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ORIGINAL ARTICLE

Alloimmunization and Autoimmunization in Multi-Transfused Thalassemic Patients: A Single Center Study

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ABSTRACT

Aim: To determine frequency of alloimmunization and autoimmunization among the patient of thalassemia with history of multiple blood transfusions.
Methods: This study was conducted by consecutive sampling in six months duration from September 2020 to August 2021 at the department of Hematology and blood Transfusion Medicine, of University of Child Health Sciences, The Children's Hospital Lahore. Any patient with a positive Direct antiglobulin test (DAT) was labeled as auto-immunized and a positive indirect antiglobulin test (IAT) was labeled as alloimmunized.
Results: Total 90 cases were enrolled with 62% male and 38% females with a mean age 6.04 + 3.3 years (range of age: 7 months to 14 years). Antibody screening and auto-antibodies were positive in 4.4% (n= 4) cases each, and allo-antibodies in 6.7% (n=6) cases (anti-E in 2, anti-C in 2, anti-K and anti-e in 1 each). Among all these patients of beta thalassemia, spleen was enlarged in 71% cases; around half of these were <5 years old. Around 96% had first transfusion <2 years. The frequency of patients positive on DAT, IAT and on anti-body screening was significantly related to the frequency of blood transfusion
Practical Implications: Autoantibodies produce a positive direct antiglobulin test (DAT), resulting in hyperhemolysis of red cells in thalassemic patients, which exacerbates the existing alloantibodies factor in children who have received several blood transfusions. The gap between transfusions will be shortened. Routinely, greater emphasis is placed on the identification of allo-antibodies, but auto-antibodies should also be evaluated so that this phenomena may be better understood and handled. This study was conducted to examine the link between a greater transfusion demand and the existence of auto or allo antibodies in Thalassemia patients. I
Conclusion: We found Alloimmunization and autoimmunization together not very uncommon in transfusion dependent patients of Thalassemia. The frequency of transfusions is affected in co-existing states. Extended matching, as well as early diagnosis and control of Auto and allo-antibodies both can improve the efficacy of blood transfusion.
Keywords: Autoantibodies, IAT, DAT, Coomb's test, Thalassemia, Allo-immunization

INTRODUCTION

Thalassemia are a diverse category of hereditary anemias caused by gene abnormalities that impact α- or β - globin chain production. Anemia is a recurrent symptom of thalassemia syndromes, produced by inadequate erythropoiesis and hemolysis, both of which are caused by an imbalance in globin chain synthesis. The main type of assistance for severe thalassemia is frequent red cell transfusion. One of the most significant side effects of transfusion is the formation of alloantibodies and in certain cases, autoantibodies against red blood cell (RBC) antigens[1].

The response of the body toward blood transfusion leading to development of alloimmunization to the antigens on RBCs is one of the immunological responses that are frequently caused by RBC transfusion and during pregnancy. It is one of the known facts that IgG antibodies can develop against the donor RBCs following blood transfusions. These IgG antibodies attach to the transfused RBCs and may cause them to be destroyed[2]. Several investigations have found varying levels of RBC allo-antibodies in beta thalassemia major patients[3,4].

Furthermore, patients in whom splenectomy was done individuals may have a greater rate of RBC alloimmunization. The lack of a spleen may hasten the immunological response to injected foreign antigens that are not adequately screened[5].

The Direct and Indirect Coombs' tests are commonly recognized as one of the most sensitive and specific tests used for the diagnosis of hemolysis due to the identification of immunoglobulin G (IgG) and the antigens bound to the RBCs. In multi-transfused thalassemic individuals, red cell allo- and auto-immunization is a well-known issue[6].

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The rationale of this study was that there is still limited local data available regarding the frequency of combined alloimmunization and autoimmunization among the patients of thalassemia with multiple blood transfusions. Autoantibodies result in positive direct antiglobulin test (DAT), leading to hyper-hemolysis of red cells in thalassemic patients which aggravate the existing alloantibodies factor in multi transfused children. The transfusion interval will be lessened. In routine more focus is on allo-antibodies detection but there should be auto antibodies workup done side by side so that this phenomenon can be better understood and managed. We have designed this study to determine the correlation between increased transfusion requirement and presence of auto or allo antibodies in Thalassemic children. Furthermore, with the detection of allo- and auto- antibodies treating physicians will be able to take decisions regarding need of antigen negative blood transfusions.

MATERIALS & METHODS

In this cross-sectional study, subjects who met the operational definitions were asked to sign a consent form or their parents were asked to provide informed permission and the Ethical Review Committee approved the trial. Objective of the study was explained to the participants. All these patients were seen from Sep 2020 till August 2021 in the Department of Hematology & Transfusion Medicine, University of Child Health Sciences, The Children's Hospital Lahore.

A child was diagnosed with -thalassemia if he or she had a history of numerous transfusions before the age of two, with or without clinically visible splenomegaly and high fetal hemoglobin (Hb-F) on Hb electrophoresis. Patients with thalassemia who have had more than 20 blood transfusions are said to have multi-transfused beta thalassemia.

Multi-transfused beta thalassemia patients having autoantibodies diagnosed on DAT were labeled as having autoantibodies due to auto-immunization. And those positive on saline indirect Anti-globulin test IAT were labeled as Allo-immunized.

With the help of the WHO Sample size calculator, 90 cases of multi transfused beta thalassemia were included. Expected frequency of antibodies (-allo and –autoantibodies) in multi-transfused thalassemic children to be 17.3%[7], Confidence level = 95% and absolute precision = 8%. All those patients already diagnosed with hemolytic anemia, known cases of any malignancy disease and patients on immunosuppressive medications were excluded from the study.

A 3-5 mL plain vial (clotted) and a 2 mL ethylene diamine tetra acetic acid (EDTA) anticoagulant sample were obtained for each subject. After being separated into three aliquots, the serum was frozen at -80°C before being thawed to room temperature and used for antibody screening. The EDTA sample was used to perform DAT. The antibody screening will be performed by the conventional tube technique (CTT). To determine the specificity of the alloantibody, alloantibody-positive samples were tested utilizing an eleven-red cell detection panel. The patients' sera were frozen at -80° C and will be tested following thawing. As a result, autoantibodies were only discovered by DAT during pre-transfusion testing with polyspecific anti-human globulin (AHG). CTT was also used to differentiate autoantibody positive patients utilizing mono-specific anti-IgG AHG and anti-C3d AHG.

The procedure and use of research study was explained to the guardians of patients. A detailed history and physical examinations were done. Age, gender, blood group, time since diagnosis of thalassemia, frequency of blood transfusion in a month and results of DAT and IAT were noted. All the data was noted in a predesigned data entry sheet.

After the analysis of the data using software SPSS, different variables were presented in the form of graphs and tables, showing frequencies and mean with standard deviations. Data of outcome variables was also stratified and significance was calculated using appropriate statistical test (p of less than or equal to 0.05 was be considered significant).

RESULTS

Total 90 cases were enrolled of children included were from 7 months to 14 years. Age groups were: Three of 90 were < two years old, 30 patients were of age two to five years and 36 of age group six to ten years while thirteen were of age group ten years and above.

Details of regarding time since diagnosis of beta thalassemia major, frequency of blood transfusions in a year, Total transfusions per year, presence of enlarged spleen and blood groups of these patients is shown in the table no 1. Patients of all four types of ABO blood groups were enrolled in the study. Around 91% were Rh positive and only 9% were Rh negative. As all these were multi-transfused beta thalassemic patients, 65 of 90 patients (71%) had enlarged spleen. 32 were of age < 5 years and rest 33 were of age > 5 years age, p value was not significant.

90 (96%) patients had a history of first transfusion before 2 years of age, with 54 male patients and 32 female patients. Among these 86 patients, frequency of blood transfusion was more than one transfusion per month for 56 (65%) patients (Male 34 and Females 22). And 43 of these 56 patients with very frequent blood transfusions, had splenomegaly. (Male 25, and females 18)

Splenomegaly was seen in 65 of 90 cases. Among these 65 patients, 18 patients had blood group A, 22 had blood group B, 6 had blood group AB while 19 had blood group O as per the ABO blood group system, the difference was not significant (p value of 0.380). Similarly, among these 65 patients with splenomegaly, 6 had Rh negative blood group and 59 were of positive Rh group, p value was significant (p value 0.034). Among the total Rh positive blood group patients of multi-transfused beta thalassemia patients, 59 of 82 (71.9% patients) had splenomegaly.

Among all multi-transfused beta thalassemia patients, antibody screening and allo-antibodies were positive in 6.7% (n= 6) (anti-E in 2, anti-C in 2, anti-K and anti-e in 1 each) and auto-antibodies in 4.4%(n=4) cases. Splenomegaly was found in 71% patients, 50% of which were <5 years. 95.6% had history of first transfusion before 2 years of age. DAT positive patients were further tested with monospecific reagent and all were Ig G type. All patients positive on DAT and positive on IAT and screening were diagnosed before age of 2 years and now having frequency of 2 blood transfusions per month.

Table 1: Details of all the variables with their percentage and Frequencies.

Variables	No.	%
Age < 5 years	41	45.6
> 5 years	49	54.4
Gender Male	56	62.2
Female	34	37.8
Blood groups		
A	24	27%
B	31	34%
AB	6	7%
O	29	32%
Rh Positive	82	91%
Negative	8	9%
Splenomegaly	Yes 65	72.2
	No 25	27.8
Splenectomy	Yes 1	1.1%
	No 89	98.9%
Frequency of blood transfusion (per month) n = 90		
01 transfusion per month	33	36.7%
02 transfusion per months	50	55.6%
03 transfusion per months	6	6.7%
04 transfusion per months	1	1.1%
> 12 transfusions per year	56	62%
First transfusion before 2 years of age	Yes: 86	96%
	No: 4	4%
Positive Antibody screening	6 of 90	6.67%
Allo-immunization	6 of 90	6.67%
Auto-immunization	4 of 90	4.4%
anti-E	2 of 6	33.3%
anti-C	2 of 6	33.3%
anti-K	1 of 6	16.7%
anti-e	1 of 6	16.7%

Table 2: Details of the all the positive cases on Antibody screening

Details of all cases with allo-immunization and/or autoimmunization										
Age in years	**Blood Group (ABO)**	**Rh (+/-)**	**Age at Dx in years**	**Frequency of Tx/ month**	**>12 Tx/ year**	**Total Tx /year**	**Spleen**	**Antibody screening**	**DAT**	**IAT**
7	O	+	2	2	Yes	25	Enlarged	Positive	Positive	Positive
4	B	+	1	2	Yes	26	Enlarged	Positive	Positive	Positive
4	B	+	1	2	Yes	28	Enlarged	Positive	Positive	Positive
7	O	+	2	2	Yes	15	Enlarged	Positive	Positive	Positive
9	A	+	1.5	2	Yes	13	Enlarged	Positive	Negative	Positive
8	O	+	2	2	Yes	16	Enlarged	Positive	Negative	Positive

Spleen was enlarged in 71% of the patients and among all these patients, disease started before the age of 2 in 96% cases. Table 1 showing values of various other variables of the patients.

Antibody screening, allo-antibodies and auto-antibodies were positive in 6.67%, 6.67% and 4.4%, respectively. Table no 2 showing the details of the all the positive cases on antibody screening, were found to fall in the category of auto-immunization and allo-immunization. 4 out of total 90 cases of multi-transfused bête thalassemia were positive on DAT and 6 of 90 positive on antibody screening, were also IAT positive. All these patients had significant splenomegaly. Stratification of DAT, IAT and anti-body screening for frequency of blood transfusion showed a significant p value (p value < 0.001)

DISCUSSION

Frequent transfusions among the beta-thalassemia patients have multiple adverse effects. These range from early transfusion reactions to transmission of viral infections and iron over loading. One of the consequences of frequency blood transfusion ignored is the red blood cell allo immunization and auto-immunization. Rare phenotypes for the Rh blood grouping system and Kell antigens discovered were introduced in the start of last decade for blood donors and patients receiving blood transfusions. The purpose of this study was to determine the number of patients who were multi transfused beta-thalassemic and had red cell alloimmunization and auto-immunization.

In this study, patients with all four types of ABO blood groups were included. Approximately 91% were Rh positive, whereas just 9% were Rh negative. Because these were all multi-transfused beta thalassemic individuals, 65 of 90 (71%) had enlarged spleens. Thirty-two (50%) of the 64 patients with splenomegaly were under the age of five.

Among the patients included in the study, 86 of 90 patients (96%) got their first transfusion before the age of two, including 54 male patients and 32 female patients. For 56 (65%) of these 86 patients, the frequency of blood transfusion was greater than one transfusion per month (Male 34 and Females 22). In addition, 43 of the 56 patients who required regular blood transfusions developed splenomegaly. (Men aged 25 and ladies aged 18)

According to Jain et al., 17.3% of 301 patients tested positive for antibodies (-allo and –autoantibodies). In 48 (15.9%) of the patients, the direct antiglobulin test (DAT) was positive. The number of patients with autoantibodies was more than the number of thalassemia patients in the allo-immunized group when compared with the control group of patients (60% vs 14.4%). They advocate antigen typing for both major and minor blood groups in all thalassemia major patients before beginning transfusion therapy, as well as screening for allo- and autoantibodies before each transfusion. Anti-Kell anti bodies were seen in 54% cases, anti-D antibodies in 18% cases, anti-c in 9% patients. In addition, the level of hemoglobin noted before the blood transfusion among the patients in vaccinated group was considerably lower (8.5 gm/dl vs 9.0 gm/dl; p=0.03) as compared to the cases in the non-immunized group. Our investigation did not record this pre-transfusion Hb. Based on these findings, it was advised that patients should undergo antigen typing for rare blood group types. Additionally, allo- and auto-antibodies should be screened at regular intervals before to each transfusion[7]. In a meta-analysis, the most prevalent alloantibodies found among the transfusion dependent patients were anti-K antigen and anti-E, -D, -C, and –c antibodies[8].

Thedsawad A, et al reported after collecting data of DAT and IAT tests among the thalassemia patients that 8 cases (13.6%) and 34 cases (57.6%) had positive DAT and flow cytometry, respectively while in 20 cases (33.9%) RBC alloantibodies were seen (anti-E (55%), anti-M (40%), anti-D (25%) and anti-c (15%). Splenectomy and higher transfusion requirements were strongly linked with the presence of RBC-bound IgG but not with the development of RBC alloantibody. In multi - transfused individuals with thalassemia, the total frequency of RBC alloantibody production was 33.9 percent. Flow cytometry revealed RBC autoantibody production more frequently (around 58 percent) as compared to the direct Coombs (around 14%t). The history of removal of Spleen has been linked to the generation of auto antibodies bound to the RBCs among the cases of thalassemia with more than two transfusions in a month. Presence of these auto- Antibodies, basically anti-RBC IgGs can be one of the major factors leading to rise in the blood transfusion requirement[9].

In a retrospective study, data of 160 β-thalassemic patients was collected during 9 years regularly in Morocco, and data was analyzed to compare various characteristics of allo immunized and non-alloimmunized patients. Autoantibodies were detected using direct anti-globulin tests through coombs gel cards. Prevalence of allo-immunizations was 8.75%, 17 alloantibodies were against Kell and Rh systems: 35% for Kel-1, 23% for Rh-3, 12% for Rh-1 and Kpa each, 6% for Rh-2. Autoantibodies were seen in 6 of 14 (43%) of allo immunized patients versus 12 of 146 (12%) of non-alloimmunized patients (P<0.01). Factors related to a higher rate of autoantibodies reported in the study were a transfusion rate of one transfusion in every 3 weeks and gender[10].

In a study data was collected from 36,000 patients of blood transfusions, 116 had irregular RBC alloantibodies with around 450 cases of multiple transfusions (16 units / patient). Among these cases of multiple transfusions, in 79 patients (around 18 percent) RBC allo antibodies were detected. In the remaining thirty-five thousand transfusion cases, 37 had RBC alloantibodies, the rate of RBC alloimmunisation was 0.10 percent. The most prevalent antibody detected in more than half of the multiple antibody cases was anti-E[11].

Makarovska-Bojadzieva T, et al studied the development of auto- and allo-iimunity similar to that done in our study and reported that RBC alloimmunization in the thalassemia patients when carefully cross matched for rare blood group antigens lead to a decrease in the frequency of allo-immunization from around 40% to 17%, with anti-E in 26% cases and anti-K in 13% of transfusion patients.

The RBC antigen discrepancy between donor and recipient, or between mother and fetus, causes RBC alloimmunization. Antigens (sugars and proteins) on the surface of RBCs are intrinsically related to membrane proteins or lipids. These surface molecules' ability to elicit an immune response is what makes them clinically important for blood component transfusion and tissue/organ transplantation[12].

Overall, autoantibodies can be found in up to 28% of cases of beta-thalassemia with numerous blood transfusions, up to 40% of cases of sickle cell disease, and up to 60% of cases of hereditary spherocytosis. Warm antibodies (IgG+) were found in 50% of DAT-positive individuals, but nothing is known about the Ab type. In investigations, allo-antibodies, transfusion exposure, and splenectomy have all been found as risk factors for autoAb development[13].

Review of literature was done by Motta I, et al on the incidence of autoimmune hemolytic anemia (AIHA) in patients of congenital anemia. They were of the conclusion that AIHA is difficult to diagnose and manage. Patients with congenital anemias should be evaluated regularly using hemolytic markers, marrow aspirations to see for compensatory changes, and direct coombs tests (DAT) for allo-Abs and auto-Abs. Immune-mediated hemolytic crises can aggravate congenital anemias. Autoantibodies have also been identified, albeit they are seldom linked with overt autoimmune hemolytic anemia. Allo- and auto-antibodies are both detected in persistently transfused patient and can be a cause of few but lethal complications[14].

In a study, allo-antibodies in beta thalassemia major patients were studied. Males and females made up 35.8 percent and 64.1 percent of the patients, respectively, with 63 percent

having alloimmunization. Anti-K, anti-D, and anti-E alloantibody variations were with a frequency of 13%, 6%, and 5.4%, respectively. Regular RBC antigen testing as well as problem-solving of alloantibody development by getting suitable blood for Kell and RH subgroups, are recommended for all instances of transfusion-derived thalassemia prior to RBC transfusion[15].

In a study in Egypt, 200 individuals from 2 to 37 years were enrolled to determine the percentage of auto- and allo-antibodies in multi-transfused thalassemia. Alloantibodies were found in 18% of the patients, whereas autoantibodies were found in 16.5%. The Kell (33%) and Rh (24.4%) families were the most commonly targeted by alloantibodies. Alloimmunization was shown to have a strong relationship with treatment duration and transfusion frequency (P=0.007 and 0.001, respectively). Risk factors leading to the development of autoantibodies were discussed. It was reported that frequency of auto-immunity among these thalassemia patients was seen to be affected with age, No. of blood unit transfusions, and history of splenectomy. The authors were of the conclusion that the higher rate of allo-immunization seen in their study highlighted that blood grouping and cross matching of blood before transfusion should use phenotypically matched cells for Kell and Rh subgroups and rare blood groups as well in order to decrease the chances of development of allo immunity, thus increasing the blood transfusion efficiency[16].

Individuals with diseases requiring frequent transfusions include sickle cell disease, myelodysplastic syndrome, or chronic myelomonocytic leukemia, as well as non-hematologic patients, can acquire allo- and auto-antibodies[17]. The occurrence of RBC allo-immunization can be one of the causes of hemolytic transfusion reactions. These reactions are due to mismatching of the blood groups specially the rare one and due to presence of auto-antibodies. RBC alloimmunization is one of the challenges faced by the laboratories and blood banks with cross matching serves for blood transfusions in terms of providing suitable blood for transfusion. These antibodies lead to delayed transfusion reactions in some cases.

CONCLUSION

Alloimmunization and autoimmunization is not very uncommon in transfusion dependent patients of Thalassemia. Extended matching, as well as early diagnosis and control of Auto and allo-antibodies, can improve the efficacy of blood transfusion.

Conflict of interest: I hereby declare that there is no conflict of interest for this study.

Limitation of study: A small sample size along with a small duration of study is the major limitation of this study. Further studies should be done at a larger scale and complications of auto-immunity and allo-immunity should also be noted; along with the factors other than discussed in this study which can be a cause of auto-immunity and allo-immunity.

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