Detection of red cell alloantibodies in thalassaemia patients

Ansuman Sahu¹, Pankaj Parida², Smita Mahapatra²*, Binay Bhusan Sahoo²

¹Department of Blood Bank- Transfusion Medicine, Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India
²Department of Transfusion Medicine, SCB Medical College & Hospital, Cuttack, Odisha, India

Received: 05 December 2019
Revised: 27 December 2019
Accepted: 31 December 2019

*Correspondence:
Dr. Smita Mahapatra,
E-mail: dr.smitamahapatra@gmail.com

ABSTRACT

Background: β-thalassaemia patients receive regular blood transfusion to thrive. Due to antigen disparity between the blood donors and these patients they develop red cell alloantibodies due to alloimmunization. The objective of this study is to predict the frequency of red cell alloimmunization amongst β-thalassaemia major patients receiving regular blood transfusion.

Methods: This study including 106 patients with β-thalassaemia was conducted in the department of Transfusion Medicine, S. C. B. Medical College, Cuttack for a period of 12 months. Alloantibodies to different red cell blood group antigens in multi-transfused thalassaemia patients were detected using the glass bead technology for blood group serology in the present study.

Results: Out of 106 β-thalassaemia major patients included in the study, 7.5% of patients developed alloantibodies, all being clinically significant. The alloantibodies were anti-E, anti-c, anti-e and anti-D. The rate of incidence of these alloantibodies was 3.8%, 1.9%, 0.9% and 0.9% respectively. There was a significant association between alloantibody formation with number of transfused packed red cells (Mann-Whitney Test: p value = 0.035) and age at first transfusion (p value = 0.001). The factors having no association with alloimmunization to red cell antigens are age and gender.

Conclusions: Alloimmunization to various erythrocyte blood group antigens is a common problem in multi-transfused β-thalassaemia patients. There is an association between number of transfused packed red cells and age at first transfusion with alloantibody formation in the study.

Keywords: Alloantibodies, Alloimmunization, β-thalassaemia, Blood group antigens, Multiple transfusion

INTRODUCTION

Thalassaemia is the most common genetic disorder worldwide affecting synthesis of one or more of the globin subunits of Hemoglobin leading to ineffective erythropoiesis. There is an overall deficit of hemoglobin tetramers in the Red Blood Cells (RBC) with reduction in the Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH). Pallor, splenomegaly of various severity, fever, failure to thrive and skeletal abnormalities are findings in the suffering patients. In the absence of stem cell transplantation, the treatment of beta thalassaemia major is based on regular blood transfusion from early childhood, which improves the anaemia and reduces the skeletal deformities associated with excessive erythropoiesis.¹ Regular blood transfusion though is lifesaver for thalassaemia patients but it introduces a multitude of alloantigen and living cells into the recipient’s body. Blood antigens such as human leukocyte antigens, class I and II; granulocyte-specific antigens; platelet-specific antigens and red blood cell-specific antigens can stimulate the immune system and produce alloantibodies.²,⁴

Red cell alloimmunization occurs because of antigenic differences between donor and recipient RBCs.
The present study aimed at detection of red cell alloantibodies in multiply transfused thalassemic patients and to determine the relative frequency of occurrence of different alloantibodies in the study population.

METHODS

A prospective cross-sectional study was conducted in the Department of Transfusion Medicine, SCB Medical College and Hospital, Cuttack from March 2013 to December 2014. A total of 106 regularly transfused thalassemic were enrolled in this study.

Inclusion criteria

- Thalassaemia patients who received at least fourteen units of PRBC in the past and had a history of blood transfusion at least once in every month.

Exclusion criteria

- Female patients with history of pregnancy or abortion.

Blood groups of the patients were studied by ABO grouping and Rh D typing by conventional tube method. Commercially available Ortho BioVue (Orthodiagnostic, USA) system was used for red cell serology. Basic crossmatch, screening and identification were done by using Ortho BioVue cassettes according to Standard Operating Procedure of the system. Cross matching was done taking 40 μl of patient serum and ABO and Rh D group specific 10 μl of 1% donor cell suspension followed by addition 50 μl of BLISS (Orthodiagnostic, USA) in a microcolumn of cassette along with a control. The mixture was kept in the supplied incubator Heatblock (Orthodiagnostic, USA) at 37 deg Celsius for 10 mins. The cassette was centrifuged for 5 min in the supplied centrifuge. Then the result was interpreted, and reaction was graded from 4+ to 1+ if incompatible. A 4+ reaction is indicated by a solid band of RBCs on top of the glass bead surface. A 3+ reaction displays agglutinated RBCs in the upper half of the glass bead column. A 2+ reaction is characterized by RBC agglutinates dispersed throughout the glass bead column, while a 1+ reaction shows RBC aggregates in mainly lower half of the column.

The incompatible serum was again processed for antibody screening and identification. In antibody screening and identification same steps were followed as that of cross matching, the difference being the cell suspension. 1 % cell suspension was taken from the supplied three cell panel (Sugiscreen, Orthodiagnostic, USA) for antibody screening and from the supplied eleven cell panel (Resolve, Orthodiagnostic, USA) for antibody identification instead of 1% donor cell as in cross matching. The tests were interpreted, and reactions were graded. The antibodies were identified according to their pattern agglutination in the corresponding antigram.

RESULTS

The study group included a total number of 106 β-thalassaemia cases, age ranging from 3 to 29 years. Maximum number of cases i.e. 37(34.9%) were from the age group of 6-10 years, followed by 30(28.3%) in the age group 11-15 years, 14(13.2%) in the age group ≤5 years, 13(12.3%) in the age group 16-20 years and 7(6.6%) in the age group 21-25 years. There were 5 thalassemic having age greater than 25 years (Figure 1).

![Figure 1: Distribution of patients according to their age.](image)

Mean age of all the cases was 11.9 years. Maximum numbers of alloantibodies were detected in the age group 11-15 year. Mean age of cases who developed alloantibody was 13.1 year and for those who didn’t develop alloantibody was 11.8 year. There was no significant relation between the age and development of alloantibodies (p value = 0.58). 32(73.6 %) of the cases were males as compared to 28(26.4%) females (Figure 2).

![Figure 2: Gender distribution of the study population.](image)
In the RhD typing only 04(3.8%) cases were RhD negative while 102(96.2%) cases were RhD positive (Figure 4). One of the RhD negative typed patient developed alloantibody against D antigen and rest of the alloantibodies were developed in RhD positive patients. In the study population the cases were grouped according to the number of blood transfusions received into five different clinical groups (Figure 5).

The number of cases in the ≤50 transfusions group were 17(16%), maximum cases 45(42.5%) were reported in the 51-100 transfusions group, 15(14.2%) cases received between 101-150 transfusions, 10(9.4%) cases received 151-200 transfusions, and 19(17.9%) cases received >200 unit. Maximum numbers of alloantibodies were developed in 101 to 150 transfusions group. Mean of number of transfusions given to all patients was 131.63. Mean of number transfusion given to patient developing alloimmunization was 159.13. Mean of number transfusion given to patient who did not develop alloimmunization was 129.39. The number of transfusions was significantly associated with development of RBC alloantibody (Mann-Whitney Test: \( p \) value = 0.035). Out of 106 patients 93(87.7%) had their first transfusion at age of less than 12 months and 13(12.3%) had the same at the age greater than or equal to 12 months (Table 1).

### Table 1: Distribution of patients with respect to age at 1st transfusion.

| Age at 1st transfusion | Numbers | Alloantibody developed |
|------------------------|---------|------------------------|
| < 12 months            | 93      | 1                      |
| ≥ 12 months            | 13      | 8                      |

Only 1(0.01%) case out of 93 patient developed alloantibody in whom first transfusion age was less than 12 months. 8(61.5%) out of 13 cases developed alloantibody who were ≥12-month-old at the time of first transfusion. ‘The age at first transfusion’ had shown significant association for development of alloimmunization (p value = 0.001). 08(7.5%) subjects out of 106 cases were detected to have alloantibodies against blood group antigens. Out of 8 individual 4(50%) were having Anti E, 2(25%) individuals had anti c, 01(12.5%) was having anti e and anti D was detected in 01(12.5%) in one individual. Autoantibody (Positive DAT) developed only in 7(6.6%) of the cases. None of them developed alloantibody.

### DISCUSSION

Alloimmunization against blood groups occurs following introduction of foreign red cell antigen via transfusion, pregnancy and transplantation. The factors responsible for alloimmunization are complex and involve at least three main contributing elements: (1) the RBC antigenic differences between the blood donor and the recipient; (2) the recipient's immune status; (3) the immunomodulatory effect of the allogeneic blood transfusions on the recipient's immune system.\(^3\) In thalassaemia, the main cause of development red cell alloimmunization is repeated blood transfusion. Blood selection based on the limited red blood cell antigens matching (ABO, RH) may increase the incidence of alloimmunization in thalassaemia. In institution blood matching is limited to ABO and Rh (D antigen).
The rate of alloimmunization depending on the blood bank policy varies from 4.97% to 37% in different countries. A low rate of alloimmunization may be expected when there is homogeneity of RBC antigens between the blood donors and recipients. At author’s center, most of patients and blood donor population are from nearby locality. This homogeneity between the patient and blood donor’s population may be one of the reasons for low rate of alloimmunization. Use of leucodepleted blood could have contributed to further decrease in rate of alloimmunization as found by Singer et al, which is not practiced in authors center. Compared to the present study higher rate of alloimmunization was found in studies from Taiwan (37%), Arab (30%) and by Singer et al, in thalassaemia patients of Asian descent. Compared to the this study lower alloimmunization was reported in thalassaemia patients from Pakistan- 6.84%, Southern Iran- 5.3%, Italy- 5.2%, and Northeast Iran- 2.87%. Comparing the studies carried out in India, rate of alloimmunization is found in Mumbai- 8%; Delhi- 9.8%; Bangaluru- 9.46%; Pune- 18.8% in India. Lower rate of alloimmunization was found in Chandigarh- 5.64% than the present study depicting alloimmunization in 7.5% cases. The rate of alloimmunization was lower in patients who received their first transfusion at <1 years of age (p = 0.016) which in accordance with previous studies which shows lower rate of alloimmunization in patients who receive first transfusion at <3 years of age where an immature immune system and some form of the acquired immune tolerance to allogeneic RBC antigens is held responsible for the reduced alloimmunization risk. There was no association of gender (male/female) with rate of alloimmunization in present study as seen in the studies of Ameen et al, El Danasoury et al, and Hendrickson et al. Reisner et al, who have reported a significant association of alloimmunization more with female patients, while Saied et al, found alloimmunization to be associated more with male patients. The relationship between the number of units transfused and alloimmunization is unknown in thalassaemia. Schonewille et al, Saied et al, and Ahmed et al, found no significant association between alloantibodies and autoantibodies formation and the number of transfused packed RBCs. However, some of the studies reported that alloimmunization is more likely in patients who receive more units of blood. Authors found that development of alloimmunization was associated with increase in number of transfusion (p-value =0.035). In the present study, all the detected alloantibodies belonged to Rh blood group system. Among them Anti-E (50%) was the commonest, followed by Anti-c (25%), Anti-e (12.5%) and AntiD (12.5%). Higher rates of alloimmunization against RhD antigen is reported by Sadeghian et al (88.8%). Blood transfusion from weak RhD donors to RhD negative thalassaemia patient is the contributing factor for alloimmunization against Rh D antigen. No anti-Kell antibodies were reported which is in accordance with study carried out by Pradhan et al, (Mumbai, India) and Chaudhary CN 14( Pune, India). In this study, autoantibody (Positive DAT) developed only in 7(6.6%) of the cases, none of which was interfering with compatibility testing. Higher rate of autoimmunization was reported by Dhawan et al (28.2%), Singer et al (25%) and Ameen et al (11%). Splenectomy could be the possible explanation for higher rate of autoimmunization. As there was no splenectomized patient in this study, the rate autoimmunization was low.

CONCLUSION

The rate of alloimmunization was low (7.5%) in this study. Several factors might have contributed to this finding, such as the homogeneity of the population, the age of first transfusion which was <12 months in most cases, antigenic differences between the blood donor and the recipient, the recipients immune status and the immunomodulatory effects of the allogenic blood transfusions on the recipients immune system. The study emphasizes the need for RBC antigen typing before first transfusion and issue of antigen matched blood. Early institution of transfusion therapy after diagnosis is another means of decreasing alloimmunization. Regular antibody screening should be mandatory for already alloimmunized patients to check for the disappearance of old antibodies or development of new alloantibody. In this study, detection of Anti D in one of our patients implies that more attention should be paid to quality control programs for determination of weak D positive red blood cells, and in order to decrease the rate of alloantibody synthesis. These measures will help in decreasing the incidence of RBC alloimmunization and delayed haemolytic transfusion reactions and enhancing the lifespan of these thalassaemic patients.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: Not required

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