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Study of alloimmunization in thalassemia major

[1]Dipti Shah, [2]Janki Prajapati, [3]Sucheta Munshi

[1]Associate Professor of Paediatrics, GMERS Medical College, Sola, Ahmedabad, Gujarat, India
[2]Senior Resident, Banas Medical College, Palanpur, Gujarat, India
[3]Associate Professor of Paediatrics, B J Medical College, Ahmedabad, Gujarat, India

Corresponding Author:
Dr. Dipti Shah (drdiptishah1999@gmail.com

Abstract

Thalassemia Major results in a severe anemia and require regular blood transfusion every 3-4 weeks. The regular blood transfusion regimen causes production of alloantibodies against one or more red cell antigens, which complicates subsequent transfusions.

Aims and objectives:

1. To find out incidence of various RBC alloantibodies in repeatedly transfused thalassemic patients.
2. To initiate pre-transfusion antibody screening on patient's sample before cross match.
3. To analyse factor responsible for development of antibodies.

Material and method: It was prospective observational study conducted at Civil Hospital Ahmedabad from 01/09/2018 to 31/08/2020**.**Transfusion dependent patients of age up to 12 years having more than 50 blood transfusion were included in the study. Blood samples were collected in pilot tubes (EDTA and plain). The plasma/serum was used for antibody screening and antibody identification test using 3-cell panel and 11-cell panel. Serological parameters tested were blood grouping, Direct Antiglobulin Test (DAT), Indirect Antiglobulin Test (IAT) and antibody screening and identification. Blood grouping was done by using DIAGAST (QWALYS) on Erythrocyte Magnetized Technology (EMT). Results were obtained and data was analysed.

Observation and results: In alloimmunized patients M:F ratio was 1:3. The mean of total number of blood transfusion of all the patients and alloimmunized was 127 and 233. It shows more chances of developing alloantibodies with higher number of blood transfusion. Out of total alloimmunized patients, 50% patients were splenectomised. Alloantibodies was present in 50% patients with O+ blood group, 25% with A-and 25% with B+. Anti-K alloantibody was found in 50% patients, Anti-D in 25% patient and Anti-E in 25%. Total 91% patients were having annual blood requirement <200 (ml/kg/year) and 9% having >=200 (ml/kg/year). Among them, all the alloantibodies present patients found having annual blood requirement >=200 (ml/kg/year). All alloimmunized patients were having Homozygous thalassemia.

Keywords: Alloimmunisation, thalassemia, splenectomy, alloantibody, blood group

Introduction

- Thalassemia refers to a group of genetic disorders of globin chain production in which an imbalance between α and β globin chain production leads to ineffective erythropoiesis.

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There are two basic types of thalassemia (1) α-thalassemia (2) β-thalassemia. The homozygous state, Thalassemia Major result in a severe anemia and require regular blood transfusion every 3-4 weeks, with a goal to correct the anemia to supress the hyperactive erythropoiesis and to inhibit the excessive gastrointestinal iron absorption. The regular blood transfusion regimen is confronted with numerous complications. Among them, there is a production of alloantibodies against one or more red cell antigens, which complicates subsequent transfusions [5]. A non-self-blood group antigen comes in contact with the transfusion recipient's immune system mostly via a blood transfusion and pregnancy; the recipient's immune system may react leading to formation of antibodies against these foreign red cell antigens. These antibodies are called alloantibodies and the phenomenon-allo immunization [2].

- The distribution of various blood groups antigens varies amongst individuals in any given population, therefore there is a variable degree of disparity amongst the donor and the recipients regarding the group systems other than ABO and Rh D, which are not tested before routine transfusions. As a result, at some stage during the transfusion management this disparity of blood group systems lead to allo immunization and therefore elaboration of antibodies against the immunogenic antigen system [5]. Combined data from 18 studies on 4005 sickle cell patients and 11 studies regarding 3394 thalassemia patients showed an overall allo immunization risk of 22% (range3-76) and 13% (range 5-28) respectively [2]. Hence, the present study was undertaken in our institute to determine the incidence of various RBC alloantibodies in β-thalassemia major patients who received multiple transfusions.

Aims and Objectives

- To find out incidence of various RBC alloantibodies in repeatedly transfused thalassemic patients.
- To initiate pre-transfusion antibody screening on patient's sample before cross match.
- To analyse factor responsible for development of antibodies.

Subject and Methods

- **Type of study:** Prospective observational study.
- **Study period:** 01/09/2018 to 31/08/2020.
- **Study place:** Paediatric & IHBT department, Civil Hospital-Ahmedabad.
- **Patient age group:** Upto 12 years.

Inclusion criteria

- Transfusion dependent patients of age up to 12 years having more than 50 blood transfusion.

Exclusion criteria

- Age more than 12 years.
- Whose relative has not provided consent.
- Having <50 blood transfusion.

Methodology

Permission from ethical committee of B. J. Medical College, Ahmedabad was taken prior to

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the study. Patients were enrolled in the study as per selection criteria. Blood samples were collected in pilot tubes (EDTA and plain). The plasma/serum was used for antibody screening and antibody identification test using 3-cell panel and 11-cell panel. Serological parameters tested were blood grouping, Direct Antiglobulin Test (DAT), Indirect Antiglobulin Test (IAT) and antibody screening and identification. Blood grouping was done by using DIAGAST (QWALYS) on Erythrocyte Magnetized Technology (EMT) and blood group discrepancies noted in 12 patients, were solved by conventional tube technique as per departmental Standard Operating Procedures (SOPs) [45].

- **Direct agglutination test:** A polyspecific direct antiglobulin test was performed on all patients using 0.85% patient red cell suspension and LISS Coombs gel card. In the cases of a positive DAT, further investigation using specific gel cards impregnated with monoclonal reagents Anti IgG, Anti IgM, Anti IgA and Anti C3d was carried out.
- **Antibody screening and identification**: A commercially available 3-cell antigen panel (ID Dia cell I, II, III) was used for the antibody screening procedure where the patients serum was reacted with red cells using low ionic strength solution (LISS) in Coomb's gel card. The cards were incubated at 37 °C for 15 min followed by 10 minutes of centrifugation. If antibody screening with 3-cell antigen panel was positive, an extended 11 cell panel was used for antibody identification in LISS [46].
- Dia cell I contains the following antigens:
  D, C, e, C^W, k, Kp^b, Fy^b, Jk^b, Le^a, P, N, S, s, Lu^b and Xg^a

- Dia cell II contains the following antigens:
  D, E, c, k, Kp^b, Fy^b, Jk^a, Le^b, M, S, Lu^a, Lu^b and Xg^a

- Dia cell III contains the following antigens:
  c, e, K, k, Kp^b, Fy^a, Jk^b, P_1, M, N, s, Lu^b and Xg^a

Sera tested positive for screening (with Dia cell I or II or III or all) were examined by an identification test using the Dia panel, which consists of 11 different group O red cells, each having variable antigens of Rh, Kell, Duffy, Kidd, Lewis, P, MNS, Lutheran and Xg blood group system (D, C, E, c, e, C^W, k, K, Kp^a, Kp^b, Js^a, Js^b, Fy^a, Fy^b, Jk^a, Jk^b, Le^a, Le^b, P, M, N, S, s, Lu^a, Lu^b and Xg^a). [Note: the antigens of different blood group systems on red cells differ with different lot number]
Statistical analyses were done using SPSS software.

Observation and Discussion

In present study, total 104 patients were included, out of which 4 patients (4%) were found with alloantibodies. In present study, incidence of alloimmunization was 4% which was comparable to the study by Sangeeta Pahuja *et al.* [6], who found alloimmunization 3.79% and Ravi kumar Jeenger *et al.* [5] study found 6.67% alloimmunization. X^2=10.33; p value=0.01.
Amongst the alloimmunized patients, there were no patients < 5 years of age, 1 (25%) was between 5-10 years, 3 (75%) patients between 10-12 years of age. The mean age of present study was 6.57 years while Jeenger *et al.* [5] had mean age of 7.64 years. Pahuja *et al.* [6] had a higher mean age of 10.4 years. The mean age of alloimmunized patients of present study was 10 years. This is comparable to age of alloimmunization Jeenge *et al.* [5] of 11.9 years and Pahuja *et al.* [6] of 9.4 years.X^2=1.55; p value= 0.21.
Alloantibodies were found in 1 (25%) male and 3 (75%) females and M:F ratio was 1:3.

Age at first initiation of transfusion

X^2=8.86; p value= 0.03

In the study, 1 (1%) patient in <6 months group, 82 (79%) patients were in the 6-12 months age, 18 (17%) were in 1-3 years and 3 (3%) in >3 years age at first transfusion. This shows that majority patients between 6-12 month of age had initiation of first transfusion and it also shows that majority of patients who had initiation of first transfusion were < 3 years of age.

Out of 4 alloimmunised patients, there were no patients < 6 month of age, 1(25%) patient was between 6-12 months, 2(50%) patients between 1-3 years and 1 patient in >3 years age was at first transfusion.

In present study, mean age at initiation of blood transfusion was 8.42 month. Jeenger *et al*. [5] and Pahuja *et al*. [6] have reported and initiation age of transfusion was 13 and 10 month respectively. The mean age of initiation of transfusion of alloimmunized patient was 7 month. This is lower than Jeenger *et al*. [5] of 27.4 month and pahuja *et al*. [6] of 16.5 month. However all patients were less than 3 year of age at the time of initiation of transfusion. In various study [47, 48] have shown lower risk of alloimmunization with early age of initiation. However present study didn't show similar finding. The present study however is comparable to Jeenger and Pahuja *et al*. study.

Total number of blood transfusion

p value= <0.0001 (Fisher exact test)

In present study, 19(18%) patients had taken < 100 transfusion, 66(63%) had 100-150, 14(13%) had 150-200 and 5(6%) had taken >200 transfusion. Total number of blood transfusion in 4 (100%) patients with alloantibody were >200.

Mean of total number of blood transfusion

Mean of total number of blood transfusion	Present study	Ravi Kumar Jeenger *et al*.
Overall	127	98
Alloimmunized	233	164.30

In present study, mean of total number of blood transfusion was 127, while in Ravi Kumar Jeenger *et al.* [5] was 98. The mean of total number of blood transfusion of alloimmunized was 233, while in Ravi Kumar Jeenger *et al.* [45] was 164.30 and also Aveen *et al*. [9] reported mean of total number of blood transfusion was 206. The present study shows that mean of total number of blood transfusion was higher with comparison to Ravi Kumar Jeenger *et al*. [5] The present study shows that more chances of developing alloantibody with higher number of blood transfusion.

Splenectomy status

Status	Total Patients (n=104)	Alloantibody Present (n=4)
Splenectomised	6 (6%)	2 (50%)
Non-Splenectomised	98 (94%)	2 (50%)

X^2=7.70; p value= 0.005

Out of total 104 patients, 6 (6%) were splenectomised and 98 (94%) were non-splenectomised. In present study, there are 4 alloimmunied patients. Incidence of alloimmunization in splenectomised was 50%. The above findings shows that the splenectomised patients had

increase rate of developing alloimmunization.

Comparison based on splenectomy status

Comparison	Present study (n= 4)	Ravi Kumar Jeenger *et al*. (n= 10)	Pahuja *et al*. (n= 9)
Alloimmunization in splenectomized patients	50%	30%	13%

In present study, the splenectomised patients there were 50% had developed alloimmunization. While in Ravi kumar Jeenger *et al*. [5] was slightly less (30%) and in Pahuja *et al*. [6] was more less (13%) reported.

Blood groups

X^2=11.03; p value = 0.14 in the present study, 42(40%) patients with O+ blood group, 2(2%) with O-, 26(25%) with A+, 2(2%) with A-, 21(20%) with B+, 1(1%) with B-, 10(10%) with AB+ and 0 with AB-. The alloantibodies were present in 2 (50%) patients with O+ blood group, 1 (25%) with A-and 1 (25%) with B+.

Comparison of blood group

In present study, 2 (50%) patients with O+ blood group, 1 (25%) with A-and 1 (25%) with B+. While in Ravi Kumar Jeengar *et al*. [5] alloantibodies found in 2(20%) O+ patients, 1(10%) O-, 1(10%) A+, 3(30%) B+ and 3(30%) AB+ patients and in pahuja *et al*. [6] alloantibodies found in O+ 1(11%) patients, A+ 3(33%), A-1(11%), B+ 3(33%), B-1(11%). No correlation was found between blood group and alloimmunization.

Alloantibody type

Type of Antibody	Number of Patients	Percentage (%)
Anti-C	0	0
Anti-D	1	25
Anti-E	1	25
Anti-Lea	0	0
Anti-K	2	50
Anti-Kpa	0	0
Anti-K, S, Cw	0	0
Anti-Kpa, K, E	0	0
Anti C, K	0	0
Anti E, Cw	0	0
Anti E, Jka	0	0
Anti E, Cw, K	0	0
Anti K, N, Cw	0	0
Anti E, C, K, Cw	0	0

In the present study, Anti-D alloantibodies were found in 1 (25%) patient, Anti-E in 1 (25%) and Anti-K in 2 (50%) patients. Sangeeta Pahuja *et al*. [6] study found Anti-K; Anti-D; Anti-Kp^a; Anti-C^w, Anti-E; Anti-C; Anti-E; Anti-E, Anti-Jk^a and autoantibodies in alloimmunized patients. In Ashu Dogra *et al*. (49) study, all alloantibodies belonged to Rh system (i.e. 50% Anti-E, 16.66% Anti-D and 33.34% Anti-K) which is similar to our study. Suvro Sankha Datta *et al*. [7] observed that around 78.5% of alloantibodies detected were against the antigens of Rh

system. Thus above finding shows that the greater alloantibodies against Rh system. In different studies [50, 51] it is found that the transfusion dependent patients with the single alloantibody are at risk of developing multiple alloantibodies in further course of time.
The present study had reported only single alloantibody and required further follow up.

Based on type of blood transfusion

Type of blood transfusion	Total Patients (n=104)	Alloantibody Present (n=4)
leukocyte depleted blood	90 (87%)	2 (50%)
Mixed	14 (13%)	2 (50%)

X^2=0.002; p value= 0.96In present study, 90(87%) patients with leukocyte depleted blood transfusion and 14(13%) with mixed blood transfusion. The alloantibodies found in 2 (50%) patients with leukocyte depleted blood transfusion and 2 (50%) with mixed blood transfusion. No significant association between type of blood transfusion and alloimmunization was found in this study. There was no comparable data available from other study.

Annual blood requirement

Annual blood requirement (ml/kg/year)	Total Patients (n=104)	Alloantibody Present (n=4)
<200	95 (91%)	0 (0%)
>=200	9 (9%)	4 (100%)

p value: 0.00002 (fisher exact test) In present study, total 95 (91%) patients were having annual blood requirement <200 (ml/kg/year) and 9 (9%) having >=200 (ml/kg/year). Out of them, all 4 (100%) alloantibody present patients having annual blood requirement >=200 (ml/kg/year). There was no comparable data available from other study.

High Performance Liquid Chromatograghy (HPLC) Report

HPLC	Number of Patients (n=104)	Alloantibody Present (n=4)
Homozygous Thalassemia	101 (97%)	4(100%)
Thalassemia-sickle	2 (2%)	0 (0%)
Thalassemia HbD	1 (1%)	0 (0%)
Thalassemia HbE	0 (0%)	0 (0%)

In present study, based on HPLC report 101(97%) homozygous thalessemia. Mix hemoglobinopathy was thalassemia-sickle in 2(2%) and thalassemia HbD 1(1%), no other hemoglobinopathy was found. All alloimmunized patients were having Homozygous Thalassemia. This parameter has not been analyzed in other study. There were no corelation between homozygous thalassemia and other hemoglobinopathies for developing alloimmunization.

Sr. No.	Age (year)	Gender	Type of alloantibody	Splenectomy status	Age at detection of Antibody (year)	No. of BT required before detection of Antibody	No. of BT required after detection of Antibody	Age at 1st Transfusion (year)	Total No. of Transfusions	HPLC
1	12	F	Anti-E	Yes	6	100	134	0.9	234	Homozygous Thalassemia
2	10	M	Anti-D	Yes	4	96	124	1.5	220	Homozygous Thalassemia

3	12	F	Anti-K	No	6	122	130	3.2	252	Homozygous Thalassemia
4	6	F	Anti-K	No	3	88	136	1.3	224	Homozygous Thalassemia

Patients who developed alloantibodies

The above table shows details of patients who developed alloantibodies. It also includes the comparison between the number of blood transfusion required before detection of antibody and after detection of antibody.

Summary

- Overall mean age of all the patients and alloimmunized patients found 6.57 years and 10 years respectively.
- M:F ratio of overall patients was 1.97:1. i.e. 66% male and 34% female. While alloimmunized patients M:F ratio was 1:3. i.e. 25% male and 75% females.
- The mean age at initiation of blood transfusion of all the patients and alloimmunized patients was 8.42 months and 7 months respectively.
- The mean of total number of blood transfusion of all the patients and alloimmunized was 127 and 233. It shows more chances of developing alloantibody with higher number of blood transfusion.
- In present study, 6% patients were splenectomised and 94% were non-splenectomised. Out of total alloimmunied patients, 50% patients were splenectomised. It shows that there are more chances of alloimmunization in splenectomised patients.
- Alloantibodies were present in 50% patients with O+ blood group, 25% with A- and 25% with B+.
- In the present study, Anti-K alloantibody was found in 50% patients, Anti-D in 25% patient and Anti-E in 25%.
- Total 87% patients belonged to leukocyte depleted blood transfusion (50% found with alloantibody) and 13% found with mixed blood transfusion (50% found with alloantibody).
- Total 91% patients were having annual blood requirement <200 (ml/kg/year) and 9% having >=200 (ml/kg/year). Among them, all the alloantibodies present patients found having annual blood requirement >=200 (ml/kg/year).
- In present study, there were 101(97%) homozygous thalessemia. Mix hemoglobinopathy was thalassemia-sickle in 2(2%) and thalassemia HbD 1(1%), no other hemoglobinopathy was found. All alloimmunized patients were having Homozygous thalassemia.

Conclusion

Red cell alloimmunization is an important development in patient with transfusion dependent thalassemia. The females and the patients in the 10-12 years age group showing more frequency of alloimmunization. Patient's age at the initiation of transfusion and the total number of blood units transfused may also affect the immune response.

In the present study, significant association was observed between splenectomy and the development of alloantibodies. Majority patient were having alloantibodies against Rh system. Proper counselling to all alloantibody positive patients required so that they get antigen negative blood transfusion only. Whenever possible phenotype matched blood (at least Rh & Kell) should be administered.

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Limitations

The patients with age of >12 years were not included in present study.
We have identified type of alloantibody present in alloimmunized patients but due to unavailability of device, we couldn't calculate titre of these alloantibodies.
Because of limited study duration, there may be few patients who can generate alloantibody in near future but couldn't be covered in present study.

List of abbrevations

RBC: Red blood cell.
MCV: Mean corpuscular volume.
MCH: Mean corpuscular hemoglobin.
RDW: Red cell distribution.
TIBC: Total iron binding capacity.
NTDT: Non-transfusion dependent thalassemia.
TDT: Transfusion dependent thalassemia.
APC: Antigen presenting cell.
AHTR: Acute hemolytic transfusion reaction.
HPLC: High performence liquid chromatography.
NESTROFT: Naked eye single tube red cell osmotic fragility test.
RES: Reticuloendothelial system.
CMV: Cytomegalo virus.
HIV: Human immunodeficiency virus.
CBC: Complete blood cell.

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