ORIGINAL ARTICLE

Study of Production of Alloantibodies in Multiple Transfused Thalassemia Patients in Pediatric Age Group

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ABSTRACT

Introduction: Thalassemia is a heterogeneous group of genetic disorders in which blood transfusion is life saving for thalassemia patients. Repeated transfusion causes multiple unexpected antibodies other than anti-A or B.

Aim: To detect and subtype the irregular antibodies in sera of thalassemia patients in Jaipur.

Materials and methods: A total of 100 patients of proven thalassemia were included in the study at Mahatma Gandhi Medical College and Hospital and JK Lon Hospital, Jaipur, Rajasthan, India. Information on transfusion history was recorded, 5 mL of blood was collected from each subject, and plasma was separated. These samples were subjected to direct Coomb's test (DCT) by column gel agglutination (CGA) technique.

Results: In the present study, the alloantibodies which were most common among the seven positive samples out of 100 are anti-D, anti-K, anti-E, and anti-MN, with an incidence of 28.57, 28.57, 28.57, and 14.3% respectively. Blood group O had maximum number of alloantibodies (57%). According to number of transfusions, alloantibodies of thalassemia major patients showed statistically significant increase with increase in number of transfusion.

Conclusion: From the current study, it can be concluded that alloantibodies to minor group antigens are more frequent among thalassemia patients, which need more frequent blood transfusions.

Keywords: Alloantibodies, Direct Coomb test, Multiple transfusion, Thalassemia major.

INTRODUCTION

The word thalassemia is derived from Greek *Thalassa*—sea and *emia*—blood. The thalassemias are a heterogeneous group of genetic disorder of hemoglobin chain characterized by reduced or absent production of one or more globin chains. Clinical severity varies widely, depending on the degree to which the synthesis of the affected globin is impaired, altered synthesis of the other globin chains, and coinheritance of other abnormal globin alleles. The type of thalassemia usually carries the name of the chain that is not produced.[1]

Although blood transfusion is life saving for thalassemia patients, it may be associated with some complication, such as iron overload, platelet, red blood cells (RBCs) alloimmunization, and infection; therefore, screening for irregular antibody should be a part of all pretransfusion testing.[2]

Unexpected antibodies are antibodies other than naturally occurring anti-A or B. Such antibodies are found in 0.3 to 2.5% of population, depending on the group of patients or donor studied and the sensitivity of the test method used.

Immunization to foreign RBC antigen may result from pregnancy or transfusion or following deliberate injection with immunogenic material. In some instance, the immunizing event is unknown.[3]

Antibody specificity and its ability to react *in vitro* at 37°C are two serological characteristics that have been most helpful in predicting *in vivo* significance.

According to the Food and Drug Administration, the following antigens present on red cells are permitted for antibody screening: D, C, E, c, e, M, N, S, s, P, Le, Le, Jk, Jk, Fy, Fy, K, k. For the detection of these antibodies, DCT and indirect Coomb's test are done; cell panel is prepared in Coomb positive cases.

With the screening and identification technique, the alloantibodies should be identified and patients should be given corresponding antigen-negative donor unit. This will help to minimize the antibody-mediated destruction of transfused red cell. Ultimately, the desired effect of transmission to the patients may result in reduction of transfusion needs for the patients.[4] Less number of transfusion reduces the psychological and financial burden on the family and will increase compliance of the patients.

AIMS AND OBJECTIVES

- To detect irregular antibodies in sera of about 100 thalassemia major patients in Jaipur.

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- To subtype such antibodies with particular reference to anti-Rh, anti-Duffy, anti-Kell, anti-Kidd, anti-Lewis, and anti-Mn group.
- To know the relationship between number of transfusion and alloantibodies.

MATERIALS AND METHODS

- Type of study: Observational cross-sectional study
- Number of patients: 100
- Study place: Mahatma Gandhi Medical College (MGMC) and Hospital and JK Lon Hospital, Jaipur, Rajasthan, India
- Duration of study: 1 year

Inclusion Criteria

- Male and female, both up to 18 years.
- Patients consenting for the study.
- Patients with multiple blood transfusion (>2 per year).

Exclusion Criteria

- Patient with single blood transfusion.
- Patient above 18 years.
- Patients who did not give consent for the study.

Ethical Clearance

Institutional Ethical Committee for research on human subjects approved my study and clearance was given to the study protocol. Written informed consent of parents or guardian and assent (for those >12 years) was obtained for participation in the study prior to evaluation of their child.

Selection of Patient

Enlisting patients with transfusion-dependent beta-thalassemia major attending thalassemia ward for regular blood transfusion in MGMC, Jaipur. This study was done on 100 previously diagnosed thalassemia major patients. Information on transfusion history was recorded; 5 mL blood was collected from each subject and plasma was separated. These patients were subjected to DCT by CGA technique.

Protocol for DCT

Preparation of cell suspension: Add 50 mL of packed RBC of test sample (washed once in saline) in 1 mL of normal saline solution and mix uniformly.

Procedure

Specimens found positive for Coomb's test were subjected to screening using three-cell panel by CGA. Panel cells have the known antigens, consisting of antigens, such as Rh, Kell, Duffy, Lewis, P1MNS, LUTH. Results were

Flow Chart 1
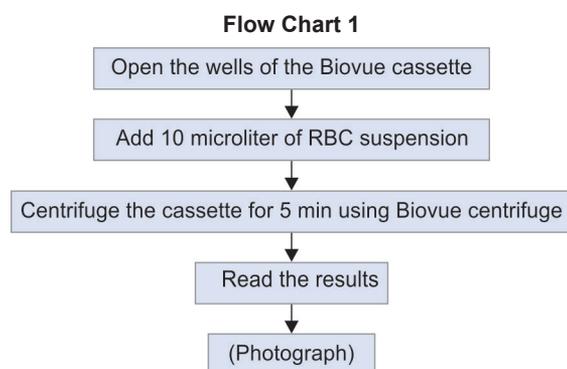
interpreted and prevalence of alloantibody was calculated. Chi-square test was used to test the association between different study variant and alloimmunization status.

RESULTS AND OBSERVATIONS

Of the 100 patients, 56% were males and 44% were females, with 62% receiving 10 to 40 number of transfusion, followed by 25% receiving 40 to 70 number of transfusion, and 13% received 70 to 100 number of transfusion. The ABO blood groups of the 100 patients were as follows: 33% of patients had blood group A, 27% had blood group B, 31% had blood group O, 9% had blood group AB, 79% were Rh positive, and 21% were Rh negative. With regards to the spleen state, none of the patients had splenectomy. Frequency of transfusion means how many blood transfusions were given per year. Out of 100 thalassemia major patients, 53% were receiving more than 12 blood transfusion/year and 47% were receiving less than 12 transfusion/year.

The most common alloantibodies were anti-D, anti-K, and anti-E, followed by anti-Mn. The incidences were 2 (28.57%), 2 (28.57%), 2 (28.57%), and 1 (14.3%) respectively of total 100 beta-thalassemia major patients (Table 1).

In our study out of 100 patients, 7 patients were having alloantibody. Out of 44 female patients, 4 females have alloantibody (57.1%) and out of 56 male patients, 3 have alloantibody (42.9%). Out of 7 alloantibody patients, 4 were Rh+ (57.1%) and 3 were Rh− (42.9%). In respect to blood group, O has maximum number of alloantibody (57.1%) followed by A (28.6%) and B (14.3%). Gender, Rh, and blood group showed statistically nonsignificant results (Table 2).

Out of 7 alloantibody patients, 4 (57.1%) patients had more than 50 transfusions as compared with 42.9% of

Table 1: Frequency of alloantibodies

Type of antibody	n (%)
Kell	2
E	2
D	2
Mn	1

Table 2: Comparison of alloantibodies of thalassemia patients among study variables

		Absent	Present	χ^2	p-value
Gender	FCH	40 (43)	4 (57.1)	0.52	0.46 NS
	MCH	53 (57)	3 (42.9)		
Rh	+	0	4 (57.1)	0.11	0.34
	–	0	3 (42.9)		
Blood group	A	31 (33.3)	2 (28.6)	2.83	0.41
	AB	9 (9.7)	0		
	B	26 (28)	1 (14.3)		
	O	27 (29)	4 (57.1)		

NS: Not significant; FCH: Female child; MCH: Male child

Table 3: Comparison of alloantibodies of thalassemia patients with number of transfusion

	Absent	Present	χ^2	p-value
<50	72 (77.4)	3 (42.9)	4.14	0.04 S
>50	21 (22.6)	4 (57.1)		

S: Significant

Table 4: Comparison of alloantibodies of thalassemia patients with number of transfusion

	Absent	Present	χ^2	p-value
<12	52 (98.1)	1 (1.9)	4.52	0.03 S
>12	41 (87.2)	6 (12.8)		
Total	93	7		

S: Significant

patients who had done less than 50 transfusions. According to number of transfusion, alloantibodies of thalassemia patients showed statistically significant results (Table 3).

Mean transfusion frequency per year is 12. Out of 100 thalassemia major patients, 53 received <12 units/year; out of these 53 patients, only 1 (1.9%) patient came with alloantibody. Out of 100 thalassemia major patients, 47 were received >12 units/year. Out of these 47 patients, only 6 (12.8%) came with alloantibody. Alloantibody with number of transfusion shows significant result (Table 4).

DISCUSSION

Only few studies in the world have investigated the frequency and cause of alloimmunization. In the present study, we studied the frequency of alloimmunization in Indian native children and look for the best solution to reduce alloimmunization in thalassemia patient. This study was carried out on 100 previously diagnosed thalassemia major patients who were receiving multiple transfusion in MGMC Hospital and Government Medical College Hospital, Jaipur, Rajasthan, India. These samples were examined for the presence of antibodies with particular reference to anti-Rh, anti-Duffy, anti-Kell, anti-Kidd, anti-Lewis, and anti-Mn groups.

As observed, the reported alloimmunization rates in thalassemics from other parts of India vary from 3.79 to 9.48%.[5] In the present study, frequency of alloimmunization was 7%, which is comparable to other centers in different countries like India (Pimpaldara et al[6]), Iran, Pakistan, Malaysia, and Italy. In the present study, anti-Kell, E, and D were the most common antibodies, followed by anti-Mn. Agrawal et al[7] revealed alloantibodies in only 2.9% cases, which is less than the present study (7%). This may be because of large sample size and adult patients. Also, other illness patients who received multiple transfusion were also taken in this study. Prevalence was 1.44% for antibody C and K. In Pakistan and Iran, anti-K was also the most common antibody. Other studies showed higher prevalence of anti-E in Asian region. Elhence et al[8] did not find significant differences in E (2%) and C (2%) antigen frequency between thalassemia patients and local donor population. The study has included only thalassemia patients, and both antibodies E (2%) and C (2%) were same in frequency. According to El Sewefy et al,[9] only 1 (0.5%) patient was reported of autoantibodies together with anti-Kell and anti-C. However, many previous studies observed higher results exceeding 25%.

Most studies stated that the relationship between the number of units transfused and alloantibody was unknown in thalassemia. Moreover, it was reported that the interval between transfusions did not appear to play a significant role in antibody development as similar interval was observed between all the patients. However, the interval shortened after the development of the antibodies due to decreased survival of foreign RBCs.

In the present study, using the mean number of transfused blood units as a cutoff, it was found that the mean frequency of blood transfusion was more than 12 units/year in alloantibody patients (p < 0.03). We also found that the frequency of transfusion could be better judged by the transfusion index, wherein the annual blood requirement as volume of transfused blood per kg of body weight was calculated.

The development of red cell antibodies (allo as well as autoantibodies) occurs in a variable number of multiple transfused patients. In such circumstances, transfusion therapy may become significantly complicated. Effects of autoantibodies may include difficulty in finding compatible RBC units because of the presence of clinically significant RBC antibodies, transfusion reactions, or platelet refractoriness. The present study is an effort to characterize blood group alloantibody formation in the patient population.

Our study revealed a statistical significant result between the frequency of blood transfusion and presence of alloantibodies. Similar results were found by Hassab et al[10] in Egypt. This is due to the fact that RBCs

alloimmunization is a common unwanted transfusion effect that occurs in up to 40% of patients depending on the number of transfusion events.

CONCLUSION

- Present study concluded that alloantibodies to minor group antigens is a more frequent finding among thalassemia major patients, which need more frequent blood transfusion.
- Incidence of detected antibodies were: Anti-Kell > anti-D > anti-E and mostly against the Kell and RH groups.
- With increased mean frequency of transfusion (>12 units/year), patients were at higher risk of developing alloantibodies.
- This problem is due to late onset of first blood transfusion and blood is transfused without screening for minor group antigen.

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