Antibody Screening in Patients With Thalassemia Major

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Background: The development of hemolytic alloantibodies and erythrocyte autoantibodies complicates transfusion therapy in thalassemia patients.

Methods: The frequency causes and prevention of this phenomenon in 90 transfused thalassemia patients were evaluated at Fatemeh Zahra Hospital in Bushehr in a cross-sectional study.

Results: In our study, the age of onset of symptoms ranged from 40 days to 12 years (1.72 ± 1.88 years). Hemoglobin (Hb) levels per transfusion in these patients were 8.40 ± 0.82%. Red cell alloantibodies were detected in 9 patients (10%). The red cell antibodies developed in this report were mainly Kell and C system. Our data showed that alloimmunization to minor erythrocyte antigens and erythrocyte autoimmunization of significant clinical variables are frequent findings in transfused thalassemia patients.

Conclusion: There is no relation between the number of blood units transfused and antibody formation in thalassemia, but it is an important factor for increased alloimmunization in these patients.

Keywords: antibody screening, thalassemia major, alloantibodies, autoantibodies

β-thalassemia major is a congenital hemolytic anemia caused by defects in β-globin chain synthesis.1 Beta thalassemia is considered to be the most common autosomal single-gene disorder worldwide. It can be found in more than 60 countries with a carrier (heterozygote) population of up to 150-200 million people or 4.5% of the world population, and at least 300,000 lethally affected homozygotes are born annually.2 Iran, with more than 18,000 affected individuals, represents 1 of the areas in the world with an unusually high prevalence of beta thalassemia. Provinces around the Persian Gulf and the Caspian Sea with a gene frequency of more than 10% constitute the thalassemia major zones in Iran.3 The excess globin chain produced is responsible for ineffective erythropoiesis and shortened RBC survival.4,5 The recommended treatment for β-thalassemia major is a regular blood transfusion every 3-4 weeks. This treatment aims to correct the anemia both by significantly suppressing the hyperactive erythropoiesis and by inhibiting the excessive gastro-intestinal iron absorption.6

Alloimmunization to erythrocyte antigens is 1 of the major complications of regular blood transfusions, particularly in patients who are chronically transfused. The factors for alloimmunization are complex and involve 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.7,8 The development of anti-RBC antibodies (alloantibodies or autoantibodies) can significantly complicate transfusion therapy. Some alloantibodies are hemolytic and may cause various hemolytic transfusion reactions and limit the availability of further safe transfusion. Others are clinically insignificant. Erythrocyte autoantibodies appear less frequently, but they can result in clinical hemolysis and in difficulty in cross-matching blood. Patients with autoantibodies may have a higher transfusion rate and often require immunosuppressive drugs, a splenectomy, or alternative treatments.8,9 Alloimmunization is often a less significant problem in patients whose transfusion is initiated before the age of 3.4 A centralized system of RBC alloantibody records is available, which provides a valuable opportunity to evaluate the frequency of alloimmunization and autoimmunization to RBC antigens in multi-transfused thalassemia major patients.10

Due to the importance of these complications and their life-threatening results, we conducted this study to evaluate the frequency of alloimmunization and erythrocyte autoimmunization, as well as patterns and factors influencing red cell immunization secondary to multiple blood transfusions in patients of β-thalassemia major in our thalassemia center in Fatemeh Zahra Hospital in Bushehr, Iran.

Materials and Methods

Patients

Clinical and transfusion records of 90 thalassemia patients who had received regular transfusions were analyzed.

Abbreviations

Hb, hemoglobin; SCD, sickle cell disease; DAT, direct antiglobulin test

Abstract

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The transfusion records of all patients were examined for age, sex, age of onset of symptoms, age at the first blood transfusion, time interval of transfusion, Hb levels per transfusion, exposure to leukodepleted blood, and the presence of alloimmunization or autoimmunization.

**Samples**

Blood samples were obtained for detection of anti-red cell antibodies. Blood was allowed to clot, and the serum was separated and stored in labeled test tubes at -20°C until the tests were performed in batches.

**Laboratory Investigation**

Red cell antibodies were detected using standard blood bank methods (saline, albumin, and Coomb’s phases) for detection of new antibodies to RBC antigens. In each case, serum was mixed with saline suspended red cells, and incubated for 1 hour at 37°C. Initially, a cell antigen pool was used for detection of unexpected antibodies. If the screen was positive, an extended (11-cell) panel was used to identify the antibody by antiglobulin testing.

**Results**

**Patient Characteristics**

All of the patients in this study had thalassemia major (mean age: 17.70 ± 8.20 years; range: 2-39 years). There were 39 (43.30%) males and 51 (56.70%) females.

All of them received leukodepleted blood for various time periods. The patients were managed by regular transfusions of packed cell/whole blood, considering ABO and Rh ‘D’ blood groups. No patient phenotypes other than these blood groups were known.

The frequency of transfusions in these patients ranged from 10 days to 15 weeks (21.88 ± 12.53 days). Age of onset of symptoms in our patients ranged from 40 days to 12 years (1.72 ± 1.88 years) and age at the first blood transfusion ranged from 40 days to 12 years (2.14 ± 2.40 years). Hemoglobin levels per transfusion in these patients were 8.40 ± 0.82%.

**Red Cell Alloantibodies**

In a total of 90 cases studied, red cell alloantibodies were detected in 9 (10%). Demographic data of alloimmunization patients in β-maj or were collected in Table 1 and Table 2. Out of those detected with alloantibodies, 2 (22.22%) were males and 7 (77.78%) were females. As shown in Table 3, the anti-K antibody was the most common, followed by the anti-C antibody. In 1 patient, antibody detection was ±, while in the others, the species of antibodies was not identified.

We found that there is no relation between the age at the first blood transfusion, time interval of transfusion, Hb levels per transfusion, and the presence of alloimmunization or autoimmunization (P>0.05).

**Discussion**

Immunologic complications of repeated RBC transfusions include RBC alloimmunization, difficulties obtaining compatible blood, development of autoantibodies, acute or delayed hemolytic transfusion reactions, and hemolytic disease of the newborn. These complications must be taken into account when planning transfusion schedules.

**Table 1_Demographic Data of Alloimmunization Patients in β-Thalassemia Major in Fatemeh Zahra Hospital in Bushehr, Iran**

<table>
<thead>
<tr>
<th>Case Number</th>
<th>Sex</th>
<th>Current Age (Years)</th>
<th>Age of the First Display Symptoms of Disease</th>
<th>Age of the First Blood Transfusion</th>
<th>Time Interval of Transfusion</th>
<th>Hb Levels Per Transfusion</th>
<th>Leukodepleted Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>24</td>
<td>Month 3th</td>
<td>Month 3th</td>
<td>Every 6-7 weeks</td>
<td>7%-8%</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>19</td>
<td>First year</td>
<td>First year</td>
<td>Every 3 weeks</td>
<td>7.5%-8%</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>16</td>
<td>Month 6th</td>
<td>Month 6th</td>
<td>Every 2 weeks</td>
<td>6%-7%</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>28</td>
<td>Fifth year</td>
<td>Fifth year</td>
<td>Every 3 weeks</td>
<td>8.5%-9%</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>18</td>
<td>Month 6th</td>
<td>Month 6th</td>
<td>Every 2-3 weeks</td>
<td>9%-10%</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>10</td>
<td>Third year</td>
<td>Third year</td>
<td>Every 21 weeks</td>
<td>8.5%-9.5%</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>18</td>
<td>Month 6th</td>
<td>Month 6th</td>
<td>Every 1-2 weeks</td>
<td>5%</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>18</td>
<td>Second year</td>
<td>Fifth year</td>
<td>Every 4 weeks</td>
<td>8%-8.5%</td>
<td>Yes</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>6</td>
<td>Month 6th</td>
<td>Month 6th</td>
<td>Every 2-3 weeks</td>
<td>7.5%-8%</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**Table 2_Summarization of Demographic Data of Alloimmunization Patients in β-Thalassemia Major in Fatemeh Zahra Hospital in Bushehr, Iran**

<table>
<thead>
<tr>
<th>Case Number</th>
<th>Current Age (Years)</th>
<th>Age of the First Display Symptoms of Disease (Years)</th>
<th>Age of the First Blood Transfusion (Years)</th>
<th>Time Interval of Transfusion (Days)</th>
<th>Hb Levels Per Transfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>17.25</td>
<td>1.53</td>
<td>1.92</td>
<td>21.12</td>
<td>7.78%</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>7.00</td>
<td>1.70</td>
<td>2.09</td>
<td>10.03</td>
<td>1.47%</td>
</tr>
<tr>
<td>Minimum</td>
<td>6.00</td>
<td>0.25</td>
<td>0.25</td>
<td>10.00</td>
<td>5%</td>
</tr>
<tr>
<td>Maximum</td>
<td>28.00</td>
<td>5.00</td>
<td>5.00</td>
<td>42.00</td>
<td>9.5%</td>
</tr>
</tbody>
</table>
consideration by blood banks with limited technical facilities and scarce donor resources when planning long-term transfusion support for transfusion-dependant patients.12

This study was conducted in order to demonstrate the frequency of red cell alloantibodies in patients with thalassemia major. We observed the presence of anti-red cell alloantibodies in 10% of our cases. This prevalence rate is in concurrence with a previous study,13 which reported a 9.2% frequency of red cell alloantibodies in patients with thalassemia.10 Patients suffering from beta thalassemia. Red cell alloantibodies developed in the previous study were mainly Rhesus and Kell systems,13 while our study revealed red cell alloantibodies mainly from Kell and C systems.

Hassan and colleagues reported a 22.7% prevalence of alloantibodies in 75 multi-transfused patients with thalassemia.14 In a study by Karimi and colleagues, blood sampling was performed for 711 β-thalassemia patients in Dastgheib Hospital in Shiraz, and autoantibodies and alloantibodies were observed among 1.7% and 5.3% of the patients, respectively.15 In another study, the overall frequency of alloantibody formation was found to be 22.06% in 68 multi-transfused patients (38 thalassemia and 30 sickle cell anemia). The most frequently detected alloantibodies were anti-K, anti-E, and anti-C.12 Moreira and colleagues reported an RBC alloimmunization rate of 12.9% in Brazilian sickle cell disease (SCD) patients and a calculated risk of alloimmunization per transfused RBC unit of 1.15%.16 Spanos and colleagues found a high frequency of red cell alloimmunization in 220 (22.6%) out of 973 thalassemic patients receiving blood matched for ABO and RhD antigen. Alloantibodies belonged to the following systems: Rhesus, Kell, MNS, Kidd, Bg, Lewis, Duffy, and P.4

In a study by Bashawri, 48 of 350 patients had developed alloantibodies (13.7%). The most common alloantibodies detected were anti-E alone (18.8%), nonspecific (12.5%), inconclusive (12.5%), anti-K (10.4%), and anti-c (6.3%).16 In 2004, Bhatti reported red cell immunization in 6.84% of 161 patients suffering from beta thalassemia. Red cell alloantibodies were detected in 4.97% patients and a direct antiglobulin test (DAT) was positive in patients (1.87%) with increased hemolysis.5 In a 2003 study, Ameen and colleagues reported that 57 (30%) patients developed RBC alloantibodies. The most common clinically significant alloantibodies were directed against in the Kell and Rh system. Red blood cell autoantibodies developed in 21 (11%) patients.10

Table 3. Distribution of Various Anti-Red Cell Antibodies in β-Thalassemia Major in Fatemeh Zahra Hospital in Bushehr, Iran

<table>
<thead>
<tr>
<th>Antibodies Detection</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>2</td>
<td>22.22</td>
</tr>
<tr>
<td>S, K, E</td>
<td>1</td>
<td>11.11</td>
</tr>
<tr>
<td>C, D</td>
<td>1</td>
<td>11.11</td>
</tr>
<tr>
<td>C, Lua</td>
<td>1</td>
<td>11.11</td>
</tr>
<tr>
<td>c, Jsa, Fyb, Lua</td>
<td>1</td>
<td>11.11</td>
</tr>
<tr>
<td>Cold Antibody</td>
<td>1</td>
<td>11.11</td>
</tr>
<tr>
<td>±</td>
<td>1</td>
<td>11.11</td>
</tr>
<tr>
<td>Unidentified alloantibody</td>
<td>1</td>
<td>11.11</td>
</tr>
</tbody>
</table>

There have been a number of studies focused on the Asian population. Singer and colleagues found that 14 (22%) of 64 patients (75% Asian) became alloimmunized. The most common alloantibodies detected were: c, S, K, and Fyb antigens, which are all potentially hemolytic antibodies.8 But another study conducted in Hong Kong among patients of Asian descent by HOR-Kung Ho and colleagues showed a total of 9 in 68 (7.4%) patients developed alloantibodies. The red cell alloantibodies found were anti-E, anti-M, anti-HLA, anti-BG, and anti-BW.17 In the study by Wang and colleagues, 11 (37%) of the 30 patients tested had alloantibodies, and anti-Mia (3 cases) and anti-E (7 cases) were the most common alloantibodies found in hospitalized patients in Taiwan.18 Chang and colleagues reported the frequency of red cell alloantibodies in a general hospital population to be 2.7%.19

Ansari and colleagues studied alloimmunization and erythrocyte autoimmunization among 80 transfusion-dependent thalassemia patients. Alloimmunization was found in 3 cases and belonged mainly to the Rh system, and red cell autoimmunization was 18.8% (15 cases).20 Sadeghian and colleagues reported that the frequency of alloimmunization in 313 thalassemia patients in northeastern Iran was 2.87%. All of these antibodies were against the Rh system (anti-D, anti-C, anti-E).21

Similarly, in the study by Santos and colleagues, among 5690 patients tested, 120 (2.1%) had alloantibodies.22 Cunningham and colleagues reported that in 502 thalassemia patients, alloantibodies and autoantibodies were detected in 104 (21%) and 46 (9.2%) subjects, respectively. Presence of both were reported in 26 (5.2%) individuals.23

The relation between the number of blood units transfused and antibody formation is unknown in thalassemia, but it is an important factor for increased alloimmunization in patients who receive multiple transfusions.20 Patients may have serologic findings of clinically significant alloantibodies without evidence of a hemolytic transfusion reaction.24

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