ORIGINAL ARTICLE

Frequency of Red Cell Alloimmunization in Multitransfused Thalassemia Patients

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ABSTRACT

Background: Thalassemia major patients managed by regular transfusion regimen may develop alloimmunization against foreign red cell antigens. If the alloantibodies are hemolyzing, transfusion reaction can occur, and provision of blood thereafter requires phenotypically matched blood in addition to 'ABO" and Rh 'D' typing.
Aim: To determine the rate of occurrence of red cell alloimmunization in regularly transfused Thalassemia patient.
Setting: Hematology department King Edward Medical University and affiliated hospitals
Duration of the study: Six months after the approval of synopsis From: 18th November 2016 to 18th May 2017
Study design: Descriptive study
Methodology: This study was conducted in King Edward Medical University and its affiliated hospitals on patients with diagnosis of Thalassemia. After informed consent, a detailed transfusion history was taken using a set questionnaire which mentioned the name, age and sex, identification number, blood group, transfusions till date, diagnosis, transfusion history (date, frequency of transfusion and blood component types), total number and duration from last transfusion, any relevant drug history, and result of serological testing like DAT test and IAT was noted. If indirect coombs test is positive then antibody screening and identification is done (using panel of 11 cells).
Results: Out of 138 cases, 42(30.30%) were between 2-10 years of age, 69(50.30%) were between 11-20 years of age and 27(19.39%) were between 21-30 years of age, mean±sd was calculated as 7.71±5.84 years, 84(61%) were male and 54(39%) were females. The occurrence of red cell alloantibodies in regularly transfused thalassemic patients was recorded in 29(21.01%). Frequency of specific types of most frequent alloantibodies in multi-transfused thalassemia patient shows that 22(76%) had Anti-Rh (Anti-D 8(27.5%), Anti-C 5(17%), Anti-c 1(3.4%), Anti-E 2(7%), Anti-e 4(14%), Anti-C^w 2(7%), 6(21%) had Anti-Kell (Anti-K 4(14%), Anti-kp^a 2(7%), 1(3.4%) had Anti-MNS (Anti-N3. 1(4%) type of alloantibody.
Conclusion: Rate of frequency of red cell alloantibodies occurrence is higher in frequently-transfused Thalassemic patients as compared to thalassemia patients with less no of transfusions while Anti-Rh was the commonest type of antibody detected. These findings are helpful for thalassemic patients receiving multiple transfusions,so these patients must receive cross-matched units and ideally their rbcs should be phenotyped to prevent alloimmunization.
Keywords: Thalassemia, multi-transfusion, red blood cell alloantibodies

INTRODUCTION

Thalassemia is an inherited disorder, due to partial or complete deficiency in formation of α or β-globin chains**.** The alternative treatment option for thalassemia (stem cell transplantation is not available) is by regular red cell (RBC) transfusion to keep the hemoglobin (Hb) concentration between 9 and 11.5 g/dL. One of the many risks associated with regular transfusions is development of antibodies against foreign red cells antigens. This genetic disparity between donor and recipient is the main reason of alloimmunization[1].

Alloimmunization is defined as formation of antibodies in the recipient against red cell antigens of donor because of genetic dissimilarity between donor and recipient belonging to same species.[3]It is frequently seen in regularly-transfused patient[2].

Production of red cell (RBC) alloantibodies and autoantibodies can cause transfusion difficulty. If alloantibodies are hemolytic, hemolytic transfusion reactions can occur and lowers the compatible RBC units availability for transfusions difficult while the others are clinically insignificant. Autoantibodies are rare but if present can result in clinical hemolysis and cross-match difficulties[4].

The incidence of development of alloantibodies in thalassemia patients varies from 5-30% in different regions of world. The main reason behind alloimmunization is minor blood group antigens. 22% incidence of alloantibodies has been seen in asians[5].

Thalassemia is a heterogeneous group of genetic disorders of globin chains of haemoglobin. Every year in india about 8000-10000 new thalassemics (homozygous)are reported and rate of occurrence of beta thalassemic gene is found more abundantly in sindhis, Bengalis, Punjabis and Gujratis.[1]

Certain factors are associated with increased chances of alloimmunization of which frequency of blood transfusion and inflammatory state of recipient are most important[6-7]. Female gender and pregnancy are other risk factors. Racial difference between donor and recipient is also important factor causing alloimmunization.[8] Majority of the individuals develop alloantibodies after 14 days of blood transfusion[9].

A study conducted by Philip, Joseph in western India and according to this study the overall rate of occurrence of RBC alloantibodies was 5.5%.Most of the alloantibodies (72.7%) belonged to the Rh blood group system.[10]

In a study conducted in Egypt, out of 272 patients, 62 patients (22.8%) were found to have alloantibodies. Rh-related alloantibodies were frequently seen in these patients[11]

In another study, 8.5% patients (6/70) were found to have red cell alloimmunization and only one autoantibody was found (1.42%). All detected alloantibodies were from Rh system (i.e. anti-E , anti D) and Kell system (anti-K).Increased production of alloantibodies was seen in frequent transfusions and in patients with regular transfusions above 1 year of age.[4]

In a study conducted in Rawalpindi, 75 cases of thalassemia major were detected to have alloantibodies by IAT, using 3 cell panel and 11 cell panel. 17 (22.7%) patients found to have red cell alloantibodies. the commonest red cell alloantibody was Anti-Kpa, followed by Anti-e, anti-E and anti-K antibodies, respectively. One patient each have Anti-Rh'D', -K, -Kpb, -CW, -Fyb and –c.[12]

The purpose of this study is to determine prevalence and specificity of red cell alloantibodies in regularly-transfused Thalassemia patient. This will help to determine whether Thalassemia patients having regular blood transfusions are more prone to develop alloantibodies or not and which type of alloantibody is the most common. This study will emphasize the risks associated with alloimmunization and importance of proper cross-matching and typing of minor blood groups especially in multitransfused patients such as Thalassemics.

The aim of this study was to determine the rate of occurrence of red cell alloimmunization in regularly transfused Thalassemia patients.

OPERATIONAL DEFINITIONS

Alloimmunization: Alloimmunization is defined as formation of antibodies (alloantigens) in the recipient of blood transfusion received from donor of the same species by DAT and IAT 15 days after last blood transfusion.

Alloantibodies: These are antibodies that are formed against genetically dissimilar antigens and include Anti-Rh, Anti-K, Anti-kidd, Anti-D, Anti-Lewis, Anti-MNS alloantibodies.

Thalassemia: It is an inherited genetic disorder diagnosed either on electrophoresis, Hb estimation or PCR, that results from deficient synthesis of either α or β globin chain causing excessive hemolysis of RBC's and anemia.

Multi-transfused patient: Patients who have received more than 5 blood transfusions in 2 years time.

MATERIALS AND METHODS

This descriptive study was conducted in Hematology Department King Edward Medical University and affiliated hospitals during a period six months after the approval of synopsis from 18th November 2016 to 18th May 2017. Sampling technique used was non-probability purposive sampling. Sample size of 138 patients is estimated by using 95 % confidence level. 7 % margin of error with expected percentage of alloantibodies in multi-transfused patients as 22.8 %

Inclusion Criteria:

1. Diagnosed Thalassemia patients on Hb electrophoresis(as per operational definitions)
2. Patients of both genders
3. Patients age between 2-30 years
4. Patients requiring blood transfusion who have been transfused more than 5 times(in last 2 years) with last transfusion given 14 days prior as identified from medical record.

Exclusion Criteria:

1. Patients with known alloantibody as per medical record.
2. Patient with any autoimmune disease (Systemic lupus erythematous, rheumatoid arthritis, etc.) as per medical record.

Data collection procedure: This study was conducted in King Edward Medical University and its affiliated hospitals on patients with diagnosis of Thalassemia. After informed consent, a complete transfusion history was taken using a questionnaire including the name, age, gender history, diagnosis, blood group, transfusions till date, identification number, total number and time period from last transfusion, any relevant drug history, and result of serological testing like DAT (using patient's RBC's and Coomb's reagent that determined alloantibody) and IAT (using patient's serum and O positive cells and Coomb's reagent that determined alloantibody) was noted.

Data analysis procedure: The data was entered and analyzed by statistical package for the social sciences (SPSS) version 22. Quantitative variable like age was presented as mean ± Standard deviation. Qualitative variable like gender and RBC alloantibodies with various common types were presented as frequency and percentages. Results were discussed in tabulated form with reference to effect modifiers like age, gender, number of blood transfusions.

RESULTS

A total number of 138 patients that fulfilled the inclusion/exclusion criteria were selected to know the rate of occurrence of red cell alloantibodies in regularly-transfused Thalassemic patients and to know the specific types of most common alloantibodies in them.

Age distribution of the patients show that 42(30.30%) were between 2-10 years of age,69(50.30%) were between 11 to 20 years of age and 27(19.39%) were between 21 to 30 years , mean±standard deviation was calculated as 7.71±5.84 years (Table 1).

Gender distribution shows that 84(61%) were male and 54(39%) were females (Table 2).

Incidence of red cell alloantibodies in regularly-transfused thalassemia patient was recorded in 29(21.01%) whereas 109(78.98%) had no findings of the morbidity (Table 3).

Frequency of specific types of most frequent alloantibodies in multi-transfused thalassemia patient shows that 76%(n=22) had Anti-Rh (Anti-D27.5% (n=8), Anti-C17%(n=5), Anti-c3.4%(n=1), Anti-E7%(n=2), Anti-e14%(n=4), Anti-Cw7%(n=2), 6(21%) had Anti-Kell (Anti-K14%(n=4), Anti-kpa7%(n=2)),3.4%(n=1) had Anti-MNS(Anti-N3.4%(n=1) type of alloantibody (Table 4).

The results were discussed in tabulated form with reference to effect modifiers like age, gender, number of blood transfusions (Table 5-7).

Table 1: Distribution of age (n=138)

Age(years)	n	%age
2-10	42	30.30
11-20	69	50.30
21-30	27	19.39
Total	138	100
Mean±SD	7.71±5.84	

Table 2: Frequency of red cell alloantibodies in multi-transfused thalassemia patients (n=138)

RBC Alloantibodies	n	%age
Yes	29	21.01
No	109	78.98
Total	138	100

Table 3: Frequency of specific types of alloantibodies in multi-transfused thalassemia patients (n=29)

Specific group of most frequent alloantibodies	Types of alloanti-bodies	n	%	Total
Anti-Rh	Anti-D	8	27.5	22(76%)
	Anti-C	5	17	
	Anti-c	1	3.4	
	Anti-E	2	7	
	Anti-e	4	14	
	Anti-Cw	2	7	
Anti-Kell	Anti-K	4	14	6(21%)
	Anti-kp^a	2	7	
Anti MNS	Anti-N	1	3.4	1(3.4%)

Table 4: Stratification for prevalence of red cell alloantibodies in regularly transfused thalassemic patients with regards to age(n=138)

Age (in years)	RBC Alloantibodies	
	Yes	No
2-10	7	35
11-20	12	57
21-30	10	19

Table 5: Stratification for prevalance of red cell alloantibodies in regularly transfused thalassemic patients with regards to gender (n=138)

Gender	RBC Alloantibodies	
	Yes	No
Male	20	64
Female	9	45

Table 6: Stratification for prevalance of red cell alloantibodies in regularly transfused thalassemic patients with regards to no. of transfusions(n=138)

No. of transfusions	RBC Alloantibodies	
	Yes	No
5-10	18	76
>10	11	33

DISCUSSION

Anti-red cell alloimmunization may develop in thalassemia major patients on regular transfusion treatment. The alloantibodies are usually hemolyzing, due to which transfusion reaction may occur requiring extended phenotypially matched blood. We planned this study to determine the frequency and specificity of red blood cell alloantibodies in multi-transfused Thalassemia patient. This may help to determine whether Thalassemia patients receiving multiple transfusions are at a high risk of developing alloantibodies or not and which type of alloantibody is the most common. This study is also helpful for creating awareness in the clinicians of risk of alloimmunization and importance of proper cross-matching and typing of minor blood groups especially in multitransfused patients such as Thalassemics.

Out of 138 cases, 42(30.30%) were between 2-10 years of age, 69(50.30%) were between 11-20 years of age and 27(19.39%) were between 21-30 years of age, mean±sd was calculated as 7.71±5.84 years, 84(61%) were male and 54(39%) were females. Incidence of red cell alloantibodies in regularly-transfused thalassemia patient was recorded in 29(21.01%).

Frequency of specific types of most frequent alloantibodies in multi-transfused thalassemia patient shows that 22(76%) had Anti-Rh(Anti-D27.5%(n=8),Anti-C17%(n=5),Anti-c3.4%(n=1),Anti-E7%(n=2),Anti-e14%(n=4),Anti-C^w7%(n=2),21%(n=6) had Anti-Kell(Anti-K14%(n=4),Anti-kp^a7%(n=2)),3.4%(n=1) had Anti-MNS(Anti-N3.4%(n=1) type of alloantibody.

In our study the frequency of alloantibodies came to be 21.01%(n=29) that means just crossmatching the recipient blood with donor is not enough ideally all multitransfused patients should be genotyped or atleast phenotyped to minimize the chances of red cell sensitization with foreign antigen.

The frequency of specific type of most frequent alloantibody detected in multitransfused thalassemic patients was anti-Rh 76%(n=22) with anti-D 27.5%(n=8) being the commonest alloantibody detected.Rh is a high prevalence antigen 85% population express this antigen,so there are increased chances of alloantibody to form against high prevalence antigens as most of the donors are Rh positive and this antigen may be missed on blood grouping if there is weak expression of Rh antigen(Du).

We compared our results with a study conducted in Egypt, alloimmunization incidence was 22.8% in 272 patients, the findings of our study. The most frequent alloantibody was Rh-related (37.4%)[11]

In a study conducted in Rawalpindi, 75 cases of thalassemia major were detected to have alloantibodies by indirect antiglobulin test, using 3-red cell panel, and sometimes 11-red cell panel. 17 (22.7%) patients found to have red cell alloantibodies. the commonest red cell alloantibody was Anti-Kpa, followed by Anti-e, anti-E and anti-K antibodies, respectively. One patient each have Anti-Rh'D', -K, -Kpb, -CW, -Fyb and –c.[12]The findings of our study correspond to these results.

In another study, red cell alloimmunization was found in 8.5% patients (6 out of 70) and only one autoantibody was found (1.42%). All detected alloantibodies were from Rh system (i.e. anti-E, anti D) and Kell system (anti-K).Increased production of alloantibodies was seen in frequent transfusions and in patients with regular transfusions above 1 year of age.

In last three studies the frequency of alloimmunization is low as compared to our study, either the target antigens show dosage effect so the antibody detection is missed or

the donors used for transfusion are totally cross-matched with the recipients so there are less chances of alloantibody to form.

The above discussion reveals that the incidence of red cell alloantibodies varies in regularly transfused Thalassemic patient which highlights the importance of research studies in different populations. However, the findings of our study are primary in our population which needs validation through meta analysis. The conclusion is, all the patients that need multiple transfusions to survive should ideally be phenotyped to prevent alloimmunization.

CONCLUSION

We concluded that the incidence of red cell alloantibodies is higher in regularly transfused Thalassemic patients while Anti-D was the commonest type of antibody. These findings are helpful for thalassemic patients receiving multiple transfusions.

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