Frequency of red cell allo- and autoimmunization in patients with transfusion-dependent beta thalassemia and affecting factors

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In this study, we aimed to determine the frequency of red cell allo- and autoimmunization and analyze the factors responsible for the development of antibodies in patients with transfusion-dependent thalassemia. This cross-sectional study was conducted on 139 patients with thalassemia major and intermedia who received leukodepleted RBC transfusions on a regular basis. Patients with a positive antibody screen were further tested for antibody identification by a gel method. Red cell alloantibodies were found in 9 (6.4%) patients, and autoantibodies were found in 17 (12.2%) patients. The most common alloantibodies detected were those against Rh and Kell antigen systems. The alloantibody development rate was higher in thalassemia intermedia patients, in Rh(-) patients, in patients with an initial transfusion age >2 years and in patients with a transfusion interval >3 weeks ($p<0.05$). The autoantibody development rate was found to be higher in adult and splenectomized patients ($p<0.05$).

Data from this study demonstrate that the RBC antibody development rate is high in our region. RBC antigen phenotyping and crossmatching with Kell and Rh subgroups may reduce alloimmunization in chronically transfused beta-thalassemia patients.

Key words: thalassemia, transfusion, alloimmunization.

Early and regular blood transfusion therapy in patients with homozygous beta-thalassemia decreases the complications of severe anemia and prolongs survival. In the long term, however, it may be associated with complications such as organ damage resulting from iron overload—a consequence of the body's limited capacity to excrete iron—and contagious infections originating from blood and its products. Although iron chelation therapy has successfully improved survival, thalassemic patients continually present new clinical challenges[1,2]. The repetition of transfusions for the treatment of thalassemia major provokes the patient's immune system and produces anti-erythrocyte antibodies: alloantibodies and/or autoantibodies. Erythrocyte autoantibodies appear less frequently, but they can result in clinical hemolysis and difficulty in crossmatching blood. Alloimmunization against red blood cell antigens increases the need for transfusion, and this can be a significantly complicated transfusion therapy. Some alloantibodies are hemolytic, causing hemolytic transfusion reactions and limiting the possibility of further safe transfusion, while others have been found to be clinically insignificant[3,4].

Early diagnosis and detection of allo- and autoantibodies are very important, as blood transfusion is essential for survival in thalassemia patients[5]. A few studies concerning this subject exist in the literature from our country. Nevertheless, more information is needed on erythrocyte alloimmunization and autoimmunization in transfusion-dependent Turkish β thalassemia patients[6-7]. In conducting this study, our purpose was to identify these allo- and autoantibodies and the factors that

might contribute to their development.

Material and Methods

We retrospectively examined the clinical and transfusion records, extending over a 5-month period from September 2011 to February 2012, of 139 patients with β thalassemia major and intermedia who had chronic transfusions with leukodepleted and relatively fresh (maximum 7 days) packed RBCs at the Division of Pediatric Hematology of Tepecik Children's Hospital in İzmir. Transfusions were not performed from relatives or family members. Subgroup-matched transfusion was performed in alloantibody-positive patients. The patients' age, sex, ABO and Rh blood group, symptoms, history of splenectomy, age at first blood transfusion and years of blood transfusion were recorded. The diagnosis of thalassemia was confirmed by standard hemoglobin electrophoresis and measurements of HbA, A_2 and F. The study was approved by the local ethics committee.

Laboratory Investigations

Using standard blood bank methods, serum was analyzed prior to each transfusion to detect new antibodies to RBC antigens. All pretransfusion sera were also tested to confirm their phenotype for the following blood group systems: ABO; Rhesus (D, C, E, c and e); Kell (K, k), Kidd (Kpa, KpB) and Duffy (Fya, Fyb). Immunological examinations were done with a gel test (ID-Micro Typing System, DiaMed AG, Cressier sur Morat, Switzerland). Dia Cell I, II and III were used to screen for the presence of red cell alloantibodies and the Dia Panel to identify these alloantibodies. Descriptive statistics and a chi-square test were performed; a p value <0.05 was considered significant. The results were analyzed using SPSS (Statistical Package for the Social Sciences, version 15, SPSS Inc., Chicago, IL).

Results

Patient characteristics are shown in Table I. A total of 139 patients, 67 children and 72 adults, were included in this study. The mean age was 18.3±8.66 years. The number of male and female patients was 76 (54.7%) and 63 (45.3%), respectively. Of the patients, 109 (78.4%) had β-thalassemia major, and 30 (21.6%) had β-thalassemia intermedia. The frequency of transfusion was less than 3 weeks in 109 (78%) patients, and over 3 weeks in 30 (22%) patients. A history of splenectomy was present in 28.3% of the pediatric patients and 77.3% of the adults.

Table II indicates the age, sex, age at start of transfusion, transfusion frequency, history of splenectomy, blood type, Rh factor and type of red blood cell alloantibody formation for each of the β-thalassemia patients in whom such antibodies formed. Alloantibodies were detected in 9 (6.4%) out of 139 patients. Six of these patients were female and 3 were male; 4 of them were children, and 5 were adults. A total of 11 alloantibodies were detected in 9 patients. Two patients were found to have more than one alloantibody (anti-C and anti-D). The incidence of anti-K, anti-C, anti-D, anti-jka, and anti-E was 27%, 27%, 18%, 18%, and 9%, respectively.

The incidence of anti-jka, anti-C and anti-D was 40%, 40% and 20%, respectively, in children. In adults, the incidence of anti-K was 50%,

Table I. Demographic and Clinic Data of Patients with β Thalassemia

Patients	n (%)
Age group	
Pediatric	67 (48.2)
Adult	72 (51.8)
Thalassemia type	
T. major	109 (78.4)
T. intermedia	30 (21.6)
Transfusion interval	
≤ 3 weeks	109 (78.4)
> 3 weeks	30 (21.6)
Sex	
Female	63 (45.3)
Male	76 (54.7)
Age at the start of transfusion	
≤ 2 years	102 (73.3)
> 2 years	37 (26.7)
Splenectomized	
Yes	75 (53.9)
No	64 (46.1)

with anti-D, anti-C and anti-E all having an incidence of 16.6% (Table II).

A comparison between the groups of β-thalassemia patients revealed a highly significant association in terms of alloantibody formation between β-thalassemia major and β-thalassemia intermedia (p=0.01). However, no correlation was found for alloantibodies between age and sex (p=0.816, p=0.184) (Table III).

A history of splenectomy was present in 66.6% of patients with alloantibodies. We found that 50% of β-thalassemia major patients and 80% of β-thalassemia intermedia patients were splenectomized. However, there was no association between the incidence of splenectomy and alloantibody formation (p=0.921) (Table III).

An evaluation of patients with and without alloantibodies revealed that the incidence of Rh factor was significantly less in alloantibody-positive patients (13% and 44%) (p=0.005) (Table III).

Detailed findings of patients with autoantibodies are shown in Table III. Seventeen (12.2%) of the patients developed autoantibodies. Autoantibody formation was significantly correlated with age group and the presence of splenectomy (p=0.001 and p<0.001, respectively). In addition, splenectomized adult patients had a higher incidence of autoantibody development than non-splenectomized adult patients (p<0.001). Immunosuppressive agents (steroids and rituximab) were needed in only one patient. Except in the case of this patient, no need was found for additional transfusion. No diversity was determined in the direct antiglobulin test results of the patients with auto- or alloimmunization.

Discussion

Worldwide, several studies have investigated the frequency and causes of allo- and autoimmunization in transfusion-dependent β thalassemia[8-9].

In the present study, the frequency of allo- and autoimmunization was 6.4% and 12.2%, respectively. The patients' age, sex, ABO and Rh blood group, symptoms, history of splenectomy, age at first blood transfusion, and years of blood transfusion were evaluated, in addition to the determination of the frequency and specificity of the alloantibodies. Alloimmunization was found

Table II. Laboratory and Clinical Findings of Alloimmunized Patients

Patient number	Age (years)	Sex	Type	Age at start of Tx (months)	Tx (U/year)	Splenectomized	Blood group	Rh	Ab
1	20	F	TM	24	34	No	B	+	K
2	30	M	TM	12	12	Yes	B	+	K
3	26	F	TI	96	5	Yes	0	-	C,D
4	12	F	TM	3	26	No	0	-	C
5	20	F	TI	72	14	Yes	A	+	E
6	24	F	TM	12	31	Yes	A	+	K
7	10	F	TI	36	14	No	A	-	C,D
8	9	M	TI	30	6	Yes	B	+	Jka
9	9	M	TI	30	6	Yes	AB	-	Jka

F: Female, M: Male, TM: Thalassemia major, TI: Thalassemia intermedia, Tx: Transfusion, U: Unit, Ab: Antibody .

Table III. Factors Affecting Antibody Formation in Transfused β Thalassemia Patients

	n	Alloantibody(+) n (%)	Autoantibody(+) n (%)	*p* value Allo.	Auto.
Total patients	139	9 (6.4)	17 (12.2)		
Thalassemia type					
T. major	109	4 (3.6)	15 (13.7)	0.010	0.294
T. intermedia	30	5 (16.6)	2 (6.6)		
Sex					
Male	76	3 (3.9)	8 (10.5)	0.184	0.501
Female	63	6 (9.5)	9 (14.2)		
Age at start of Tx					
≤2 years	102	3 (2.9)	13 (12.7)	0.005	0.758
>2 years	37	6 (16.2)	4 (10.8)		
Splenectomized					
Yes	75	5 (6.6)	16 (21.3)	0.921	<0.001
No	64	4 (6.2)	1 (1.5)		
Age group					
Adult	72	5 (6.9)	15 (20.8)	0.816	0.001
Pediatric	67	4 (5.9)	2 (2.9)		
Tx interval					
≤ 3 weeks	109	3 (2.7)	15 (13.7)	0.001	0.294
> 3 weeks	30	6 (20)	2 (6.6)		
Rh factor					
Negative	19	4 (21)	2 (10.5)	0.005	0.807
Positive	120	5 (4.1)	15 (12.5)		

Allo: Alloantibody, Auto: Autoantibody, Tx: Transfusion

to be associated with age at first transfusion, thalassemia type and presence of Rh factor. Autoantibody development was found to have a relationship with age and the presence of splenectomy.

In a recent study in Turkey, the incidence of autoantibodies and alloantibodies in patients with thalassemia major was 5.6% and 10.5%, respectively[10]. Thalassemia patients have been found to be at high risk for alloantibody formation because of frequent blood transfusions. Globally, the incidence of alloantibody formation has been found to be between 3% and 40%, with differences attributed to the age range, rate of transfusion of incompatible blood, genetic variability of the population and rate of transfusion of non-leukodepleted blood[4-23]. Compared with the global population, our study showed a low prevalence of alloimmunization in multi-transfused thalassemic patients. One of the reasons for the low incidence of alloimmunization may be the transfusion of leukodepleted blood in all patients.

There is great variability in the types of alloantibodies reported in the literature, with most of them being potentially hemolytic alloantibodies. Most studies agree in finding that the most common alloantibodies to develop are those against antigens of the Rh subgroups and the Kell system[5,9,11,12]. In our study group, all patients received compatible blood for ABO and Rh D. The most frequent alloantibodies were those against the Kell antigen (27%) and

the C antigen of the Rhesus system (27%), which are potentially hemolytic antibodies.

Previous studies have focused on elucidating the association between auto- and alloimmunization and the clinical and demographic characteristics of transfusion-dependent thalassemia patients. However, the results obtained from different investigations are still insufficient and contradictory[4-23]. This study found alloimmunization not to have a statistically significant correlation with age and sex. Schonewille et al.[13] also detected no relationship between alloimmunization and age. However, Azarkeivan et al.[5] and El Danasoury et al.[14] did report a relationship between age and the development of alloantibodies. The same two authors found no relationship between sex and alloimmunization. It has been argued that transfusion at an early age may offer a certain degree of protection against red cell alloimmunization in young children[4, 15]. Reisner et al.[16] reported a higher risk of alloimmunization in females.

This study found a significant difference in the frequency of alloantibody presence between patients who started blood transfusions before 2 years of age and those who started transfusions after 2 years of age (p=0.005). The first group had a lower incidence of red cell alloimmunization. Karimi et al.[9] detected no relationship between the variables of age and alloimmunization. In thalassemia major patients, red cell antibody production usually occurs after the age of 6 years, following multiple transfusions. This is perhaps due to immune tolerance developed by periodic blood transfusion started at an early age.[15]

Reports in the literature indicate contradictory results in association with a history of splenectomy. Singer et al.[4] and El Danasoury et al.[14] reported that patients who underwent splenectomy had a higher incidence of alloimmunization. They found that the absence of a spleen may further enhance the immune response to infused foreign antigens that are not effectively filtered. In contrast to these studies, our findings were similar to those of a study performed in Hong Kong suggesting that splenectomy did not affect the incidence of alloimmunization[12]. Despite a higher risk of alloimmunization, splenectomy may decrease transfusion rates in immunized thalassemia patients.

This study unearthed an association between the type of thalassemia and alloimmunization. Thalassemia intermedia patients developed more alloantibodies (p=0.01). Azarkeivan et al.[5] reported an association similar to that in this study; however, they detected no relationship between the type of thalassemia and the number of alloantibodies. The present study noted that thalassemia intermedia patients receive their first transfusions at a relatively late age, after the maturation of the immune system, which may be the cause of the high incidence of autoantibodies in these patients. Alloantibodies increase the need for transfusion as well as the risk of side effects in thalassemic patients[5,17,18]. Despite this finding, alloimmunization incidence was found to be less in the group receiving transfusion at intervals >3 weeks, because most of the alloantibodies were detected in thalassemia intermedia patients, who needed transfusion less often than thalassemia major patients.

Some reports have found the most common antibodies to be those against the Rh subgroups and Kell antigen. Consequently, the present study evaluated the association between Rh factor and the development of alloantibodies, and found that the development of alloantibodies was significantly higher in the Rh negative group (p=0.005). Under normal conditions, crossmatching is performed only for the D antigen of the Rh system. Other antigens (C, c, E, e) are also present in the Rh system. This study found anti-C to be one of the most common alloantibodies (Table II). The present study thus suggests that in order to decrease hemolysis and the need for transfusions, packed cells should be crossmatched for minor blood groups—in our country, for Kell and Rh C in particular—as well as for major blood groups.

This study found autoantibodies in 17 patients (12.2%); the number of these autoantibodies was found to be significantly higher in splenectomized and older patients (p<0.001 and p=0.001, respectively). Recently published trials obtained similar data by assessing the correlation between high risk of autoimmunization and splenectomy. This can be explained with reference to the filtering system of the spleen, which eliminates damaged erythrocytes. Senescent erythrocytes and impaired blood cells, more common in patients without a spleen, expose new antigens and

promote or enhance autoimmune reactions[4,19]. This paper reports the results of a study carried out at a tertiary hospital in the Aegean region in order to determine the prevalence of RBC allo- and autoantibodies and the factors that could affect their development. Compared to the findings of some other studies, our data showed a lower rate of RBC immunization to red cell antigens. This can probably be explained by the use of leukodepleted and relatively fresh packed red cells.

In conclusion, prediction of RBC antigen phenotypes before the first transfusion takes place is recommended for thalassemia patients. We also recommend that the types of alloantibodies present in the population of a given region should be identified, and that thalassemia patients should receive compatible blood for Rh and Kell subgroups taking these specific alloantibodies into consideration, for more effective prevention of alloantibody development.

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