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The Prevalence and Main Determinants of Red-Cell Alloimmunization among Iranian Beta-thalassemia Patients; A Multicentric Tertiary Hospital Study

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Abstract

Background: The production of alloantibodies as an immunological reaction to blood cell transfusion is a therapeutic challenge in patients with beta-thalassemia. Determining the rate of this event and its related factors is very important in managing blood transfusion. This study aimed to assess the prevalence rate and main determinants of red-cell alloimmunization in Iran's selected sample of patients with beta-thalassemia.

Methods: This cross-sectional study was performed on 265 beta-thalassemia patients scheduled for regular blood transfusions in Imam Khomeini Complex Hospital and Children's Medical Center as tertiary hospitals in 2021 and 2022. Along with baseline characteristics, special laboratory techniques also tracked the types of alloantibodies to red cell antigens.

Results: The overall rate of alloantibody positivity in thalassemia patients was 17.4%. The most common alloantibodies were anti-K in 10.6%, anti-E type in 4.5%, and anti-C in 2.3%.

Conclusion: Overall, 17.4% of beta-thalassemia patients in our population may face red-cell alloimmunization. Phenotyping and blood matching for K, E, and C antigens in patients with transfusion-dependent thalassemia could reduce the rate of RBC alloimmunization.

Keywords: Beta-Thalassemia, Blood group antigens, Blood transfusion, Clergy, Erythrocytes, Iran, Prevalence, Thalassemia

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Introduction

Thalassemia is a major health genetic-based problem and the most common hereditary hemolytic anemia in almost all countries, particularly in the Mediterranean regions (1). In this disease, not only is the homozygous genotype the cause of the disease and its related complications, another important problem is the carrier of the heterozygous form, which remains unknown in many cases and will be the cause of the transmission of the disease to the next generations (2). According to the literature, the heterozygous variant of thalassemia includes 4.5% of apparently healthy people in society (3). One of the potential limitations related to the management of the disease is the need for regular and periodic blood transfusion, which itself can lead to the occurrence of alloimmunization to erythrocyte antigens. The production of alloantibodies against the foreign red blood cells, or alloimmunization, is a major problem in patients suffering from thalassemia due to prolonged multiple transfusions (4,5). Such events can shorten the life span of red blood cells and thus in more patients' dependence on blood transfusion (6,7), ultimately leading to management of blood transfusions in these patients. Also, the development of alloantibodies may lead to the need for the prescription of more immunosuppressive drugs or even a higher likelihood of splenectomy surgery that will make the management of the disease twice as difficult (8). The overall prevalence of alloimmunization among thalassemia patients has been estimated to be 2.5 to 37% (9). More interestingly, the rate of this reaction and the production of alloantibodies are also dependent on the type of surface antigens of red blood cells that, according to the literature, the immunization rate varies from 0.5% for the Duffy antigen to up to 70% for Rh "D" antigen (10,11). In other words, depending on the types of red cell antigen profiling (Duffy, Kell, Lewis, Kidd, MNS, *etc*.), the rate of alloimmunization can be widely different. In the present study the prevalence and main determinants of red-cell alloimmunization in a selected sample of patients with beta-thalassemia in Iran to reduce alloimmunization in chronic transfusion-dependent patients is studied.

Materials and Methods

This cross-sectional study was performed on 265 thalassemia patients scheduled for regular blood transfusions. All thalassemia patients referred to Imam Khomeini Complex Hospital and Children's Medical Center as tertiary hospitals in 2021 and 2022 were evaluated. Patients with available data and documents were included in the research, and those with uncertain information were excluded. The baseline information, including demographic information, blood group, the type and dosage of blood transfusion, and the time interval between transfusions, were collated by the patients' interviewing and a review of patients' medical records. After obtaining the ethical code from the ethical committee at Tehran University of Medical Sciences (IR.TUMS.IKHC.REC.1399.026) and written consent from the participants, the peripheral blood sample was extracted. ABO grouping and Rh (D) typing and antibody testing were routinely performed. Antibodies and albumin were purchased from Immunodiagnostic Co. (Germany).

Antibody screening assay was performed according to standard protocol using IMMUCOR Screening Reagent Cells (USA).

The tests were performed according to a standard protocol. All antisera and antibody screening vials used were tested for quality control, and the results were acceptable. The negative results of the anti-human globulin step were verified using control cells and were acceptable.

If the screening test is positive, the sample is sent to a blood transfusion facility to check the type of specific antibody by using BIO-RAD caseds, including both the 3-cell and 11-cell panels. The agency reported the precise type of antibody found in the serum using the full panel. For patients with transfusion reactions or suspected antibody typing, red blood cell phenotypes were also determined and reported.

The study endpoint was to determine the prevalence value of each alloantibodies to red cell antigens in the study population and their correlates of baseline characteristics. For statistical analysis, the quantitative variables were expressed as mean±SD and the categorical variables as numbers (percentage). The association of baseline parameters with the alloimmunization state was assessed using the chi-square test or independent t-test. p-values of ≤0.05 were considered significant. The statistical software SPSS version 28.0 for Windows (IBM, Armonk, New York) was used for statistical analyses.

Table 1. Baseline characteristics of the study population

Gender, %	
Male	115(43.4%)
Female	150(56.6%)
Mean age, year	24.71±10.87
Blood groups, %	
A+	78(29.4%)
A-	10(3.8%)
B+	50(18.9)
B-	6(2.3%)
O+	94(35.5%)
O-	12(4.5%)
AB+	14(5.3%)
AB-	1(0.4%)
The number of units transfused	
1	47(17.8%)
2	210(79.2%)
3	8(3.0%)
Time intervals between transfusions, days	28.33±1.82

Table 2. The frequencies of different alloantibodies in patients with thalassemia

Alloantibody positivity, %	46(17.4)
K	28(10.6)
E	12(4.5)
C	6(2.3)
D	4 (1.5)
c	2(0.8)
jka	2(0.8)
Cw	1(0.4)
jkb	1(0.4)
S	1(0.4)

Results

In total, 265 patients with thalassemia (115 men and 150 women) with an average age of 24.71±10.87 years were included in the study. Baseline characteristics are shown in table 1. The most frequent blood groups included O+ (35.5%) followed by A+ (29.4%). All packed cells used for transfusion were Leukoreduced (LR) type, and according to checked phenotypes of patients, some specific packed cells with antigen-negative were used. The patients with anti-K received some types of packed cells, including K-negative, and patients with anti-E received some types of packed cells, including E-negative, and also patients with anti-C and D comply with this rule. Overall, 82.2% of patients received more than one unit of blood for transfusion. The mean time interval between transfusions was also 28.33±1.82 days.

Regarding the frequency and types of alloantibodies released in the study population (Table 2), the most common alloantibodies were anti-K in 10.6%, anti-E type in 4.5%, and anti-C in 2.3%. In total, the rate of alloantibody positivity in thalassemia patients was found to be 17.4%.

Comparing the subgroups with and without alloantibody positivity toward transfusions (Table 3) showed no difference between the two groups in terms of demographics, type of blood group, the number of units transfused, and the time between transfusions (p-value >0.05). In the present study, 93 patients checked for RBC phenotype and showed one or more antigens negative on red blood cells but

Table 3. The main determinants of alloantibody positivity

Parameter	Alloantibody positivity (+)	Alloantibody positivity (-)	p-value
Gender, %			0.196
Male	17(37.0)	65(29.7)	
Female	18(39.1)	71(32.4)	
Mean age, year	29.76±11.28	24.27±10.76	0.159
Blood groups, %			0.133
A+	14(30.4)	64(29.2)	
A-	1(2.2)	9(4.1)	
B+	9(19.6)	41(18.7)	

Contd. table 3.

B-	3(6.5)	3(1.4)	
O+	11(23.9)	83(35.5)	
O-	3(6.5)	9(4.1)	
AB+	4(8.7)	10 (4.6)	
AB-	1(2.2)	0(0.0)	
The number of units transfused			0.274
1	6(13.0)	39(18.0)	
2	40(87.0)	170(78.3)	
3	0(0.0)	8(3.7)	
The time between transfusions, days	25.63±12.19	28.92±2.18	0.492

only 46 (49.5%) patients produced antibodies against absence antigens, and 47 (50.5%) patients still didnot produce antibodies against absence antigens or the antibody titer may be lower than the level that can be detected by the methods used. The average age of patients with antigen-negative phenotype without antibody production was 28.8 years, and the interval between transfusions was 31.4 days (p-value >0.05).

Discussion

Continuous blood transfusion is a supportive vital treatment for thalassemia patients; however, one of the most difficult side effects related to this performance is alloimmunization against blood units transfused, and access to effective blood can be very difficult in these patients. Increasing the likelihood of acute and delayed hemolytic reactions is a dilemma for such patients. Based on the studies, depending on the type of blood unit injected, the time of injection, and the type of blood group of the patient, the production rate of alloantibodies may be completely different. Therefore, determining the production rate of these antibodies in the first place and then evaluating the relationship between background indicators and the risk of this immune reaction will be very important in preserving the lives of patients.

BIO-RAD caseds offer a range of blood typing and antibody screening reagents. The caseds typically include 3-cell and 11-cell panels, which provide a variety of red blood cells with different antigen profiles to detect a broad range of antibodies. Even with an 11-cell panel, gaps in the antigen profile could exist (*e.g.*, some rare antibodies may still not be identified if the panel does not contain the appropriate red blood cells). This can lead to false-negative results or inconclusive tests, especially in cases of very rare or weak antibodies. Cross-reactivity between antibodies or weak reactions could produce false positives, requiring further testing (*e.g.*, enzyme treatment, elution, or extended panels). False negatives could also occur if antibodies are not present in detectable titers. The use of larger panels (*e.g.*, the 11-cell) increases costs, both in terms of reagent purchase and laboratory time. This could limit its use in some settings. While the caseds are standardized, interpretation still requires a high level of skill, especially when dealing with weak reactions or antibodies with complex characteristics.

In the present study, initially the production of alloantibody in the studied patient population had a rate of 17.4%, which was close to the expected statistics in previous studies in Iran, but different compared to other societies. Also, according to the present study analysis, 93 patients were checked for RBC phenotype, and 49% of them produced antibodies against absence antigens. The most prevalent types of alloantibodies were anti-K and anti-E. Other patients with abnormal phenotyping without antibody production are predisposed to producing antibodies and need close observation. For this reason, antigen-negative packed cells were also used in most of these patients. Antibodies production in these patients may be related to age and interval time of transfusion, but in the present study, this was not established and needed more patients to find the probable reason. In a systematic review and meta-analysis by Rostamian *et*

al in 2022 (12), on Iranian populations, the prevalence of alloimmunization was 13% [anti-D (25%) and anti-K (25%)] and was most prevalent among Iranian β-thalassemia patients. In a study by Senavirathna *et al* in Sri Lanka in 2022 (13), unexpected red blood cell antibodies were present only in 1.5% of patients, and the identified antibodies were Anti E, Anti K, Anti D, Anti C, Anti S, Anti Jkb. In a study by Chao *et al* on Chinese thalassemia patients in 2013 (14), 9.4% developed RBC alloantibodies, mostly as Anti-E and Anti-C. It seems that in different societies of the world, the reports regarding the prevalence and type of alloantibody produced are completely different. Therefore, it is very important to determine the risk factors related to the production of this type of antibodies against blood transfusion to reduce this immune reaction. A review of studies shows that the risk factors associated with this reaction have been completely different in different studies. In a study by Kababi *et al* in Morocco in 2019 (15), the prevalence of alloimmunizations was 8.75%, and a transfusional interval of less than 3 weeks was associated with a high rate of red cells alloimmunization. In a study by Zaidi *et al* in Pakistan (16), no independent risk factor of baseline characteristics associated with alloimmunization was reported, which was similar to the present study's findings. Ahmed AM *et al* (17), in a study on Egyptian patients with β-thalassemia showed that the male gender, the number of units transfused, and splenectomy were associated with a higher alloimmunization rate. In another study in Greece by Spanos *et al* (18), the alloimmunization rate was closely associated with the type of blood group in which anti-Kell was most often identified (28.5%). They also showed that alloimmunization appears considerably lower in patients in whom blood transfusion is started before the age of 3 years than in those in whom it is started after that age. Therefore, in various studies, different underlying factors have been proposed regarding the risk of this immune reaction. The BIO-RAD caseds, including both the 3-cell and 11-cell panels, are valuable tools for antibody screening and identification. They offer a practical solution for detecting a broad spectrum of antibodies, with the 11-cell panel providing enhanced sensitivity for rare antibodies. However, limitations such as antigen gaps, potential for false results, and cost considerations must be weighed when choosing the appropriate testing method. Ensuring that laboratory personnel are adequately trained and using confirmatory tests as needed will enhance the accuracy of results and improve patient care.

Of course, the conditions of designing and conducting the study, such as the number of samples studied, the age range of the patients, the previous history of blood transfusions, or even other underlying diseases of people may be effective in the occurrence of this reaction, which should be considered in the implementation of subsequent studies. The prevalence of alloantibodies in β-thalassemia patients in Iran was higher than in similar studies in other countries. Clinically significant alloantibodies can impact the life quality and overall survival of patients. The findings of the present study implicate that further research and awareness of clinicians to prevent alloimmunization is necessary.

Conclusion

It can be finally concluded that the rate of alloimmunization against red blood cells in the present study population is estimated to be 17.4%.

The most common allontibodies are anti-K and anti-E. Appropriate preventive strategies, such as phenotyping for patients before beginning transfusion, could be implemented to avoid complications in thalassemia patients.

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The ethical code was obtained from the ethical committee at Tehran University of Medical Sciences (IR.TUMS.IKHC.REC.1399.026).

Conflict of Interest

There was no conflict of interest in this manuscript.

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