive)	1 (1.0)
The variables are presented in frequencies and percentages.	

Table III: Comparison of the blood groups in terms of alloantibody production

Variable	***Alloantibody***		***P-value***
	Positive (n = 26)	***Negative (n = 79)***	
	Main blood groups: n (%)		
A	9 (34.6)	26 (32.9)	0.22
B	2 (7.7)	20 (25.3)	
AB	1 (3.9)	4 (5.1)	
O	14 (53.8)	29 (36.7)	
RH (Positive)	19 (73.1)	71 (89.9)	0.03*
C (Positive)	18 (69.2)	68 (86.1)	0.05*
cc (Positive)	22 (84.6)	65 (82.3)	0.78
E (Positive)	7 (26.9)	32 (40.5)	0.21

The variables are presented in frequencies and percentages. Independent t-tests were used for the quantitative comparison of the two groups.
****: It is statistically significant.***

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Table IV: Comparison of the blood groups in terms of alloantibody production

Variable	Alloantibody		P-value
	Positive (n = 26)	Negative (n = 79)	
D (n = 103)			0.10
positive	19 (73.1)	67 (87.0)	
negative	7 (26.9)	10 (13.0)	
ee (n = 88)			0.24
positive	15 (68.2)	53 (80.3)	
negative	7 (31.8)	13 (19.7)	
Kell (n = 104)			0.79
positive	1 (3.8)	4 (5.1)	
negative	25 (96.2)	74 (94.9)	
Fya (n = 101)			0.26
positive	22 (88.0)	59 (77.6)	
negative	3 (12.0)	17 (22.4)	
Fyb (n = 100)			0.88
positive	20 (80.0)	61 (81.3)	
negative	50 (20.0)	14 (18.7)	
Jka (n = 96)			0.18
positive	19 (76.0)	62 (87.3)	
negative	6 (24.0)	9 (12.7)	
Jkb (n = 101)			0.90
positive	17 (65.4)	50 (66.7)	
negative	9 (34.6)	25 (33.3)	
Duffy (n = 50)			0.76
positive	1 (7.7)	2 (5.4)	
negative	12 (92.3)	35 (94.6)	
Kidd (n = 42)			0.07
positive	1 (10.0)	0 (0.0)	
negative	9 (90.0)	32 (100.0)	
Independent t-tests were used for the quantitative comparison of the two groups.			

Table V: The association of alloantibody with several variables

Variable	P-value
Age	0.31
Gender	0.28
Age at the start of the first transfusion	0.776
Splenectomy	0.37
Transfusion frequency	0.67
Blood unit frequency	0.57
Main blood groups	0.22
Rh group	0.03*
****: It is statistically significant.* Chi-square test or Fisher's extract tests were used to test the association.**	

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Discussion

The aim of the present study was to determine the frequency of major and subgroup antigens and their phenotypes in thalassemia major patients. Based on the findings, of 105 patients participating in this study, 26 (24.7%) had detectable alloantibodies in their serum. had a significant difference between the two groups of patients with positive and negative alloantibodies were significantly different in terms of Rh and C blood groups ($P < 0.05$). Moreover, no remarkable association was found between the existence of alloantibody and age, gender, spleen conditions and the time of first transfusion ($P > 0.05$). Thalassemia patients are among the high-risk individuals in the society in terms of the formation of alloantibodies. Red blood cell surface antigens are numerous, but, for blood transfusion, ABO and Rh groups are controlled. Since thalassemia patients constantly undergo blood transfusion, they may develop antibodies against other red blood cell antigens. This can cause blood reactions in the next transfusions. Among blood group antigens with subgroups D, E, e, C and c, Rh and Kell groups have more antigenic properties and are, therefore, able to stimulate the immune system (17).

In a study by Koochakzadeh et al. (16), the prevalence of anti-red cell alloantibodies was 24.7%, which is the same as the findings of the present study (24.7%). This rate is much higher than the alloimmunization prevalence in thalassemia cases in Malaysia (8.6%)(18) and Pakistan (6.8%) (19). However, it is not comparable to the prevalence of alloimmunization among the known high-risk population in Taiwan (37%)(20). This disparity suggests that something other than geographical environment is involved in the high rate of alloimmunization.

Bhatti et al. (3) observed alloantibodies against red blood cells in 97.4% of their patients, and most of these antibodies were related to Rh system antigens as well as anti-Kell, anti-JSb and anti-Jka types. In a similar study, Norol et al. (21) showed the prevalence of alloantibodies in 8.2% of thalassemia patients. The antibodies were generally against Jka and Jkb, Fya and S groups, and two types of antibodies were observed in one patient. Also, in a study in Greece, Spanos et al. (22) reported the highest amount of alloantibody were of anti-D and anti-Kell types.

In the present study, 24.7% of the patients had alloantibodies the highest amount of which was anti-E (6.6%), anti-Kell (5.7%) and anti-D (3.8%). This is in line with the results gained by Spanos et al. (22).

Consistent with our findings, Koochakzadeh et al. (16) found that age, gender, and blood type had no significant association with alloantibody positivity. Some previous studies, however, indicated an increase in the rate of alloantibodies with age through repeated blood transfusions. It should be noted that the role of age is of significance more at the first blood transfusion session.

Based on the results, the ABO blood type had no considerable effect on the increase of alloantibodies, which is in agreement with the results of a study by Eghbali et al. (23) conducted on an Iranian population. It seems that the main blood groups have no significant role in the generation of alloantibodies.

According to the results of this research and compared to other centers in the world, there are similarities among all the studies regarding the production of antibodies and the occurrence of blood reactions in patients who receive blood repeatedly.

In the current study, 17.9% of the participants had undergone splenectomy, but splenectomy did not make a statistically significant difference in alloantibody classes detected in the tests (P

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= 0.369), which is consistent with the study of Tahannejad et al. (24).

It seems that the role of the spleen in the phenomenon of alloimmunization is a dual and contradictory one. A group of researchers believe that splenectomy increases the production of alloantibodies in the serum because the center for collecting and removing foreign antigens is removed and the immune complexes are no longer cleaned (25, 26).

On the contrary, another team of researchers (27)not only failed to find such an association between the state of the spleen and the increase of alloantibody but even argued that splenectomy is not harmful because red blood cells are no longer removed from the bloodstream, and it reduces the need for transfusion. It is even suggested that splenectomy is a solution at the very beginning of blood collection (28).

Based on the findings, the age in the first transfusion had no statistically significant relationship with the detection of alloantibodies (P = 0.776). In this regard, Tahannejad et al. (24) reported no notable association between the time of the first transfusion and the occurrence of alloantibodies, which is in line with the present study. In contrast, as Hiradfar et al. (25) showed, alloimmunization had a remarkable relationships with the age at the beginning of transfusion, which is not consistent with the findings of this study.

The other factors involved in the production and spread of alloantibodies include the age of patients at the time of blood collection, the time interval between blood collections, and the number of transfusions. At the first glance, it may seem that aging and the number of transfusions induce an increase in the production of alloantibodies due to the increase in the amount of antigens entering the blood and sufficient time for immune stimulation and secondary responses. However, there is no evidence for this direct relationship (26), although such a relationship has been reported in some studies (18, 29).

To justify the contradictory behavior in the above discussion, some researchers mention the phenomenon of tolerance as a mechanism that reduces the prevalence of alloantibody. Thus, if foreign antigens enter the body at the very beginning of infancy and when the child's immune responses have not yet developed, the immune system develops tolerance instead of responding to antigens; even in future encounters, it does not trigger a serious immune response. As another important point, the difference in the prevalence of alloantibody can be due to the non-standardization of some antibody screening methods and the poor techniques of alloantibody identification and interpretation. One of the limitations of this study was the small sample size. Multicenter studies with bigger sample sizes suggest formulating evidence-based transfusion guidelines for thalassemia patients in Iran.

Conclusion

Considering the incidence of alloimmunization in thalassemia patients, most cases of which occur in Rh subgroups especially E, D and Kell groups, and based on a newly diagnosed case in a thalassemia patient, it is recommended to evaluate not only ABO and Rh groups but Kell, Kidd and Duffy groups as well. Determining the complete phenotype of the red cells in beta-thalassemia patients who are candidates for long-term treatment with blood before starting the treatment and performing screening tests for antibody(s) before each blood draw is currently the most practical way to prevent or reduce the formation of alloantibodies in patients with beta thalassemia major.

Conflict of interests

There is no conflict of interests.

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