

Red Cell Alloimmunization in Thalassaemia Patients

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Objectives: To determine the frequency of red cell alloantibodies in a thalassaemia patient and to identify the common alloantibodies.

Methods: A retrospective review of blood bank records for all thalassaemia patients. The records included, request received for blood group typing, antibody screen, antibody identification and cross-matching during the period between October 1st, 1997 to September 30, 2002. The history of blood transfusion was reviewed and the frequency rate for alloantibodies was determined.

Results: Out of 76 thalassaemic patients, nine (11.8%) had developed alloantibodies. Alloantibodies detected include: nonspecific antibodies 3 (33.3%), anti E and nonspecific 2 (22.2%), anti-K together with non specific antibodies 1 (11.1%), anti-E 1 (11.1%), anti E together with anti K and nonspecific alloantibodies 1 (11.1%), anti-Le^a 1 (11.1%).

Conclusion: Red cell alloantibodies developed in 11.8% of thalassaemic patients. The most common alloantibodies were Rhesus and Kell antibodies, which are present in 33.3% and 22.2% of these patients respectively. Alloimmunization is not an uncommon problem facing blood banks and finding compatible units for regularly transfused thalassaemic patients may be very difficult. In order to reduce alloimmunization a policy for performing extended red cell phenotyping on these

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patients is essential and at least antigen Kell and E negative blood should be provided for transfusion to these patients.

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The mainstay of therapy for severe beta-thalassaemia syndromes is periodic blood transfusions. They are generally given to correct the anemia, prevent ineffective erythropoiesis and improve growth and development. Alloimmunization to red cell antigens is an immune response often stimulated by the transfusion of blood products and by fetal maternal bleeds¹. It is one of the important complications of blood transfusion. Several studies demonstrated various frequencies and percentages of alloantibody development. The frequency of alloimmunization to red cell antigens in thalassaemia patients has varied from 5-10% to 19%-25% and 30%¹⁻⁵. The different reports associated the frequencies of sensitization (alloimmunization) to age, number of transfusions and the age when transfusions started. It was shown that the frequency of alloimmunization was significantly lower in patients less than 5 years when blood transfusion started⁶. This was attributed to a form of immune tolerance, and in general alloimmunization increased with the number of blood transfusions¹⁻⁴. Experience also showed it is more likely when transfusion is intermittent and begun beyond early childhood.

The most common alloantibodies identified in most of the reports were those of the Rhesus (Rh) and Kell systems³⁻⁵. Some studies identified alloantibodies of the Kidd and Duffy systems in addition to the Rh and Kell systems^{1,2,6}. Sensitization to red cell antigens; however, is not confined only to thalassaemia and sickle cell anemia patients, it occurs in the general transfused population with a frequency of 1-3%, and in those multitransfused due to different clinical disorders, the frequencies were found to be 11.8%, 6%, 2.9% and 9%⁽⁷⁻¹³⁾. Transfusion dependent thalassaemia patients may also develop red blood cell autoantibodies, in addition to red blood cell alloantibodies^{5,14,15}. Although autoantibodies develop less frequently than alloantibodies, they may cause clinical hemolysis, difficulty in cross-matching blood and finding compatible blood^{14,15}.

The main objectives of this study were to assess the frequency of alloimmunization in thalassaemia patients and to identify the common alloantibodies.

METHODS

All thalassaemic major patients managed in KFHU were checked against blood bank transfusion records. A total of 76 thalassaemic patients records were reviewed.

Laboratory Investigations

After ABO and Rh blood grouping by the standard tube method, the following were done:

Antibody screening

Prior to every transfusion, sera are tested for the presence of alloantibodies using two-cell panel with a homozygous expression of the antigens (Ortho-clinical Diagnostics,

Inc, U.S.A. and Dia Med, ID-Micro Typing System, Morat Switzerland). Any pretransfusion sera with a positive antibody screen were subjected to antibody identification.

Antibody Identification

Antibody specificity was performed by using a commercial RBC panel with known antigens (Resolve Panel A and B, Ortho Clinical Diagnostics Inc., USA, and Dia Med – ID-Dia Panel P) against patient’s serum.

Direct Antiglobulin Test (DAT) was performed using 3-5% of patients RBCs and appropriate controls. A polyspecific antiglobulin reagent is used. The results were read macroscopically and microscopically, all negative results were confirmed by adding control cells.

Crossmatch

A crossmatch is performed prior to transfusion, after the selection of the appropriate blood from results of blood group, antibody screening and identification. First a saline procedure that is performed at room temperature then at 37⁰C, followed by an antiglobulin test using albumin. Blood lacking the antigen for the corresponding alloantibody identified is chosen and blood is transfused if crossmatch is negative.

RESULTS

During the study period total of 76 thalassemic patients were reviewed. Nine of them (11.8%) were found to have alloantibodies. Six were Saudis (66.7%) in whom 4 were females and 2 males and three were non-Saudis (1 Pakistani, 1 Palestinian, 1 Sudanese) (33.3%), (1 male and 2 females). The mean age was 18.44 ± 8.02 years, the oldest 28 years and the youngest 2 years. The alloantibodies detected in rank order include: non-specific antibodies 3 (33.3%), anti-E together with non specific antibodies 2 (22.2%), anti-K with non specific antibodies 1 (11.1%), anti E 1(11.1%), anti-E together with anti-K and nonspecific 1 (11.1%), anti Le^a 1 (11.1%) (Table 1).

Table 1. Alloantibodies detected in thalassemia patients studied

Alloantibody specificity	Number of patients	Percentage %
Non Specific (NS)	3	33.3
Anti E & NS	2	22.2
Anti E	1	11.1
Anti K & NS	1	11.1
Anti Le ^a	1	11.1
Anti E & Anti K& NS	1	11.1
Total	9	100

Twenty-one of the thalassaemia patients (27.6%) had a persistent positive direct antiglobulin test (DAT). Two of the 9 patients (22.2%) with alloantibodies were among the 21 patients with a positive DAT.

DISCUSSION

Hemoglobinopathies and thalassemia are common hematological disorders in the eastern region of Saudi Arabia^{16,17}. The frequency of alloimmunization in our study of (11.8%) is relatively low and is similar to many previous studies. Some international reports showed different frequencies, 22.6% in a study by Spanos et al, 11% in a study by Coles et al, Sirchia et al reported 5.2%, and Michail Merianou reported a frequency rate of 23.43%¹⁻⁴. Ameen in a study in Kuwait showed a higher frequency (30%)⁵. In general the frequency ranges between 5-30%.

Our data concur with results of many studies that the overall prevalence of red cell alloimmunization is lower in thalassemia major patients, than in patients with other diseases. This low prevalence has led to performing extended red cell antigen phenotyping on all thalassemia major patients. The specificity of RBC alloantibodies detected were against the Kell, Rh blood group systems which is also similar to all the previous studies. It is known that these blood group systems are strong immunogens, and because most of the different studies have had alloantibody specificity to the Kell and Rh systems, it was advocated by many that thalassemia patients be transfused with blood matched at least for Rh and Kell system antigens, and that would lead to a much lower rate of alloimmunization, but the potential to form RBC alloantibodies to unmatched antigens still exists.

Twenty-one thalassemia patients studied had a positive direct antiglobulin test (DAT) without evidence of autoimmune hemolytic anemia. The phenomenon of development of autoimmunization as a result of frequent blood transfusions and alloantibody development has been known for many years but has received little attention^{5,18,19}. Apparently the positive DAT didn't cause any problems in finding compatible blood. Fortunately, we always found compatible blood for these patients. However, the possibility of "missed" delayed hemolytic transfusion reactions cannot be excluded, as they may have been masked by the features of their underlying disease i.e. anemia and jaundice.

To prevent alloimmunization to RBC antigens there has been recommendations to develop programs that provide antigen-matched RBC transfusions to all thalassemia and sickle cell disease patients. It is obvious that providing antigen matched blood will effectively prevent alloimmunization. However, the cost effectiveness of establishment of these programs for chronically transfused patients has been a debatable and controversial. It is difficult also to establish and maintain a donor pool for each patient. Moreover, the costs involved in storing the collected units i.e. cryopreservation may be needed. The complications of alloimmunization that may occur are many; some antibodies may become non-detectable over time endangering future transfusions and placing the patient at risk for anamnestic antibody production, which may lead to delayed hemolytic transfusion reactions. They may even present with a delayed transfusion reaction that may go unrecognized and/or be masked by features of their underlying disease.

Also some patients may present as emergency and may have multiple alloantibodies, making difficult to find compatible blood, and the difficulty increases when these patients present to some hospitals for the first time with no previous records.

Routine extended red cell phenotyping be done for all chronically transfused thalassemia and sickle cell disease patients before starting RBC transfusions. A nationwide standard transfusion policy is needed as well as a national registry for these patients, because many of the patients are managed at different hospitals.

Leucocyte poor blood be provided for these patients, as a number of studies have shown the role of leucodepletion in preventing alloimmunization and autoimmunization^{20,21}. Storage of RBCs at 1 to 6⁰C will induce apoptosis in white blood cells (WBCs), this will lead to release of potentially immunostimulatory antigens and soluble biologic mediators from dying cells, and these will sensitize the immune system of transfusion recipients and therefore, lead to development of autoimmune disease. Also another study demonstrated that erythrocyte alloimmunization is not absolutely necessary for autoantibody formation, despite the strong association, as 19 out of 76 of our patients (25%) developed autoantibodies without alloantibodies. It is possible therefore, that certain persons are genetic responders with an increased tendency to develop erythrocyte antibodies. Another alternative explanation is that repeated destruction of erythrocyte membrane and abnormal interaction with the endothelium may lead to exposure of erythrocyte neoantigens that induce IgG autoantibody formation²².

CONCLUSION

The above recommendations must be taken into consideration to prevent alloimmunization, autoimmunization and the complications that can occur in these chronically transfused patients. We conclude that the issue of alloimmunization in thalassaemia patients is quite important in both clinical and laboratory practice.

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