

Red Cell Alloimmunization in Repeatedly Transfused Thalassemia Major Patients

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Thalassemia major patients managed by regular transfusion regimen may develop anti-red cell alloimmunization. If the alloantibodies are hemolyzing in nature, transfusion reaction may occur, and provision of blood thereafter requires matching of the relevant blood group in addition to 'ABO' and Rh 'D' matching. We investigated 75 cases of multiply transfused thalassemia major patients for development of alloantibodies against red cells by indirect antiglobulin test, using 3-red cell panel, and when required 11-red cell panel. Anti-red cell alloantibodies were detected in 17 (22.7%) patients. Anti-Kp^a antibodies were the commonest, followed by Anti-e, anti-E and anti-K antibodies, respectively. Anti-k, -C^W, -Fy^b, -Kp^b, -Rh 'D' and -c were detected in one patient each. It is concluded that in multiply transfused patients, alloantibodies develop in a significant number of patients. The hemolyzing nature of antibodies should be determined in patients who develop these antibodies, and transfusion should be arranged accordingly

Key words: Red Cell Alloimmunization; Alloantibodies; Thalassemia Major; Multiple Transfusions.

Introduction

The recommended treatment for β -thalassemia major is regular blood transfusion every 3-4 weeks, with a goal to correct the anemia to significantly suppress the hyperactive erythropoiesis, and to inhibit the excessive gastro-intestinal iron absorption.¹ The regular blood transfusion regimen is confronted with numerous complications. In almost every patient, the transfusion requirement slowly increases over the years. Various factors which contribute towards this increased requirement include: Development of hypersplenism; Alloimmunization against various blood group antigens; Chronic infections; Folate deficiency (if not continually corrected by regular life time folate intake); Progressive bone marrow fibrosis as a result of toxic effect of free elemental iron; Aplastic crises and Hemolytic crises, etc.

The distribution of various blood groups antigens varies amongst individuals in any given population. Therefore, there is bound to occur a variable degree of disparity amongst the donors and the recipient, as regards the group systems other than 'ABO' and Rh 'D', which are not tested for before routine transfusions. As a result, at some stage during the transfusion management, this disparity of blood

group systems can lead to alloimmunization, and therefore elaboration of antibodies against the immunogenic antigen system(s).

As regards the risk of allosensitization to clinically important blood group antigens, all childrens' ABO, Rhesus, Kell, Kidd and Duffy systems, etc. should be typed at the outset. In every transfusion, thereafter, a matching with donor's blood should be ascertained in at least the ABO, Rhesus and Kell systems.¹ It is important to understand that, with an extended phenotyping of patient's red cells, it is possible to determine which alloantibodies could develop as a result of previous transfusions. For example, if a patient is JK^a, it is unlikely to develop anti-JK^a antibodies.²

In Pakistan, an elaborate data on various blood group systems, particularly the groups other than ABO and Rh 'D' (e.g., Kell, Duffy, Kidd, MNS, Lewis, etc.) is lacking. Therefore the prevalence rates of these groups and any statistical differences amongst various racial groups are not known. A wide discrepancy in the distribution of certain blood group antigens between donor and recipient can be responsible for a higher risk of alloimmunization in multiply transfused patients.³ However, many of the antigens present on the erythrocytes infrequently give

rise to alloimmunization even when injected into patients lacking the antigen; the rate of immunization ranges from 70% for Rh 'D' antigen to as low as 0.5% for the Duffy antigens.⁴ It is also interesting to observe that the ability to react to alloantigens varies greatly from person to person. Some individuals will not become immunized to any antigen despite repeated transfusion, whereas others will become immunized, when transfused, to many of the antigens that they lack.⁴

The present study was carried out to determine the seroprevalence of anti-red cell alloantibodies in multiply transfused thalassemia major patient in our setup.

Patients and Methods

A total of 75 patients of Thalassemia Major who had already received multiple transfusions (>10 times) were randomly selected. Forty of these patients were registered for transfusion management at Children Hospital, Pakistan Institute of Medical Sciences, and the remaining 35 at thalassemia management centre of Holy Family Hospital, Rawalpindi. A verbal consent was taken in all the cases. The study was carried out from October 2003 to December 2003.

Clinical data was entered in a proforma, with special reference to: age; age at diagnosis; frequency of transfusions; present clinical status; and any increase in the transfusion requirement, etc.

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

Red cell antibodies were detected using standard blood bank methods (saline, albumin and coomb phases). In each case, serum was mixed with saline suspended red cells, and incubated for one hour at 37°C. Initially, a 3-cell antigen panel was used. If the screen was positive, an extended (11-cell) panel was used to identify the antibody by antiglobulin test.

Results

Patient Characteristics

In a total of 75 cases of thalassemia major studied in this series, 48 were males and 27 females,

with a male: female ratio of 1.77:1.

The age at the time of study ranged from 2 yrs 5m to 21 yrs with a mean age of 6.5 yrs. Age at the time of diagnosis ranged between 6m and 2 ½ yrs, with a mean age of 1 year and 4 months.

Hepatomegaly was observed in all the patients, whereas an enlarged spleen was noted in 73 cases (97%); in two patients, there was a history of splenectomy for hypersplenism.

These patients were poorly iron-chelated (Ferritin level: 669-6325 ug/l with mean \pm SD: 3390 \pm 135.6 ug/l) due to various reasons.

The patients were being managed by regular transfusion of packed cell/whole blood keeping in consideration only ABO and Rh 'D' blood groups. In none of the patients phenotypes other than these blood groups were known. Further, it is not a practice in any thalassemia management center in Pakistan, at present, to give a phenotypically matched blood for Cc, Ee, Kell, Duffy, Kidd or any other minor group antigens to patients on regular transfusion regimens.

All the patients were being transfused a non-leukodepleted blood.

The frequency of transfusions in these patients ranged from 7 days to 5 wks. In 54 cases (72%), there was an evidence of increase in the transfusion requirement during the past few weeks to months.

Red Cell Alloantibodies

In a total of 75 cases studied, red cell alloantibodies were detected in 17 (22.7%). As shown in table 1, anti-Kp^a antibodies were the commonest, followed by anti-e, anti-E, anti-K antibodies. Anti-k, -C^w, -Fy^b, -Kp^b, -Rh 'D' and -c antibodies were detected in one patient each.

Table 1: Distribution of Various Anti-Red Cell Antibodies in 75 Multiply Transfused Thalassemia Major Patients

Antibodies Detected	No of Patients	Percentage
Anti-E	2	11.8
Anti-e	3	17.6
Anti-Kp ^a	4	23.5

Anti-K	2	11.8
Anti-k, -C ^w , -Fy ^b , -Kp ^b , -, -Rh 'D', -c	1 each	5.9 each

Discussion

The development of red cell antibodies (allo as well as auto-antibodies) occurs in a variable number of multiply transfused thalassemia major patients. In majority of them, these antibodies are non-hemolytic; however, in others, they may be hemolytic. In such circumstances, transfusion therapy may become significantly complicated, as the patient may develop transfusion reaction. If red cell antibodies are autoantibodies, immuno-suppression may have to be given.⁵

We observed the presence of anti-red cell alloantibodies in 22.7% of our cases. This prevalence rate is in concurrence with a previous study⁶ (p>0.05), which reported a 20% prevalence of alloantibodies in 64 multiply transfused Asian patients of thalassemia and thalassemia-HbE, who had received transfusion predominantly from white donors. However, the prevalence of these antibodies was far less in white thalassemics who had received transfusions from white donors. Another previous study of 161 thalassemia major patients has shown a drastically lesser prevalence (6.8%) of anti-red cell antibodies in multiply transfused thalassemia major patients in our setup⁷ (p<0.001).

β-Thalassemia is prevalent in Pakistan.^{8, 9} The patients are unfortunately poorly managed, and develop all types of complications.¹⁰ Data regarding development of anti-red cell antibodies is scanty. Likewise statistics relating to Rh (CcDdEe) and minor group antigens (Kell, Kidd, Duffy, Lewis, MNS, Luthern, Ii, P, etc.) on mass scale is not available. In our cases, as well, information relating to blood groups other than ABO and Rh 'D' groups was lacking, mainly for want of funds and lack of facilities available in the relevant centers.

In our patients, the pre-transfusion donor vs. recipient matching was targeted only for ABO and Rh 'D' antigens. It has been documented that if blood were matched only for ABO and Rh 'D' groups, a high rate of allo-immunization would be expected.¹¹ On the other hand, a low rate of allo-immunization (5-10%) would be expected if there is less heterogeneity of red cell antigens between donors and recipients, as has been reported in studies from Italy and Greece.^{12, 13} It is also noteworthy that, in Pakistan, very few centers offer the facility of anti-red cell antibody detection.

Therefore, even if suspected, the presence of these antibodies is not verified in most of the cases. In our setup, the donors are from every walk of life, including a diverse racial and ethnic background. Therefore it is expected that various groups of donors may show a disparity in their minor blood group systems. In our cases, a detailed phenotype of red cells pertaining to minor groups was never performed, and this is an area, which needs to be explored on priority basis.

Apart from disparity between blood group antigens in the donors and recipients, some other factors are also worth mentioning:

1. A vulnerable immune status of the recipient may predispose to altered or increased immune response to various antigens. This aspect of immune status has never been explored in our patients, and requires to be looked into.
2. Allogeneic blood transfusion may have an immuno-modulatory effect on recipient's immune system, e.g., the transfused white cells may have an immuno-suppressive effect on the recipient's immune system. CD4:CD8 ratio deviates from normal in patients who get multiply transfused with non-leuko-depleted or leuko-reduced blood with a greater propensity to develop allo- and autoantibodies.^{14, 15} It is possible that in our patients who, by and large, get transfused with non-leuko-depleted blood, the immuno-modulatory effect of transfused blood may be significantly contributing to the problem of red cell antibody development. Further, the frequency of hemolytic vs. non-hemolytic alloantibodies remains to be evaluated.

The number of Thalassemia major patients in Pakistan is on the increase, and if the prevalent transfusion practices are continued, the problem of alloimmunization will significantly increase.

It has been observed that patients' age at the start of transfusion management may have a bearing on the frequency of development of anti-red cell alloantibodies, as an earlier start of transfusions may impart immune tolerance in some patients.¹¹ Further, the total number of transfusions is also expected to have an effect on frequency of alloimmunization.⁴

The prevention of red cell alloimmunization is difficult. However, transfusion of packed red cells after matching for all major antigens associated with clinically significant antibody production can to a great extent ameliorate the problem. If the antibodies have already developed, the minimum requirement is

transfusion of blood matched taking into account at least the antibodies, which have already developed. This exercise is however very laborious and expensive, particularly because the majority of alloantibodies and autoantibodies are non-hemolytic. Therefore, the recommended preventive measures would be beneficial for only a few patients who would develop these problems.

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