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ORIGINAL ARTICLE

Detection and Identification of Red Cell Alloantibodies in Multiply Transfused Thalassemia Major Patients: A Prospective Study

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Abstract Life long red blood transfusion remains the main treatment for β thalassemia major patients. The development of alloantibodies complicates transfusion therapy in thalassemia patients. Alloimmunization to red cell antigens is one of the most important immunological transfusion reaction and causes delayed type of transfusion reaction. A prospective study was conducted from January 2007 to January 2010. This was a cohorts of 115 patients were selected from regular transfusion group and they were followed for two and half year. They were followed up for the effect of transfusion during study period. There was a decline in patient number from 115 to 96 due to mortality and transfer of patient. A total of 96 multiply transfused thalassemia patients were prospectively included in this study and three consecutive samples collected after every 6 months and investigated for the development of alloantibody to red cell antigens. Tests for antibody screening and identification were performed on preserved sample to investigate prevalence and development of red cell alloimmunization by standardized laboratory techniques by same person at Prathama Blood Centre. A total of 96 patients were included in the study. 63 patients were males and 33 females. A total of five single alloantibodies were formed in five patients out of them four (80 %) belonged to Kell blood group system and one (20 %) from Rh system. It was observed that two (1.92 %) of new thalassemia patients developed red cell alloantibodies during study period. Red cell alloimmunization should be kept in mind in the patients receiving multiple transfusions. In present study, alloimmunization rate was 5.21 %. Mean transfusion duration in these patients was 23.90 days, probably due to presence of alloantibody. RBC alloantibody detection on regular interval and corresponding antigen negative blood transfusion is strongly recommended in transfusion dependent thalassemia patients.

Keywords Red cell alloimmunization · Thalassemia major

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Introduction

Thalassemia also known as "Cooley's anemia" is an inherited disease of the red blood cells classified as a hemoglobinopathy. Appropriate and regular red cell transfusion remains the main treatment choice for a large number of patients with thalassemia major. The natural course of the disease is dramatically altered by transfusion side effects, which need to be monitored and treated throughout life. These patients who are maintained on hyper transfusion regimen can develop various complications due to multiple transfusions, one of them being allosensitization to red cell antigens. As blood is routinely matched with respect to major blood group antigens i.e.

ABO and Rh D antigen, there is a high probability that the donor will have minor blood group antigens not present in the recipients which will result in alloimmunization [1].

Life long red blood cell (RBC) transfusion remains the main treatment for severe thalassemia. Transfusion therapy could be complicated with the development of anti RBC antibodies (alloantibodies and/or autoantibodies). Some alloantibodies are hemolytic and may cause hemolytic transfusion reactions and limit the availability of further safe transfusion [2]. The most frequent detected antibodies are directed against Rhesus (Rh), Kell (K), Duffy (Fy), Kidd (Jk) system antigens, in order of frequency [3]. The reported incidence of alloimmunization in multiply transfused thalassemics patients was 3–30 % [1–18].

Principally the focus is on red cell, because present study is on red cell alloimmunization. The risk of alloimmunization depends on recipient exposure to foreign antigen and its immunogenicity. Human red cell determinants vary considerably in their ability to act as antigen. Few of them are strongly antigenic, as measured by the frequency with which individual exposed to them respond by producing antibodies. Alloimmunization to red cell antigens is one of the most important immunologic transfusion reaction and causes delayed type transfusion reaction. Alloimmunization significantly concerns the Rh, K, Fy and Jk system which are clinically significant. They can cause, not invariably hemolytic transfusion reactions and limit the ability of safer transfusion while, others are clinically insignificant [19].

Management part of red cell alloimmunization cases includes detection, identification and finally providing antigen negative blood for further transfusion. It produces exceedingly difficult situation while selecting a compatible blood unit for patient who has developed alloantibodies against "high frequency" antigens (public antigens). Members of the patient's family, especially siblings, are usually the most promising sources of potential donor. It is often very helpful to know the ethnic group of the patients with antibodies to a high incidence antigen, because the chances of finding a compatible donor may be greatly enhanced if search efforts are targeted. It produces less difficult situation in selection of compatible blood unit if alloimmunization is against "low frequency" antigens (private antigens).

This study was designed to determine the rate of red cell alloimmunization, development of red cell alloimmunization and prevalence of in multiply transfused thalassemia patients during the study period.

Materials and Methods

This prospective study was cohorts of 115 patients were selected from regular transfusion group and they were followed for two and half year. They were followed up for the effect of transfusion during study period. There was a decline in patient number from 115 to 96 due to mortality and transfer of patient. A total of 96 multiply transfused thalassemia patients were prospectively included in this study and three consecutive samples collected after every 6 months and investigated for the development of alloantibody to red cell antigens. Five to seven ml of blood was collected in plain tube and serum was separated. Separated serum was taken in two aliquots, labeled properly & stored in two different boxes at −30 °C in deep freezer, till the antibody screening and identification performed. Tests for antibody screening and identification were performed on preserved sample to investigate prevalence and development of red cell alloimmunization by standardized laboratory techniques by same person at Prathama Blood Centre. Antibody screening was carried out on serum employing commercial three-cell panel using standardized blood bank techniques. If patients were found to have an irregular red cell alloantibody then the antibody identification was performed using commercial 11 cell panel cells (Diamed). The thalassemia patients were received regular transfusion from Prathama Blood Centre Ahmedabad, Jeevandeep hospital Ahmedabad and Red Cross Blood Centre Ahmedabad were included in this study and investigated for the development of alloantibodies.

Statistical Analysis

While evaluating the data whether it is significant or not, different types of statistical tests were applied according to requirement. The data were evaluated by applying the '*Z*' test and '*t*' test. If the '*p*' value was less than 0.05 then it was assumed that the comparison was significant. If the '*p*' value was less than 0.01, the comparison was considered as highly significant.

Results

The present study of detection and identification of clinically significant red cell alloantibodies in multiply transfused thalassemia patients was carried over a period of 2 years from January 2007 to January 2009.

Repetition of transfusions for the treatment of thalassemia major provokes the patient's immune system and produces anti-erythrocyte antibodies (alloantibodies and/or autoantibodies). A total of five alloantibodies were formed in five patients which were IAT positive and three of them were DAT positive in the present study.

Erythrocyte autoantibodies appear less frequently, but they can result in clinical hemolysis and in difficulty in cross-matching blood. Alloimmunization against red blood

Table 1 Incidence of red cell alloantibodies in three consecutive set of samples of multiply transfused thalassemia patients

S. no.	Set of sample (no. of patients)	Antibody detected in patients	Percentage (%)
1.	First set (S-1 = 96)	3	3.13
2.	Second set (S-2 = 96)	5	5.21
3.	Third set (S-3 = 96)	5	5.21

cell antigens increases the need for transfusion and can be significantly complicated transfusion therapy. Some alloantibodies are hemolytic and may cause hemolytic transfusion reactions and limit the availability of further safe transfusion. Others are clinically insignificant.

Development of alloantibody during the study period was carried out using commercially prepared reagent screening cell panels. Once alloantibody was detected by screening cell panels identification panel were used to identify alloantibody.

Red Cell Alloantibody Detection and Identification

Incidence

Serum of all thalassemia patients was first screened for red cell alloantibody using commercially prepared three-cell panel. The samples giving positive reaction on screening were further tested with 11-cell identification red cell panel obtained commercially from M/S Diamed, Switzerland. In both screening and identification, procedure IAT tube technique was used. Table 1 shows the detection of red cell alloantibodies in three consecutive set of samples in 96 multiply transfused thalassemia patients. Table 2 shows the details of such alloimmunized patients with specificity of alloantibodies.

The overall incidence of red cell alloimmunization in multiply transfused thalassemia patients was found to be 5.21 %. The incidence of red cell alloantibodies in three consecutively collected set of samples of multiply transfused thalassemia patients were 3.13, 5.21 and 5.21 % respectively (Table 1).

In the first set of sample of 96 patients only three patients were showed presence of alloantibody (incidence 3.13 %). In the second and third set of sample (96 patients), five patients were positive for alloantibody (incidence 5.21 %). It was observed that 1.92 % of new thalassemia patients developed red cell alloantibodies during study period (Table 1).

Specificity

Serum samples those were positive on screening were tested with 11-cell identification panel obtained from M/S DiaMed. Table 2 shows the results. The number of transfusion received by these red cell alloimmunized patients ranged from 50 units to about 196 units. Majority of the red cell alloantibodies belonged to K blood group system followed by the Rh blood group system. Total five samples showed presence of alloantibody, four (80 %) belonged to K blood group system and one (20 %) from Rh system (Table 2).

Red Cell Alloimmunization According to the Gender of the Patient

The gender of the patient might influence the rate of red cell alloimmunization. The association of alloantibody development and gender of thalassemia patients was studied. In the present study, the rate of red cell alloimmunization in females was found higher (9.10 %) than males (3.17 %) and the difference was significant. In present study the overall incidence of red cell alloimmunization in multiply transfused thalassemia patients was found to be 5.21 % (Table 3).

Proportion of Single and Mixed Red Cell Alloantibodies

Red cell alloantibody development may be single or multiple in thalassemia patients. A total of five alloantibodies were formed in five patients which were IAT positive and three of them were DAT positive in the present study. In the study group patients it was single alloantibody in all patients.

Table 2 Development of red cell alloantibodies with specificity in three consecutive samples of multiply transfused thalassemia patients

Pts.	Tx no. at 1st sample	Antibody specificity	Tx no. at 2nd sample	Antibody specificity	Tx no. at 3rd sample	Antibody specificity
RK/M/4	38	No	44	Yes (−K)	50	Yes (−K)
SH/M/9	88	No	94	Yes (−K)	102	Yes (−K)
BK/F/12	132	Yes (−E)	138	Yes (−E)	144	Yes (−E)
PG/F/13	149	Yes (−K)	155	Yes (−K)	160	Yes (−K)
RR/F/18	172	Yes (Kp^a)	184	Yes (Kp^a)	196	Yes (Kp^a)

Table 3 Distribution of patients with alloantibodies according to the gender

Gender	Number	Alloantibody positive	% of positivity
Male	63	2	3.17
Female	33	3	9.10
Total	96	5	5.21

Red Cell Alloantibody and Blood Group Antigen System

In all blood centers, the thalassemia patients had received blood transfusion matched only for ABO & Rh (D). It was important to know what types of alloantibodies were common in multiply transfused thalassemia patients so as to establish cross-matching strategies. A total of five alloantibodies were formed in five patients in the present study. It was further investigated that which were the common blood group systems against which red cells alloantibodies were encountered in the present study. Majority (80 %) of red cell alloantibodies belonged to the K system and 20 % were directed against Rh blood system. No other clinically significant red cell alloantibodies were detected in the present study, such as belonging to the blood group system Duffy, Kidd or MNS.

In K blood group system, alloantibodies against K and Kpa antigen constituted the majority (80 %) followed by antibodies against E antigen in 20 % of cases. In K blood group system, anti-K alloantibodies in 60 % of cases and anti-Kpa alloantibody in 20 % of cases were seen in the present study. In Rh blood group system, alloantibodies against E antigen were encountered in 20 % of cases in present study (Table 4).

Discussion

The development of red cell alloantibodies is a known and common complication of chronic transfusion therapy. Various centers all around the world have reported different frequencies of alloimmunization. The frequency of red cell alloimmunization ranging from 3.1 to 30 % [1–18]. The some of these reports have a high rate of alloimmunization [2, 6, 8, 15] but majority of centers have reported low rate of alloimmunization [3, 12, 13, 16–18, 20, 21]. Most of this published data have similar frequency of red cell alloimmunization in present study.

Table 4 Distribution of alloantibodies according to the blood group system

Blood group system	Antibody (n)	%
Rh	1	20
Kell	4	80
Total	5	100

The thalassemia patients are transfused with ABO and Rh (D) matched packed red blood cells. Although, all patients are phenotyped for minor red cell antigens before transfusion regimen is planned to form a base line, the extended phenotype matched blood is not provided. Out of 96 thalassemics studied, the red cell alloantibodies detected in five patients (5.21 %) using commercially prepared reagent screening cells. The incidence of red cell alloimmunization in the present study (5.21 %) was almost similar to other studies. A total of five alloantibodies were formed in five patients which were IAT positive and three of them were DAT positive in the present study. Total five patients showed presence of alloantibody, four (80 %) belonged to K blood group system and one (20 %) from Rh system. All of the red cell alloantibodies detected in the present study belonged to K and Rh blood group system, which was in accordance with the results of other studies [6, 12, 15, 18].

The incidence of red cell alloimmunization in thalassemia major patients varies from as low as 3.1 % in a study from Italy [9] to 22.6 % from Greece [6] and as high as 30 % from Iran [15]. Various factors affect the rate of alloimmunzation, such as racial factors & disparity between antigenic frequency of donors and the recipient.

Majority of Asian centers have reported low rate of alloimmunization [12, 13, 16–19, 21]. Most of this published data have similar frequency of red cell alloimmunization as in present study. A low rate of red cell alloimmunization has been observed in India, by Choudhary et al. [12] and Pradhan et al. [13] ranging from 5 to 8 % including the present study (5.21 %), which can be explained by homogeneity between the donor and the recipient population. A low rate of alloimmunization may be expected when there is homogeneity of red cell antigens between the blood donors and the recipients [21].

A study from Italy by Sirchia et al. [3] reported a low rate of alloimmunization (5.2 %) in multiply transfused thalassemia major patients. The red cell alloantibodies were found in 74 patients out of 1432 patients. A total of 136 alloantibodies were found in 74 patients who were entirely confined to the common antigens of Rh, K, Jk, Fy and MNS system. A low rate of red cell alloimmunization has been observed by Sirchia et al. [3], Choudhary et al. [12] and Pradhan et al. [13] ranging from 5 to 8 % including the present study (5.21 %), which can be better explained by homogeneity between the donor and the recipient.

However, some studies reported a high rate of red cell alloimmunization [2, 6, 8, 10, 15, 21]. Singer et al. [2] reported the high frequency of red cell alloimmunization

(22 %) in multiply transfused thalassemia major patients. He reported that 19 red cell alloantibodies were seen in 14 out of 64 patients. Three antibodies were detected in one patient while two antibodies in three patients. Anti-K was most often identified alloantibody; the present study showed similar result as described by Singer et al. [2] regarding of specificity of alloantibody. This report also interestingly states that patients who receive blood matched for Rh and K system from their first transfusion, the rate of alloimmunization is found to be relatively low. Hence they inferred that transfusion of blood phenotypically matched for Rh and K systems compared to blood phenotypically matched for the standard ABO-RhD system could prove to be effective in preventing alloimmunization. Singer et al. [2] reported a high rate of red cell alloimmunization (22 %) which was not similar as described in present study (5.21 %).

Spanos et al. [6] also found a high rate 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: 34 % to Rh, 29.8 % to K, 7.9 % to MNS, 8.1 % to Jk, 6.6 % to Bg, 5.9 % to Lewis, 4.1 % to Fy and 1.32 % to P. Results of Spanos et al. [6] not in accordance of present study where low rate of alloimmunization (5.21 %) were reported.

Hence it concluded that there were relatively low rate of alloimmunization in our set of patients when compare the data from western world. This also concluded that red cell alloimmunization should not be overlooked in patients with thalassemia major receiving regular blood transfusion. It should always be considered if the patient repeatedly suffers from haemolytic transfusion reaction or not being able to maintain haemoglobin at a desired level in spite of regular transfusions. Regular yearly screening for red cell alloantibodies would add towards the better management of these patients.

A low rate of alloimmunization may be expected when there is homogeneity of RBC antigens between the blood donors and recipients [3, 9, 11–13, 16–18, 20, 21]. In present study a low frequency of red cell alloimmunization (5.21 %) is reported this may be due to homogeneity of RBC antigens between the blood donors and multiply transfused thalassemic patients. Present study showed similar finding as described by these observers including Indian observers.

Although some studies showed a direct relationship between the number of transfusions and the alloimmunization rate [6] others found no such relationship [12]. The relation between the number of units of blood transfused and antibody formation is unknown in thalassemia major but it is an important factor for increased alloimmunization. However, it has been said that the earliest sensitization if any, appears usually after ten transfusions [6]. The five patients who developed red cell alloimmunization in this study were already taken more than ten transfusions. The results of present study, regarding the number of transfusions and the development of alloimmunization were almost similar as described in many studies [6, 17, 20].

In thalassemia major patients, red cell alloimmunization production usually occurs after the age of 6 years after multiple transfusions. Perhaps this is due to tolerance developed by periodic blood transfusion started in early age [3]. In present study five patients were positive for alloantibody (incidence 5.21 %). Out of five, four (80 %) patients who develop alloimmunization were above the age of 6 years, only one patient was below the 6 years who was already received 44 units of red cell transfusion at the time of development of alloantibody. The reason for the development of red cell alloimmunization in this 4 year old patient was probably multiple transfusions. This Results of Sirchia et al. [3] was almost similar as described in study of Bilwani et al. [20] and in present study.

Conclusion

Present study demonstrated the rate of red cell alloimmunization was 5.21 % in multiply transfused thalassemia major patients. Regular screening for development of alloantibodies in multiply transfused thalassemia patients should be done every yearly. With the screening and identification technique, the alloantibodies should be identified and patients should be given corresponding antigen negative donor unit. This will help to minimize the antibody mediated destruction of transfused red cells. Ultimately, the desired effect of transfusion to the patient may result in reduction of transfusion needs for the patients. Less number of transfusions reduces the psychological and financial burden on the family and will increase the compliance of patient.

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