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Red cell alloimmunization and associated risk factors in multiply transfused thalassemia patients: A prospective cohort study conducted at a tertiary care center in Northern India

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Abstract:

BACKGROUND: One of the complications of chronic transfusions in thalassemia is the development of red cell alloimmunization.

AIMS: The aim of the study was to determine the frequency, specificity of red cell alloantibodies, and factors influencing alloimmunization in multiply transfused thalassemia patients.

MATERIALS AND METHODS: The study was carried out prospectively on beta-thalassemia patients over 10 months. Plasma samples were used for antibody screening and identification using the column agglutination technique. Patients' clinical, laboratory, and transfusion details were obtained from hospital information system and patient files.

STATISTICAL ANALYSIS: Continuous variables were reported as median and quartile, whereas categorical variables were provided as numbers and proportions. $P < 0.05$ was considered statistically significant.

RESULTS: Out of 255 patients, 17 (6.6%) patients developed alloantibodies. Alloimmunized patients had significantly higher median ages at their first transfusions (1 year vs. 0.5 years; $P = 0.042$) than nonalloimmunized patients. Alloimmunized patients had significantly higher conjugated bilirubin ($P = 0.016$) and serum ferritin ($P = 0.007$). The majority of alloantibodies had specificity toward K antigen, followed by E, C, D, JKa, and JKb antigens. Alloimmunized patients received more units per year than nonalloimmunized patients (median, 30 vs. 24 units; $P < 0.001$). The average transfusion interval time between two successive transfusions showed a significant difference ($P < 0.001$).

CONCLUSIONS: The prevalence of alloimmunization in thalassemia patients in North India is relatively low. Since most of the alloantibodies belong to Rh and Kell blood group system, extended phenotype-matched blood for Rh and Kell will be helpful in further preventing or decreasing the development of alloantibodies in multiply transfused thalassemia patients.

Keywords:

Blood transfusion, red cell alloantibody, red cell alloimmunization, thalassemia

Introduction

The thalassemias and hemoglobin (Hb) variants are the most common monogenic disorders worldwide. Being an important cause of morbidity and mortality, they impose a heavy burden on the families of the affected patients. India has the largest number of children with

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thalassemia major in the world – about 100000–150000, with an average frequency of carriers being 3%–4%.[1] About 10,000–15,000 babies with thalassemia major are born annually in India.[2]

To deliver normal red blood cells (RBCs), reduce the patient's inadequate erythropoiesis, and maintain the pretransfusion hemoglobin level above 9.0–10.5 g/dL, patients with thalassemia major are given lifelong blood transfusions, every 2–5 weeks.[3]

RBC alloimmunization, or the formation of antibodies against nonself-antigens on RBCs, is one of the major complications of chronic transfusion in beta-thalassemia patients.[4] These antibodies may be clinically significant, leading to delayed hemolytic or serologic transfusion reactions. Furthermore, alloantibodies make it difficult to find compatible blood, delay the provision of safe transfusions, reduce the *in vivo* survival of transfused cells, and may hasten the iron loading of tissues.[5] Depending on factors including the homogeneity of the donor-recipient population, the RBC phenotype matching strategy, and the age at which the transfusion was initiated; the incidence of alloimmunization is variable worldwide.[6] According to a study, the global red cell alloimmunization rate for people with thalassemia ranges from as low as 2.5% to as high as 37%.[7]

The mechanism of alloimmunization is less understood. However, it likely involves a number of contributing factors, such as recipient exposure to foreign donor antigens, antigenic immunogenicity of RBC, the immunological status of the recipient, age at first transfusion, and the duration of transfusions.[7] Compared to nonalloimmunized patients, alloimmunized patients showed an increase in memory B cells (CD19+, CD38+), suggesting a substantial change in the immune system induced by erythrocytes.[8] Furthermore, splenectomy may affect the alloimmunization rate. The absence of the spleen may hasten the immune response to the infused unfamiliar antigens, which are not effectively filtered.[9]

Extended RBC phenotyping and provision of antigen-negative blood for the identified alloantibodies reduces posttransfusion complications. However, these procedures are laborious and cannot be routinely performed in blood banks, especially in developing nations.[10] Some organizations only offer prophylactic Rh and K or extended matched red cells if a person has developed an alloantibody. Even in the absence of alloantibodies, an international consensus group advises preventive ABO, Rh, and K-matched red cells for patients with thalassemia to lower the likelihood of alloimmunization.[11]

However, the recommendation was based on studies of very poor quality, so the tool used for Grading of Recommendations, Assessment, Development, and Evaluation indicated that the strength of the recommendation was low.[12]

Very few studies from India have addressed the various risk factors affecting alloimmunization in thalassemia. The aim of the study was to determine the frequency, types, and factors influencing red cell alloimmunization in multiply transfused thalassemia patients at our center.

Materials and Methods

After receiving written informed consent and a waiver from the institutional ethical review board, the study was carried out prospectively on beta-thalassemia patients over 10 months (from December 2021 to September 2022). Patients with autoimmune and connective tissue illnesses were excluded from the study. All thalassemia patients were provided ABO and RhD matched, cross-match compatible, leukofilterd fresh blood (<5 days old) with the rate of 10–15 ml/kg. If any patient develops alloantibody, corresponding antigen-negative blood is provided for all future transfusions.

Blood samples in ethylenediaminetetraacetic acid that were provided for routine compatibility testing were used in the study. No additional samples were collected from the patient. Plasma was used for antibody screening and identification using the column agglutination technique and commercially available 3-cell (ID-Diacell I-II-III, DiaMed Switzerland) and 11-cell panels (ID-Diapanel, DiaMed Switzerland) panels, respectively.

Clinical details of each patient were obtained from patient files. Laboratory details and transfusion records were obtained from the hospital information system.

Statistical analysis

Version 20 of IBM SPSS for Windows was used for all statistical analyses (IBM Corp, Armonk, NY, USA). Continuous variables were reported as median and quartile, whereas categorical variables were provided as numbers and proportions. The use of binary logistics revealed the association. $P < 0.05$ was considered statistically significant.

Results

A total of 255 patients with beta-thalassemia receiving regular transfusion therapy at our center were included in the study. Of the 255 patients, 71 (27.8%) were female and 181 (70.9%) were male. Two hundred and twenty (220/255, 86.2%) patients had beta-thalassemia major and

35 (35/255, 13.7%) patients had beta-thalassemia intermedia.

Out of 255 patients, 17 (6.6%) patients developed alloantibodies. Table 1 shows the clinical details of patients with and without alloantibodies. Alloimmunized patients had significantly higher median ages at their first transfusions (1 year vs. 0.5 years; $P = 0.042$) than nonalloimmunized patients. Among the alloimmunized patients, 23.5% (4/17) were splenectomized compared to 13.4% (32/238) in nonalloimmunized patients. However, the difference was statistically insignificant ($P = 0.249$).

Table 2 shows laboratory parameters of patients with and without alloimmunization. Alloimmunized patients had significantly higher conjugated bilirubin ($P = 0.016$) and serum ferritin ($P = 0.007$) than patients without alloimmunization.

Out of 17 patients that were alloimmunized, 5 (29.4%) had two alloantibodies and 12 (70.5%) had only one [Table 3]. The majority of alloantibodies had specificity toward K (6/17) antigen, followed by E, C, D, JKa, and JKb antigens.

Details of transfusion support to the patients are shown in Table 4. In the present study, alloimmunization was associated with a greater annual transfusion rate. Patients who were alloimmunized received more units per year than patients who were not alloimmunized (median, 30 vs. 24 units; $P < 0.001$). The average transfusion interval time (days) between two successive transfusions showed a significant difference ($P < 0.001$), as well.

Multiple logistic regression analysis was used to examine the association of various variables with alloantibody formation in beta-thalassemia patients [Table 5]. Alloantibody formation showed a strong association with the number of red cell units transfused per year (odds ratio [OR]: 0.927 confidence interval [CI]: 0.888–0.968), interval between two consecutive transfusions (OR: 1.205, CI: 1.105–1.314), and total consumption of red cells per year (OR: 0.993, CI: 0.988–0.998).

Discussion

One of the adverse effects of RBC transfusions in multiply transfused patient is the development of alloimmunization to red cell antigens, an immunological reaction that is typically triggered by the transfusion of blood products. The rate of alloimmunization is variable in patients with thalassemia. In our study, we demonstrated alloimmunization in 6.6% of patients, which is in concordance with other Indian Studies. In India, the prevalence of alloimmunization is variable ranging from 5.6% to 8.6% [Table 6].

The relatively lower rate of alloimmunization in our study is probably due to the antigenic similarities between donors' RBCs and the recipient's blood. Alloimmunization has been linked to antigenic discrepancy between donors and recipients. Singer *et al*.[7] reported a high alloimmunization rate among patients of Asian origin in the United States and concluded that different racial background between white donors from the United States and Asian recipients likely contributes to increased incidence of alloimmunization. Ameen *et al*.[17] have reported an even higher rate (30%) in

Table 1: Details of patients with and without alloantibodies

Variables	Patients with alloimmunization (*n*=17), *n* (%)	Patients without alloimmunization (*n*=238), *n* (%)	*P*
Age (years), median (quartile)	18 (10-24)	9 (12-21)	0.74
Gender			
Male	11 (64.7)	172 (72.3)	0.881
Female	6 (35.2)	66 (27.7)	
Diagnosis			
Major	13 (76.5)	207 (87)	0.224
Intermedia	4 (23.5)	31 (13)	
Age at first transfusion, median (quartile)	1 (0.7-2)	0.5 (0.41-1.5)	0.042
Splenectomy	4 (23.5)	32 (13.4)	0.249

Table 2: Laboratory parameters of patients with and without alloantibodies

Variables	Median (quartile)		*P*
	Patients with alloimmunization (*n*=17)	Patients without alloimmunization (*n*=238)	
Pretransfusion Hb (g/dL)	8.1 (7.7-8.35)	8.6 (7.6-9.2)	0.038
Total bilirubin (mg/dL)	1.2 (1.07-1.6)	1.28 (0.79-2)	0.622
Conjugated bilirubin (mg/dL)	0.98 (0.88-2.44)	0.81 (0.49-1.37)	0.016
Serum ferritin (ng/mL)	2640 (2305-3240)	2111 (1658-2786)	0.007

Hb=Hemoglobin

Kuwait which may be related to the heterogeneity of the population. Lower alloimmunization rates of 4.9%–10% were reported in thalassemia patients in countries with more homogeneous populations, in Greece, Italy, Iran, Pakistan, and India.[18-22]

The specificities of alloantibodies were mostly against the antigens of the Rh blood group system (12/17, 70.5%), followed by Kell (6/17, 35.2%) in the present study. This is in agreement with other studies which reported specificity of alloantibodies in the Rh and Kell blood group system.[4,6] Thus, it is apparent that if the thalassemia patients are provided with extended phenotype-matched blood for at least Rh and Kell, the prevalence of alloimmunization can be further decreased in our patient population.

In the current study, patients who received their first transfusion in early age (median 0.5 years) had a reduced alloimmunization rate (P = 0.042) as compared to late onset of transfusion (median 1 year). Previous studies have demonstrated that the risk of alloimmunization was significantly lower in hemoglobinopathy patients who began transfusion therapy at a young age compared to those who began later in life.[7,13]

The lower risk of alloimmunization has been attributed to an immature immune system and some form of acquired immune tolerance to allogeneic RBC antigens.[23] Early institution of transfusion therapy after diagnosis is an important means of decreasing alloimmunization but carries the risk of more exposure to other transfusion complications.

We found no correlation between the rate of alloimmunization and gender of the patient. Ameen *et al*.[17] also reported that gender had no significant influence on the development of alloimmunization. On the contrary, Reisner *et al*.[24] and Saied *et al*.[25] had observed that more alloimmunization in female and male gender, respectively.

Splenectomy is recommended procedure in thalassemia to reduce excessive blood consumption and consequently decreased iron overload. Among the other risk factors associated with RBC alloimmunization in patients with thalassemia, splenectomy is the most frequently reported.

According to a theory, the absence of the spleen's function in the filtering of foreign, damaged, and conformationally altered RBCs following splenectomy may further enhance the immune response to the infused antigens, so the rate of alloimmunization may also be affected by splenectomy.[9] In our study, although splenectomized patients had a higher alloimmunization rate than nonsplenectomized patients, the difference was not significant [P = 0.249, Table 1]. Other studies have also reported no difference among patients who underwent splenectomy.[22,26,27]

Alloantibodies are referred to as "clinically significant" if they result in clinical hemolytic transfusion reactions such as fever, chills, and hemoglobinuria associated with increase in the hemolytic laboratory parameters such as increased bilirubin.[28] In our study, alloimmunized patients showed significantly higher levels of conjugated bilirubin and lower Hb level compared

Table 3: Different alloantibody with their specificity

Antibody	Antibodies, *n* (%)
Anti-K	6 (35.2)
Anti-E	4 (23.5)
Anti D + Anti C	2 (11.7)
Anti-C + Anti K	1 (5.8)
Anti-E + Anti K	2 (11.7)
Anti-JK[a]	1 (5.8)
Anti-Jk[b]	1 (5.8)
Total	17 (100)

Table 4: Transfusion details of patients with and without alloantibodies

Variables	Median (quartile)		*P*
	Patients with alloimmunization (*n*=17)	Patients without alloimmunization (*n*=238)	
Number of units transfused (/year)	30 (27-36)	24 (19-28)	<0.001
Annual consumption of red cells (mL/kg/year)	240 (190.9-268.8)	155 (116-216)	<0.001
Average interval (days) between two consecutive transfusions	18 (15-21)	26 (22-29)	<0.001

Table 5: Factors associated with alloimunization

Variables	Regression coefficient (β)	OR (CI 95%)	*P*
Age at first transfusion (years)	−0.001	0.999 (.708-1.4111)	0.997
Numbers of unit transfused (/year)	−0.076	0.927 (0.888-0.968)	0.001
Interval between two consecutive transfusions	0.187	1.205 (1.105-1.314)	<0.001
Annual consumption of packed red cells (mL/kg/year)	−0.007	0.993 (0.988-0.998)	0.005
Splenectomy	0.683	1.981 (0.608-6.452)	0.257

CI=Confidence Interval, OR=Odds ratio

Table 6: Prevalence of alloimmunization in thalassemia from different regions of India

Authors	Total number of patients	Prevalence (%)	Region of India
Dhawan *et al.*, 2014[13]	319	5.64	Western
Elhence *et al.*, 2014[14]	280	8.6	Northern
Datta *et al.*, 2015[15]	500	5.6	Eastern
Sahu *et al.*, 2020[16]	106	7.5	South
Present study	255	6.6	Northern

to nonalloimmunized patients [Table 2]. As a result of this subclinical hemolysis which is going on in alloimmunized patients, they are more likely to receive higher number of transfusions. In our study, we found that alloimmunized patients received significantly higher number of blood units compared to nonalloimmunized patients [$P < 0.001$, Table 4]. Moreover, the interval between two consecutive transfusions was significantly decreased along with increased annual consumption of blood [Table 4]. This increased rate of transfusion with increased consumption of red cells resulted in increased deposition of iron. We observed a statistically significant difference ($P = 0.007$) in serum ferritin levels among alloimmunized compared to nonalloimmunized patients. On regression analysis, rate of alloimmunization was found to be associated with increased numbers of unit transfused/year, annual consumption of red cells, and decreased transfusion interval [Table 5].

Conclusions

In our study, the alloimmunization rate was 6.6%. Alloantibody formation showed strong association with number of red cell units transfused per year, interval between two consecutive transfusions, and the total consumption of red cells per year. The majority of antibodies (88.1%) were produced against the Rh and Kell blood type systems. Thus, extended phenotype-matched blood for Rh and Kell will be helpful in further preventing or decreasing the development of alloantibodies in our multiply transfused thalassemia patients.

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Conflicts of interest
There are no conflicts of interest.

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