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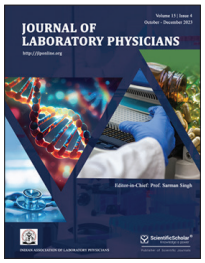

Original Article

Phenotyping of Rh and Kell blood group antigen in thalassemia and its impact on alloimmunization in a tertiary care hospital

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ABSTRACT

Objectives: Alloimmunization to red cell antigens is a dreaded complication in multitransfused patients, leading to difficulty in obtaining compatible red blood cell units and development of delayed hemolytic transfusion reactions. The objective of this study was to assess the impact of partial matched phenotype blood (for RhD, C, c, E, e, and Kell antigens) on alloimmunization in thalassemics versus non-phenotype matched blood (ABO & RhD).

Materials and Methods: This cross-sectional study was conducted over a period of two years where 250 patients with thalassemias were enrolled. They were divided into two groups, patients in Group I (n = 180) who received partial matched phenotype blood since initiation of transfusion therapy and those in Group II (n = 70) subjects who received usual matched blood.

Statistical analysis: All statistical calculations were done using statistical package for the social sciences (SPSS) 21 version. Data were described in terms of range, median (interquartile range [IQR]), frequencies, etc.

Results: The median (IQR) age of the study population was 12 (7–18) years (range 6 months–36 years). The most common Rh antibodies were anti-D (2.85%), anti-E (2.85%), anti-C (1.42%), and anti-c (1.42%), and Kell antibodies were (7.1%). It was seen that chances of developing autoantibodies (37% vs. 5%), alloantibodies 11 (15.7% vs. 0%), and transfusion reactions 25 (35.7% vs. 3.3%) were more in Group II subjects as compared to Group I. A significant difference was seen with febrile non-hemolytic transfusion reactions in between two groups 0.001 (95% confidence interval 2.98–65.73).

Conclusions: Patients with thalassemia should be typed for RhD (C, c, E, and e) and Kell antigen before initiation of transfusion, which will help in reducing the rate of alloimmunization, autoimmunization, and frequency of transfusion and will improve the overall survival rate in thalassemia.

Keywords: Alloimmunization, Autoimmunization, Febrile non-hemolytic transfusion reaction, Thalassemia

INTRODUCTION

Thalassemia is the most common inherited single-gene disorder, caused by a decrease or absence of α- or ß-globin chain production. The disorder is commonly inherited in an autosomal recessive manner and is more common in areas with high rate of consanguinity.[1-3]

The prevalence of thalassemia and hemoglobinopathy varies with geographic locations. Thalassemia and hemoglobinopathy are the major health concern in the Indian subcontinent as the prevalence rate of beta thalassemia mutations is as high as 17% in some populations.[4] It has been estimated that in India, 0.37/1000 live births have hemoglobin disorder.[5]

Thalassemia has a high frequency in a broad belt, extending from the Mediterranean basin through the Middle East (Iran), India, and Southeast Asia and South China.[6] Beta thalassemia major, also known as "Cooley's anemia," usually reveals its manifestations in the first year of life in 95% of the patients, and it has been evaluated that there are 80 million carriers with βthalassemia universally.[7]

Chronic life-long transfusions are required for patients with severe thalassemia to provide normal red blood cells (RBCs) and to suppress the patients' ineffective erythropoiesis. Cumulative exposure increases the risk for red cell alloimmunization, that is, development of alloantibodies against the foreign RBCs and subsequent delayed hemolytic transfusion reactions (DHTRs).[8] The prevalence of alloimmunization among patients with thalassemia ranges from 3% to 42%, with most antibodies directed against the Rh system.[9] Alloimmunization further complicates the transfusion therapy due to difficulty in getting compatible blood, increased incidence of additional antibodies and autoantibodies (antibodies against self-RBC antigen) development, febrile non-hemolytic transfusion reactions, and DHTR.

There are currently 44 recognized blood group systems containing 354 red cell antigens by the International Society of Blood Transfusion Working Party as of December 2022. The 44 systems are genetically determined by 49 genes.

Among the blood group systems discovered to date, the ABO and Rh systems are the most clinically significant in the field of transfusion medicine[10,11] At present, 54 Rh antigens have been discovered in the Rh system. The five major antigens are associated with commonly made clinically significant antibodies, namely, anti-D, anti-C, anti-E, anti-C, and anti-E.[12,13] The Rh system is highly immunogenic and complex, with numerous polymorphisms and clinically significant alleles. The Kell blood group system is the third-most important immunogenic blood group system. It was discovered in 1946 following the discovery of the antiglobulin test. There are more than 30 antigens in the Kell blood group system. Kell antigens are well expressed on fetal RBCs and erythroid precursors. Kell protein is important in transfusion medicine because it is polymorphic and immunogenic, and alloantibodies often produce when unmatched blood is transfused.[14] Antibodies of all blood group systems are lethal and can cause alloimmunization and hemolytic transfusion reactions (HTRs). Both systems are important due to the immunogenicity of their antigens and the potency of their antibodies. Transfusion of phenotypically matched blood for Rh and Kell antigens, compared with blood phenotypically matched for the standard ABO and D antigens, proves to be effective in preventing alloimmunization[15,16] Considering the adverse effect of alloimmunization and autoimmunization and challenges in their management, we conducted this study to evaluate the frequency of erythrocytes alloantibodies and autoantibodies and to specify the most frequent alloantibodies. The goal is to support the future consideration of applying partial better matching of the major culprit antigens responsible for alloimmunization in multitransfused thalassemia patients.

The aim of the study is to evaluate the rate of alloimmunization in multitransfused thalassemia subjects with partial phenotype versus non-phenotype matched blood.

MATERIALS AND METHODS

This is the cross-sectional descriptive study carried out over a period of two years from January 2021 to December 2022 in Dayanand Medical College and Hospital, Punjab. Patients with transfusion-dependent thalassemia (TDT) registered for regular transfusions at the center were recruited to participate in the study after obtaining approval from the Institutional Ethics Committee (IEC) - IEC No.: 2023-848. A consent waiver was obtained from the IEC. A volume of 3 mL was drawn in ethylene diamine tetra acetic acid vial as well as plain for ABO and Rh, direct antiglobulin test (DAT), antibody screening, and antibody identification. ABO and Rh blood grouping, antibody screening, phenotyping for RhD (C, C, E, and E), and Kell antigen were done on fully automated system Neo Iris Immucor automated platform that uses microplate hemagglutination technique for typing with immunoglobulin (Ig)M monoclonal commercial antiserum for Rh and Kell phenotyping.

Direct Coombs test was done with a polyspecific anti-human globulin reagent (anti-IgG+ anti-C3d) using the gel technique with a LISS/Coombs card (Diamed GmbH Switzerland).

Antibody specificity detection was performed using commercial 11-cell identification panel ((ID-Diapanel, Diamed GmbH, Cressier FR Switzerland). Antibody confirmation was done by phenotyping and using select cells.

RESULTS

Two hundred and fifty subjects with TDT registered were enrolled in the study. Among 250 participants, 168 (67.2%) were male and 82 (32.8%) were female. The median (interquartile range) age of the study population was 12 (7–18) years (range 6 months–36 years). The subjects were

Table 1: Distribution according to the blood groups.

Blood groups	Group I		Group II		Total	Chi-square value	*P*-value
	No. of cases	% age	No. of cases	% age			
A−	2	1.1	1	1.4	3	5.767	0.567
A+	26	14.4	15	21.4	41		
AB−	1	0.6	2	2.9	3		
AB+	9	5.0	3	4.3	12		
B−	10	5.6	5	7.1	15		
B+	74	41.1	25	35.7	99		
O−	3	1.7	0	0.0	3		
O+	55	30.6	19	27.1	74		
Total	180	100.0	70	100.0	250		

divided into two groups, Group I comprised of 180 patients with TDT who received partially matched phenotype leucodepleted packed RBCs; Group II comprised 70 patients with TDT who were already multi-transfused where phenotyping was not possible and were matched only for ABO and RhD. There was no significant gender difference in the prevalence of disease ($P = 0.362$).

The most common blood group in the study population was B blood group (n = 99, 39.6%) followed by O blood group (n = 74, 29.6%), A blood group (n = 41, 16.4%), and AB blood group (n = 12, 4.8%). In Rh negative group, B negative were most frequent (n = 15, 6%) as shown in Table 1.

Rh phenotyping was done in Group I subjects. The most common detected Rh antigen was E antigen (100%), and the least common was E (10.5%) and Kell antigen was negative, as shown in Table 2. The frequencies of different Rh phenotypes among Group I subjects are shown in Table 3.

Direct Antiglobulin Test (DAT) was put on polyspecific AHG (anti IgG+C3d) cards on both the groups to detect autoantibodies. Group 1 showed DAT positivity in 9 (5%) subjects with no alloantibodies. Group II showed DAT positivity in 26 (37%) subjects with 11 (15.7%) alloantibodies against Rh and Kell blood group antigens. The alloantibodies detected were Anti D 2 (2.85%), K 5 (7.1%), Anti E 2 (2.85%), Anti C 1 (1.42%) with two subjects developed dual alloantibodies Anti c 1 (1.42%), K and anti K, E as shown in Tables 4 and 5.

Table 2: Antigenic profile of Group I.

Antigen	No. of positive cases *n* (%)
e	180 (100)
D	164 (91.1)
C	153 (85)
c	113 (62.8)
E	20 (11.1)
K	0 (0)

Table 3: Classification of Group I according to Fisher Race and Weiner nomenclature.

Fisher race	Weiner	No. of cases	% age
DCe/dce	R^1r	74	41.1
DCe/DCe	R^1R^1	67	37.2
dce/dce	rr	16	8.9
DcE/dce	R^2r	6	3.3
DCe/DcE	R^1R^2	13	7.2
Dce/dce	R^0r	4	2.2
		180	100.0

DISCUSSION

Regular blood transfusion regimen among thalassemia patients produces antibodies of IgG class frequently against minor blood group systems such as Rh, Kell, Duffy, and Kidd. Alloimmunization is one of the most difficult post-transfusion complications to manage and associated with transfusion delays, shortened *in vivo* survival of donor RBCs and HTRs, which can be fatal in some cases.[17] Gaining knowledge of the frequency of RBC antigen phenotypes in patients with TDT allows the establishment of a comprehensive donor and patient blood database to minimize the risk of alloimmunization. The most frequent Rh antigen in subjects was E antigen 180 (100%), and the least common was E antigen 20 (11.1%). In Asians, the prevalence of D, c, C, E, and e is 99%, 47%, 93%, 39%, and 96%, respectively.[17] The most common Rh phenotype was R^1r 74 (41.1%) and R^1R^1 67 (37.2%), while least common phenotype was R^0r 4 (2.2%). It was observed that participants receiving partial matched phenotype blood did not develop alloantibodies while 11% of participants receiving only ABO and RhD matched, developed 11 (15.7%) antibodies, as shown in a similar study by Pujani *et al.*[18] The most commonly encountered alloantibodies in our study was against Rh (8.5%) and K (7.1%) blood groups shown in a similar study by Pahuja *et al.*[19] In another study by Al-Riyami *et al.*[20] 9.3% participants showed antibodies against anti-E and

Table 4: Comparison of alloimmunization in two groups.

	Alloimmunization rate	Total number of Alloantibodies	Rh					Kell
			D	C	c	E	e	
Group 1	-	-	-	-	-	-	-	-
Group II	15.7% (11/70)	11	2 (2.85%)	1 (1.42%)	1 (1.42%)	2 (2.85%)	-	5 (7.1%)

Rh: Rhesus.

Table 5: Results from a linear regression analysis on FHTHR, HTR, DHTR, and non-specific reaction in between two groups.

	Group I		Group II		Total	*P*-value	Odds ratio	95% CI
	No. of cases	% age	No. of cases	% age				
FNTHR	2	1.1	10	14.3	12	0.001*	14.00	2.9817–65.7342
Allergic	2	1.1	3	4.3	5	0.107	3.76	0.6146–23.0171
HTR	0	0.0	1	1.4	1	0.108	7.79	0.3136–193.5663
DHTR	2	1.1	8	11.4	10	0.001*	10.84	2.2400–52.4455
Non-specific	0	0.0	3	4.3	3	0.005*	18.72	0.9542–367.2048
Total	6	3.3	25	35.7	31	0.001*	16.11	6.2345–41.6340

FNTHR: Febrile non-hemolytic transfusion reaction, HTR: Hemolytic transfusion reaction, DHTR: Delayed hemolytic transfusion reaction, CI: Confidence interval. *P<0.05 was considered statistically significant.

anti-K and 2.87% participants developed antibodies against anti-D, anti-C, and anti-E in the study by Sadeghian *et al.*[21] Singer *et al.*[15] have shown a decrease in alloimmunization rates from 33% to 2.8% by providing phenotype matched blood for Rh and Kell antigens. Alloimmunization has been linked to antigen discrepancy between donor and recipient. Chronically transfused thalassemia patients who were given antigen-matched donor RBC units (Rh and Kell) had shown improved RBC survival and diminished frequency of transfusion. The frequency of autoantibodies was significantly higher in alloimmunized Group II as compared to non-alloimmunized Group I (37.0% vs. 5.0%). Autoantibodies production in thalassemia patients has been previously reported in the studies by Dhawan *et al.*,[22] Obaid *et al.*,[23] Guirat-Dhouib *et al.*,[24] Davari *et al.*,[25] and Jain *et al.*[26] There is a strong association between autoantibody and alloantibody development. B-cells are the cornerstone of autoimmunity and are also key elements in the initiation of alloantibody production. Dinardo[27] reported a significant prevalence of autoantibodies (6.54%) that were much higher in the group of alloimmunized patients in comparison to the non-alloimmunized group (2.32%). Transfusion reactions were more in the group receiving non-phenotype matched blood than phenotype matched blood (35.7% vs. 3.3%). There was an absolute significant difference with in FHTHR between two groups 0.001 (95% confidence interval [CI] 2.98–65.73). Similar results were obtained for D (P = 0.001) (95% CI 2.24–52.44) and non-specific reactions (P = 0.005) (95% CI 0.95–367.20) as well. Febrile, allergic, and DHTR were the most frequent reactions observed as shown in a similar study Kasraian and Karimi[28] and Savage.[29]

CONCLUSIONS

The alloimmunization rate in transfusion dependent thalassemia patients was 4.4%. The rate of alloimmunization was higher in multi-transfused patients with TDT who received non-phenotype matched transfusion (15.7%) and no alloimmunization was detected in those who received partial phenotype-matched blood. The dominant alloantibodies in the study were directed against Rh antigen (8.5%) and Kell antigen (7.1%). It is recommended that a policy of partial matched phenotype blood must be adopted in transfusion regimens for all thalassemia patients in developing countries like India to offer a balanced, cost-effective alternative to minimize red cell alloimmunization and autoimmunization. Follow-up testing and documentation of alloantibodies in the transfusion records should be done at each visit to mitigate DHTRs.

Ethical approval

The authors declare that they have taken the ethical approval from IEC (IEC No.:2023-848).

Declaration of patient consent

Patient's consent not required as patients identity is not disclosed or compromised.

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Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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