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Prevalence of Alloimmunization against RBC Antigens in Thalassemia Major Patients in South East Of Iran

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Abstract

Background: Thalassemia major is a lifelong transfusion dependent disease. Continuous blood transfusion can causes alloimmunization against RBC antigens and complicate further treatment in these patients. The purpose of this study was to determine the frequency of RBC allo and auto antibodies, the types of these antibodies and the factors that affect alloimmunization in patients with thalassemia.

Material and methods: This descriptive study was performed on 221 males and 164 females with thalassemia major referred to Ali Asghar hospital in Zahedan, Iran. Initially information sheet about age, gender, race, age on first blood transfusion, history of splenectomy and ABO & Rh blood group were filled out. For alloantibody screening, patients serum were tested in three phases (Salin, 37°C with LISS and Anti Human Globulin) by pooled cells of Biorad. In case of a positive screen, antibody identification was performed by using panel cells prepared by *Iranian Blood Transfusion* Organization. Finally obtained results were analyzed by SPSS software.

Results: Out of 385 patients (221 male &164 female; mean age, 13.8 years; range, 1-45 years), 69 cases (17.9%) had been alloimmunized. Majority of alloantibodies were directed against Rh and Kell systems. 21(5.5%) patients were positive for autoantibodies.

Conclusion: The relatively high prevalence of alloantibodies (17.9%) in our studied patients indicated the importance of cross matched blood from the beginning of transfusion in thalassemia major patients.

Keywords: Thalassemia major; Alloantibody; Panel cell

Introduction

Thalassemias are genetic heritable disorders that result from a reduced rate of production of one or more of the globin chains. Depending on the globin chain involved, they are divided in α, β and δβ-thalassemia [1,2]. Severe clinical manifestations of thalassemia major including anemia and delayed growth are apparent in the first year of life of the patients [2].

Lifelong red blood cell transfusion has remained the main treatment of severe thalassemia [3]. Repeated blood transfusions can stimulate the patient's immune system and and results in the formation of anti-erythrocyte antibodies usually IgG class [1,4]. Although autoantibodies appear with less frequency, but they can result in clinical hemolysis and complication of blood cross-matching. Alloimmunization against red blood will increase the need for blood transfusions in patients with thalassemia. Some alloantibodies are hemolytic and may cause hemolytic transfusion reactions and limit the availability of further safe transfusion but others are clinically insignificant [5,6].

In guidelines for chronic transfusions in patients with thalassemia, antigen phenotyping before the first blood transfusion, laboratory tests including CBC, cross-match and RBC antibody screening are recommended. While antibody screening is included in the compatibility testing protocol in the developed countries, it is not yet available in Iran and other developing countries [6-8].

The reported frequency of antibody formation is highly variable in different parts of the world ranging from 2.37% to 37% [9]. Thalassemia is common in Asia and there are more than one million carriers of the disease in this area. Iran also has more than twenty thousand thalassemia carriers [4,10].

In Sistan and Baluchistan province, south east of Iran, because of the high rate of consanguineous marriages, thalassemia is common. Although it is well known that the development of anti-erythrocyte antibodies lead to interference in transfusion therapy, but limited data about frequency of RBC immunization in these patients are available.

The purpose of this study was to determine the frequency of RBC alloantibodies and/or autoantibodies, the types of these antibodies and factors influencing on alloimmunization among multiple-transfused thalassemia major patients.

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Material and Methods

This descriptive study was performed on 221 males and 164 females with thalassemia major who had received regular transfusion in Ali Asghar hospital in Zahedan, Iran from Jan to Jul 2011.

At the first, the patient's age, sex, age on first blood transfusion, ABO and Rh blood group, history of splenectomy and ethnic background were recorded in questionnaires. After receiving a written consent from each patient, before transfusion, 10ml of venous blood was collected in two separated tubes, K2-EDTA added tube for auto control and without anticoagulant tube for antibody screening and antibody identification.

Patients with background of HIV, HBV, HCV or any other infectious symptoms in two recent weeks were excluded from study.

Initially, antibody screening was done by using 3-cell panel of Biorad Corporation (Biotest cell-P3), according to standard blood bank methods [11-14]. Two parts serum and one part RBCs were mixed and evaluated in three phases (RT, 37°C and combs phases) by using of LISS prepared in Biorad (LISS MBL2). All data was entered in predefined tables. Negative results were confirmed by adding check cells of Biorad (Coombs cell-E).

In case of a positive screen, antibody identification were performed in the same phases as Ab screening, by using 11-cell panel prepared in IBTO (*Iranian Blood Transfusion* Organization) (Panel cell 11IP11C41 ,11IP11C42), and finally according to presented antigram pattern of each panel, type of specific antibody against each antigen was determined. An autocontrol were also put simultaneously to determine the presence of autoantibody.

The results were analyzed statistically by SPSS software, version 16 by using of T-test and chi-square test. A p-value of less than 0.05 was considered statistically significant.

Results

From total number of 385 patients with thalassemia major, 221 persons (57%) were males and 164 patients (43%) were females with an average age of 13.8 ± 7.7 years (range 1 to 45 years) (Table 1).

In aspect of ethnicity, 308 patients were Baluch, 70 were Sistani and 7 patients were Fars. All of patients were received blood components in Zahedan Blood Transfusion Organization.

One hundred fifty-five patients (40.3%) were with O blood group, 132 (34.3%) were with B blood group, 80 (20.8%) were blood group A, and 18 (4.7%) were blood group AB. 356 patients were rhesus positive and 29 were rhesus negative. In alloimmunized patients, majority was O positive (Table 2).

The age on first blood transfusion ranged from 1 to 108 months with mean ± SD of 12.3 ± 15.5 (Figure 1).

Twenty-eight (7.3%) cases among 385 patients underwent splenectomy. There was no statistically significant association between alloimmunization and splenectomy (p=0.57).

Sixty-nine (17.9%) of 385 patients developed alloantibodies against RBC antigens that in 57 cases alloantibody were exactly determined. Majority of alloantibodies were directed against antigens in the Rh and Kell systems (Figure 2). The most common alloantibody among alloimmunized patients was Anti-E (present in 10 cases). Autoantibodies in 21(5.5%) patients were positive (Table 3). Also our findings revealed that 14.7 percent of females and 24 percent of males had evidences of alloimmunization. Chi-Square test showed significant difference (Odds ratio=1.76) between gender and alloimmunization (p=0.043).

Age(years)	Number (%)	Alloimmunized patienys (%)
<5	67 (17.4)	**11 (15.9)**
6-10	77 (20)	**11 (15.9)**
11-15	70 (18.2)	**18 (26.1)**
16-20	77 (20)	**16 (23.3)**
>20	94 (24.4)	**13 (18.8)**
Total	385 (100)	**69 (100)**

Table 1: Age distribution in studied patient

		ABO				Total
		A	B	AB	O	
Rh	Pos	9	26	0	31	66
	Neg	0	1	1	1	3
Total		9	27	1	32	69

Table 2: Frequency of ABO and Rh blood groups in 69 alloimmunized patients

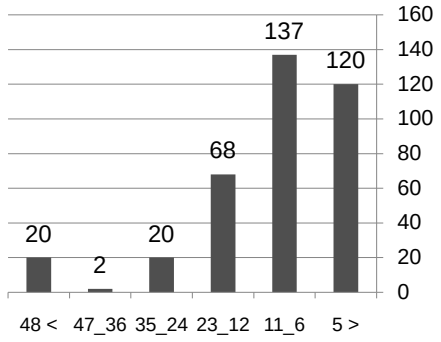

Figure 1: Ageon first transfusion in 69 alloimmunized patients (month).

In this study, Chi-Square test showed no significant differences between rate of alloimmunization and ABO (p=0.173) and Rh(p=0.21) blood groups. This differences is not statistically significant (p>0.05), but may be clinically important. Minority and majority of rate of alloimmunization was in patients with AB (1 case) and O (32 cases) blood groups respectively. Also alloimmunization was very low in Rh negative patients as compared with Rh positive ones (4.3% vs. 95.7%).

A higher rate of alloimmunization occurred among Sistani patients, compared to Baluch and other races, but this difference was not statistically significant (p=0.192).

Discussion

The present study was assessed the prevalence of alloimmunization among thalassemia patients in Sistan and Baluchistan province, southeast of Iran. The province has one of the largest groups of patients with thalassemia major in Iran. These patients underwent regular blood transfusion. Maximizing the efficacy of blood transfusions in these patients is of particular importance. Repeated blood transfusion in thalassemia patients can causes alloimmunization against different red blood cell antigens that are absent on patient's red blood cells. This alloimmunization can decreases survival of transfused red blood cell and reduces efficacy of blood transfusion. Due to the large number of thalassemia patients in Sistan and Baluchistan province, we design a study on a considerable number of patients with thalassemia major referred to Ali Asghar hospital in Zahedan.

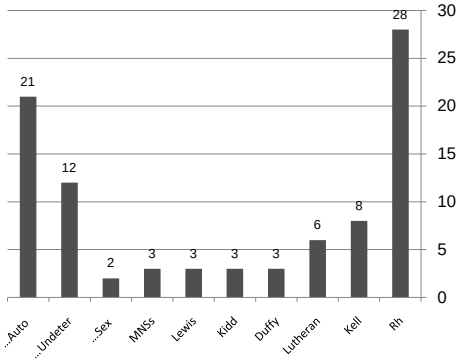

Figure 2: Frequency of different detected antibodies in patients with β-thalassemia major

Blood groups	Type of alloantibodies	Patients number	Percentage
Rh	Anti-C	6	1.6
	Anti-c	5	1.3
	Anti-E	10	2.6
	Anti-e	3	0.8
	Anti-C^w	5	1.3
Kell	Anti-K	4	1
	Anti-k	1	0.3
	Anti-Kp^a	3	0.8
Duffy	Anti-Fy^b	3	0.8
Kidd	Anti-Jk^a	2	0.5
	Anti-Jk^b	1	0.3
Lewis	Anti-Le^b	3	0.8
MNS	Anti-s	3	0.8
Lutheran	Anti-Lu^a	6	1.6
Sex Linked	Anti-Xg^a	2	0.5

Table 3: Distribution of alloantibodies in 69 alloimmunized patients

Our study revealed that sixty-nine patients had been allo immunized, but most of our patients did not revealed any evidence of hemolytic transfusion reaction. Twenty-one patients also developed auto antibodies.

The most common antibody among these patients was Anti-E (present in 10 patients). Anti-C(6 patients),Anti-Lu^a (6 patients), Anti-c (5 patients) and Anti-C^w (5 patients) were other common allo antibodies. The majority of detected allo and autoantibodies were non-hemolytic. Therefore, the recommended preventive measures would be beneficial for only a few patients who would develop these problems [12].

In a similar study that was performed by Eshghi et al. [15] in 2000, no type of alloantibody reported in thalassemia major patients of Ali Asghar hospital from Zahedan but our study showed that rate of alloimmunization against RBC antigens was 17.5% that in 57 cases alloantibodies were exactly determined [13]. Previous studies have reported that the rate of alloimmunization ranged from 5 to 30 percent in transfusion dependent thalassemia patients [8,11,14,15]. The age and the immune status of the patient may also contribute to the immune response [1]. In this study, the majority of the alloimmunized patients were between 11 to 15 years old (26.1%), followed by the age range of 16 to 20(23.2%), >20(18.8%), <5 and 6-11(15.9% each) respectively. There was no association between alloimmunization and age in this study (p=0.23). A few studies have also reported no significant relationship between age and alloimmunization in transfusion dependent thalassemia patients [7,16,17]. A number of patients in this study have developed multiple RBC alloantibodies with potential clinical significance, without documented evidence of hemolytic transfusion reaction. It seemed that further studies are needed for investigation of immune system in transfusion-dependent thalassemia patients.

In the present study, twenty-nine patients among 69 alloimmunized patients were started transfusions between 6 to 11 months. This finding is contrast with other studies. It has been reported that transfusion at an early age (< 1 year old) may lead to immune tolerance that induced by an immature immune response to repeated blood transfusions and protections from alloimmunization [1,7,8,11]. However, our results showed there was no statistically significant association between alloimmunization rates and the age at beginning of transfusion (p=0/33). The mean(SD) age at first blood transfusion in patients with alloimmunization and without alloimmunization was 12.4 and 12.7 years respectively and this difference was not statistically significant (p=0.9). The relationship between the number of transfused units and alloimmunization is unknown in thalassemia. It was reported that red cell alloimmunization is more likely in patients who received more blood units [18]. Of twenty-eight patients who underwent splenectomy, 4 patients had alloantibodies and 3 carried autoantibodies. There was no significant association between alloimmunization and splenectomy (p=0.57).

The results of this study revealed that the majority of RBC alloantibodies are against Kell and Rh blood group systems [19-22]. Since alloimmunization against minor blood groups antigens can affect the effectiveness and frequency of blood transfusions, selection of compatible blood in term of these blood groups especially for Rh(E) and kell is particularly important.

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