

Association of sex, age and ABO–Rh(D) type with unexpected antibodies among multitransfused thalassemia patients in Bikaner, Rajasthan, India

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ABSTRACT

The thalassemia has become a sensitive issue for clinical and public health owing to the morbidity and mortality caused and potential risks associated with multiple transfusions. Here, a blood bank based cross sectional analytical study was carried out during the period of three months from January 2017 to March 2017, among transfusion dependent beta thalassemia major patients. ABO–Rh(D) blood grouping and screening for unexpected red cell antibodies (other than anti–A and anti–B antibodies) were performed on a Immucor Galileo Neo System (fully automated immunohematology analyzer). Out of 56 patients, 37 (66%) were males and 19 (34%) were females with a male to female ratio of 1.95:1. Two cases (3.6%) were detected positive by antibody screening. Alloimmunization was statistically analyzed on the basis of age, sex and subjects' ABO–Rh blood group. This study underlines the need for unexpected antibody screening among thalassemic patients receiving blood transfusion therapy.

Keywords: thalassemia major, transfusion therapy, alloimmunization, unexpected antibodies

INTRODUCTION

The thalassemia is associated with a genetically determined reduction in the rate of synthesis of one or more types of normal hemoglobin (Hb) polypeptide chain. The lack of polypeptide chain results in interference in erythroid maturation, function and ineffective erythropoiesis. Beta thalassemia major is characterized by major or total suppression of beta chain synthesis in the homozygous form of the disease^[1].

Treatment modalities for patients with beta thalassemia major include chronic transfusion therapy, iron chelation, splenectomy, and allogeneic hematopoietic transplantation. The goal of long–term transfu-

sion support is to maintain the patient's hemoglobin at 9.5~10.0 g/dL, thus improving his or her wellbeing while simultaneously suppressing enhanced erythropoiesis^[2].

Alloimmunization, i.e. the development of alloantibodies against foreign red blood cells (RBCs) is one of the important complications of blood transfusions in multiple transfused thalassemia patients^[3,4]. Alloimmunization further complicates transfusion therapy due to difficulties in getting compatible blood, increased frequency of additional alloantibody and autoantibody (antibody against self RBC antigens) development, delayed hemolytic transfusion reaction (DHTR) and life–threatening hyper–hemolysis syndrome^[3–6].

The factors responsible for the development of unexpected antibodies among multiple transfusion patients are complex and need to be studied. Although the number of transfusion dependent beta thalassemia major patients in India is large, there is little available

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data on alloimmunization or autoimmunization among such patients, especially in Western Rajasthan.

The present study was conducted to determine and compare the frequency of unexpected antibodies on the basis of their age, sex and blood group amongst transfusion dependent thalassemia major patients receiving regular transfusion support at the Department of Immuno-Hematology & Transfusion Medicine of Sardar Patel Medical College & Associated Group of Hospitals, Rajasthan.

MATERIALS AND METHODS

A blood bank based cross sectional analytical study was carried out during the period of three months from January 2017 to March 2017 among transfusion dependent beta thalassemia major patients.

Information was collected, including transfusion history, age, sex type of blood transfused (whole blood or packed cells; leuko-depleted or non-leuko-depleted blood), status of splenectomy and detailed clinical evaluation.

Venous blood sample 2~3 mL was collected from each subject in (EDTA ethylene diamine tetra acetic acid) anticoagulated vials from which plasma was separated. ABO-Rh(D) blood group was determined for each sample by fully automated immunohematology analyzer (Immucor Galileo Neo System, Immucor, USA) using direct hemagglutination microstrips with reagents provided by the manufacturer.

All the samples were screened for a poly-specific direct agglutination test (DAT), auto control (AC) and indirect agglutination test (IAT) by column agglutination technology (CAT) in low ionic strength solution (LISS-as DG Gel Solution) using Diana Gel cards on a Grifols semi-automated immunohematology analyzer.

All the samples were then screened for unexpected RBC antibodies (other than anti-A and anti-B antibodies) by the Immucor Galileo Neo System based on solid phase RBC adherence (SPRCA) capture technology using commercially prepared Capture LISS, Capture-R Ready-Screen Pooled cells and 3 cell panels.

Appropriate tests of significance were applied, and the results were statistically analyzed using MS Excel and PRIMER software.

RESULTS

A total of 56 beta thalassemia major patients were enrolled for the study, who regularly received blood transfusions at 2~5 week's intervals. They received both leuko-depleted and non-leuko-depleted, ABO-Rh(D) matched, allogenic packed RBCs (with SAGM as an additive solution).

The transfusion history revealed that 85.7% (48/56) of patients received more than 50 units of transfused blood, while 14.3% (8/56) of patients received 20~49 units. All patients positive for unexpected antibody screening received more than 150 units of blood transfusion.

ABO blood group determination revealed that 37.5% (21/56), 32.1% (18/56), 16.1% (9/56) and 14.3% (8/56) of subjects were of O, B, A, and AB blood group, respectively. Four subjects (7.1%) were Rh(D) negative (one B- and three O-) and rest were positive for Rh(D). The distribution of ABO-Rh blood groups among thalasseemics is shown in **Table 1**.

3.6% (2/56) of subjects were screened positive for unexpected antibodies against RBC antigens. 1.8% (1/56) subjects of blood group O and 1.8% (1/56) subjects of blood group A were detected positive for unexpected antibodies. All the positively screened subjects were in the Rh(D) positive blood group.

Subjects screened positive for unexpected antibodies are shown in **Fig.1**, in relation to ABO-Rh(D) blood groups. The association between ABO-Rh(D) blood group and occurrence of unexpected antibodies among thalasseemics was found statistically insignificant (Chi square test; $P = 0.891$; with DOF = 5 and Yate's correction applied; NS).

Out of total 56 subjects, 37 (66%) study subjects were males and 19 (34%) were females; with a male to female ratio of 1.95:1. None of the male subjects were screened positive for unexpected antibodies. All unexpected antibody positive subjects (3.6%) were females. The association observed between sex and unexpected antibody status was deemed insignificant (Chi square value with Yate's correction applied 0.561; P value >0.05 ; NS).

The age of study subjects ($n=56$) ranged from 1 to 18 years; with mean age of (8.4 ± 4.6) years. The mean age of unexpected antibody negative subjects was (8.3 ± 4.6) years (age range 1 to 18 years) against (13.0 ± 5.7) years

Table 1 Distribution of ABO-Rh blood groups among thalasseemics

Blood group	Rh(D) Pos	Rh(D) Neg	Total	Percentage(%)	ABO distribution in general population(%)
A	9	0	9	16.07	22.52
B	17	1	18	32.14	36.72
O	18	3	21	37.50	31.63
AB	8	0	8	14.29	9.13
Total	52	4	56	100.00	100.00

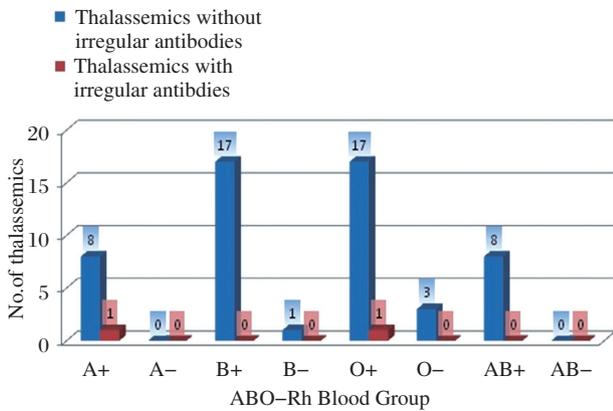


Fig. 1 ABO-Rh blood group and irregular antibodies among thalassemics.

(age range 8 to 17 years) in subjects detected positive for unexpected antibodies. The difference in the means was observed statistically insignificant (Student’s unpaired *t*-test; DOF = 54; *P*= 0.16; NS). Age distribution of the subjects is presented in **Table 2**.

Table 2 Age distribution of thalassemia major patients under study [(n)%]

Age group (Years)	Without unex-pected antibodies	With unexpected antibodies	Total
1~6	21 (37.5)	0	21 (37.5)
7~12	23 (41.1)	1 (1.8)	24 (42.9)
13~18	10 (17.9)	1 (1.8)	11 (19.6)
Total	54 (96.4)	2 (3.6)	56 (100.00)

1.8% subjects (1/56) underwent splenectomy. This subject was a female, age 13 years, ABO-Rh(D) blood group B⁺, with first transfusion at age 6 months, and duration since first transfusion 12.5 years. The splenectomized subject was found negative for antibody screening.

DISCUSSION

Fifty six beta thalassemia major patients were included in this study. These patients were transfused with both leuko-depleted and non-leuko-depleted ABO-Rh(D) matched, allogenic packed RBCs with SAGM additive solution. In this study, we found unexpected RBC antibodies among 3.6% subjects (two patients out of 56 tested positive on antibody screening). This frequency is comparatively very low when compared to the various studies conducted worldwide.

High frequency of unexpected antibodies (ranging from 19% to 43%) in thalassemia patients has been reported in various studies from Egypt (42.8%; 29.5%, and 19.5%)^[7-9], Asian descent in the USA (36%)^[10], and Hong Kong (23%)^[11]. International reports have shown wide variations in the frequency of alloimmunization following regular transfusions in thalassemics.

Studies from Pakistan (6.8%; 9.2%)^[12,13], Egypt (8%)^[14], Taiwan (9.4%)^[15], and Iran (12%)^[16] have reported alloimmunization rates ranging from 6% to

12%. Compared to these studies, lower alloimmunization rates have been reported among thalassemia patients in studies from Iran (2.9%) by Sadeghian *et al.*^[17] and Malaysia (1.6%) by Noor *et al.*^[18], which were close to the present study (3.6%).

Only a few studies from India have reported the frequency of unexpected antibodies in multiple transfused thalassemia patients, as yet none from Rajasthan have been published. A study from Pune by Chaudhari (2011)^[1] reported alloimmunization rates as high as 18.8%. Shenoy *et al.* (2013)^[19] from South India reported 9.5%, Patel *et al.* (2016)^[20] reported 8% and Dhawan *et al.* (2014)^[21] reported 5.6% alloimmunization in Chandigarh. The frequency of unexpected antibodies among thalassemics in the present study (3.6%) was found comparable with Saleem *et al.* (2015)^[2] in Mangalore (1.8%) and Dhawan *et al.* (2014)^[21] in Chandigarh (5.6%).

The differences in alloimmunization were attributed to at least three contributing factors: the RBC antigenic difference between the blood donor and the recipient, the recipient’s immune status and the immunomodulatory effect of the allogenic blood transfusion on the recipient’s immune system^[10,22].

A low rate of alloimmunization is expected in a population where there is homogeneity of RBC antigen between the blood donors and recipients^[10]; when a transfusion of extended phenotype blood group matches blood units^[5,23] and leuko-depleted blood is being practiced^[24].

Singer *et al.* reported a higher frequency of alloimmunization among thalassemics who were splenectomized which was attributed to the absence of the efficient filtering of damaged or senescent RBCs, which may reveal new antigens^[10]. Absence of spleen is also considered as one of the risk factor for alloimmunization^[10], however, the only splenectomized patient from the present study was found negative for unexpected antibodies upon antibody screening.

Even though some earlier studies have shown some correlation between development of RBC alloimmunization and factors like splenectomy status, type of blood product transfused and number of units of blood transfused, in the present study we were not able to obtain such a correlation, neither did we obtain any association in the development of unexpected antibodies with the age of the patients, gender, or ABO-Rh(D) blood group status, which could be due to low frequency or small sample size.

CONCLUSIONS

This study underlines the need for routine screen-

ing for unexpected antibodies among multi-transfused thalassemic patients. Routine pre-transfusion antibody screening is essential to ensure safer transfusions. The low alloimmunization rate observed in this study implies that there is homogeneity of RBC antigens in blood donors and recipients.

Comparable analytical studies, with larger sample sizes giving comparison with more variables are desirable in all parts of the country, in order to understand the pattern and establish associations with factors which are responsible for alloimmunization in multi-transfused patients.

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