Frequency of Red Cell Alloimmunization in Patients with β-Major Thalassemia in an Iranian Referral Hospital

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Abstract

**Objective:** Frequency of red cell alloimmunization in patients with β-major-thalassemia in Mofid children's hospital, Tehran, Iran

**Material & Methods:** This is a cross-sectional descriptive study conducted in Mofid children's hospital, March 2007. A total of 121 major thalassemia patients on regular blood transfusion were included in this study. Clinical and laboratory data were collected and analyzed to find out the frequency, pattern and factors influencing red cell immunization secondary to multiple blood transfusions in these patients.

**Findings:** Mean age of patients was 13 (6.19) from 2-26 years. They had received regular blood transfusions during periods ranging from 1 to 25.5 years. Red cell alloimmunization was found in 9 patients (7.4%). In female group, 5 out of 66 (7.6%) patients and in male group 4 out of 55 (7.3%) patients had evidence of alloimmunization. The mean age of patients with alloimmunization was 9.6 (6.5) years (range 3.7-20). Four patients (44.4%) with alloimmunization were more than 3 years old at the time of first blood transfusion. The mean age at first blood transfusion in patients with alloimmunization and without alloimmunization was 2.8 (2.4) and 1.7 (2.0) years ($P=0.1$). The differential rate of splenectomy as a risk factor between patients with and without alloimmunization (11.1% and 8% respectively) was not statistically significant ($P=0.5$). Direct or indirect antiglobulin tests were positive in 5 (62.5%) patients. The blood alloantibodies by a panel of antibodies using standardized blood bank methods were detected in 4 patients, which were of anti-K and anti-D types.

**Conclusion:** The rate of red blood cell alloimmunization is relatively low in our patients. The age at first blood transfusion and splenectomy were not statistically significant as risk factors for alloimmunization in this study.

**Key Words:** Thalassemia; Alloimmunization; Splenectomy

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Introduction

β-major-thalassemia is a congenital hemolytic anemia caused by defects in β-globin chain synthesis. The globin chain that is produced in excess is responsible for the ineffective erythropoiesis and shortened red blood cell (RBC) survival. In the absence of stem cell transplantation the disease is treated by life long red cell transfusion to keep the hemoglobin level between 9–11.5 g/dL. Regular blood transfusions are essential to maintain growth and development during childhood and also to sustain good quality of life during adulthood[1-3].

Alloimmunization to erythrocyte antigens is one of the major complications of transfusions, particularly in patients who are chronically transfused. The factors for alloimmunization are complex and involve at least 3 main contributing elements: the RBC antigenic difference between the donor and the recipient, the recipient's immune status, and the immunomodulatory effect of the allogenic blood transfusions on the recipient's immune system. The majority of patients have a long-term exposure to non leukopoer and/or suboptimally leuko-reduced blood and a higher alloimmunization (and autoimmunization) rate than the patients exposed to leukodepleted blood. Splenectomized patients may have a higher alloimmunization rate. The absence of spleen may further enhance the immune response to the infused foreign antigens, which are not effectively filtered[3].

Alloimmunization is often a less significant problem in patients whose transfusion is initiated before the age of 3[4]. This type of sensitization results in difficulty obtaining compatible blood, transfusion reactions, hemolysis and occasionally life-threatening events[1-3]. 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[1-3]. The rate of alloimmunization to minor blood group antigens occurs in about 20-30% of patients[5]. Due to importance of these complications and their life threatening results, we evaluated the frequency, pattern and factors influencing red cell immunization secondary to multiple blood transfusions in patients of β-major-thalassemia in our thalassemia center in Mofid Children’s Hospital in Tehran, Iran, in March 2007.

Material & Methods

This is a cross-sectional descriptive study conducted in Mofid children’s hospital, March 2007. A total of 121 thalassemia patients receiving multiple blood transfusions were included in this study. Diagnosis of thalassemia was confirmed by standard hemoglobin electrophoresis and measurement of HbA, HbA2 and HbF. We analyzed the transfusion records and laboratory data concerning 121 regularly transfused thalassemic children including age, sex, date of first blood transfusion, history of splenectomy, Hb level, reticulocyte count, direct and indirect Coombs test, serum level of AST (aspartate aminotransferase), total and direct bilirubin, hemoglobinuria and type of alloantibody (by a standard panel test in central blood bank) to find out the frequency, pattern and factors influencing red blood cell alloimmunization secondary to multiple blood transfusions in patients of β-major-thalassemia.

All data were statistically analyzed using t-test, Logistic Regression test and Fisher’s exact test. A P-value of less than 0.05 was considered statistically significant.

Findings

A total of 121 patients were included in the study. 66 (54.5 %) patients were females and 55 (45.5%) males. Mean (standard deviation) age of patients was 13.0 (6.1) years and age ranged from 2 to 26 years.

They had regular blood transfusions during periods ranging from 1 to 25.5 years. The
interval between blood transfusions was 18-30 days. Red cell alloimmunization was found in 9 (7.4%) patients. Demographic data of alloimmunization patients have been shown in table 1. The range and mean (SD) age of patients with alloimmunization was 3.7 to 20 and 9.6 (6.5) years, respectively. Four (44.4%) patients with alloimmunization were more than 3 years old at the time of the first blood transfusion. In the female group, 5 out of 66 (7.6%) patients and in male group 4 out of 55 (7.3%) patients had alloimmunization. Six out of 9 (67%) patients had complete evidence of clinical and laboratory tests of alloimmunization including decreased Hb, increased reticulocyte count, indirect hyperbilirubinemia, increased level of serum AST, and hemoglobinuria, but 2 out of 9 (22%) patients showed only shortening of intervals between blood transfusions and positive indirect Coombs tests. Direct or indirect antiglobulin tests were positive in 5 out of 9 (62.5%) patients. Red cell alloantibodies were detected in 4 patients, 2 of which were anti-K and the other two anti-D.

The mean (SD) age at first blood transfusion in patients with alloimmunization was 2.8 (2.4) years and in patients without alloimmunization it was 1.7 (2.0) years. This difference was not statistically significant (P=0.1). Analysis of logistic regression test for age of first blood transfusion relative to alloimmunization gave a beta index of 0.02 and P of 0.1 showing that age at first blood transfusion is not a good predictor for alloimmunization.

Ten of 121 (8.26%) patients underwent splenectomy, only 1 of them had alloantibodies and she had developed the alloantibody before splenectomy. The comparison rate of splenectomy as a risk factor between patients with and without alloimmunization (11.1% and 8% respectively) was not statistically significant (P=0.5).

All patients with alloimmunization were treated with prednisone, intravenous immunoglobulin and cyclosporine A. Beside the above drugs, azathioprine was used for three of them.

**Discussion**

This is a study about red cell alloimmunization rate among β-major-thalassemia patients on regular blood transfusion. Post-storage leukodepleted blood was used for transfusion. It is not a standard practice in our center to give phenotype-matched red cells to patients with thalassemia. The use of phenotype-matched blood is given only in patients who

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**Table 1- Features of β-major-thalassemia patients with alloimmunization in Mofid Children's Hospital, Tehran, Iran**

<table>
<thead>
<tr>
<th>Case Number</th>
<th>Sex</th>
<th>Current age (years)</th>
<th>Age of the first blood transfusion (years)</th>
<th>Age of alloimmunization (years)</th>
<th>Splenectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>5</td>
<td>0.2</td>
<td>4</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>3.5</td>
<td>2.5</td>
<td>2.8</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>6</td>
<td>3.5</td>
<td>4</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>7</td>
<td>6</td>
<td>6.1</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
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</tr>
<tr>
<td>6</td>
<td>F</td>
<td>7</td>
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</tr>
<tr>
<td>7</td>
<td>M</td>
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<td>F</td>
<td>21</td>
<td>0.4</td>
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</tr>
</tbody>
</table>
have developed alloantibodies. Red blood cell alloimmunization was found in 9 (7.4%) patients. Our patients were treated with immunosuppressive drugs including prednisone, intravenous immunoglobulin, cyclosporine A and azathioprine and now all of them are in good condition.

In previous studies, the rate of alloimmunization varies from 4.97% to 37% in different countries[2-12]. In a study by Singer et al in 2001, the incidence of red cell alloimmunization among 64 Asian patients in the United states was 22%, splenectomy was found to be a risk factor (36% vs 12.8%) and 6 (25%) patients had positive Coombs test[3]. Ammen et al in 2003 reported 57 cases of alloimmunization in 190 (30%) patients with age ranging from 2 to 10 years.

The alloantibodies were Anti-Rh and Anti-Kell[4]. The red cell alloantibodies were detected in 4 of our patients, anti-K detected in 2 patients and anti-D in 2 patients. Unfortunately the type of antibody was unknown in the other 5 of our patients. Wang LY et al in 2006 reported 30 patients, 15 females and 15 males, with a mean age of 20 (4-31) years with alloimmunization in 11 (37%). Only 1 of these patients had a history of splenectomy which was performed after alloimmunization[5]. In a study in Hong Kong in 2001 splenectomy did not affect the incidence of alloimmunization[6]. Our experience does not substantiate the results of Singer et al, that patients who undergo splenectomy have a higher incidence of red cell alloimmunization[6]. Rather, our findings were similar to the study in Hong Kong indicating that splenectomy does not affect the incidence of alloimmunization.

In our study direct or indirect antiglobulin tests were positive in 5 (62.5%) patients with alloimmunization. Bhatti FA et al in 2004 reported 161 patients with 4.97% alloimmunization and positive direct Coombs test in 3 (187%) patients. The authors recommend only ABO and Rh crossmatching for transfusion because of low rate of alloimmunization and high cost of testing[2].

The role of leukodepletion in preventing red cell alloimmunization has been evaluated in several studies showing that patients receiving leukodepleted blood appear to have a lower rate of red cell alloimmunization, suggesting that it is the removal of leucocytes that reduces immune activation due to allogenic transfusion. However, various studies have suggested that apoptosis and loss of viability of residual white cells in leukodepleted blood that have been stored before being transfused may lead to the release of immunostimulatory white cell antigens and soluble biologic mediators resulting in sensitization of the recipients[5]. In our hospital packed cell transfusion is done using bedside filter since 1999, so only 3 out of 9 patients in our study had a history of blood transfusion without using filter. (Patients 7, 8, and 9 received blood transfusions before 1999).

The relationship between the number of units transfused and alloimmunization is unknown in thalassemia[1-5]. Red cell alloimmunization is reported to be more likely in patients who receive more units of blood. It has been thought that transfusion at an early age may offer some protection against red cell alloimmunization because of immune tolerance in young children[5]. So alloimmunization appears considerably lower in patients in whom blood transfusion started before the age of 3 than in those in whom it started after that age (20.9% vs 47.5%, P<0.0001)[5]. Regarding our study 4 (44%) patients with alloimmunization were more than 3 years old at the time of first blood transfusion vs 5 (55%) patients aged less than 3 years. Comparing these 2 groups, the age of first blood transfusion in our study was not found to be a risk factor for alloimmunization.

**Conclusion**

Red cell alloimmunization is an important finding in patients with β-major-thalassemia. Several factors might have contributed to this finding, such as the heterogeneity of the population, the difference in the number of studied patients, the differences in age at first
transfusion, antigenic differences between the blood donor and the recipient; the recipient's immune status; and the immunomodulatory effects of the allogenic blood transfusions on the recipient's immune system and splenectomy. With the growing knowledge of the immune effects of current blood transfusions and limited data on the immune status of thalassemia patients, a large study addressing the complex interaction of these factors is needed. Obtaining RBC antigenic phenotype on all thalassemia patients, providing leukodepleted blood, matched for antigens of the ABO-D and Kell systems in patients who have a life-long transfusion-dependency, could be so effective against red blood cell alloimmunization.

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**References**


