Frequency Of Alloantibody In Multiple-Transfused Thalassemia Major Patients And Factors Influencing On Alloimmunization

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Abstracts: Background & Objective**:** The recommended treatment for beta thalassemia major involves regular blood transfusions, whichstimulate the patient's immune system and results in the formation of antierytrocyte antibodies usually IgG class. They can result in clinical hemolysis and complication of blood cross matching. The purpose of this study was to determine the frequency of RBC alloantibodies, the type of these antibodies, factors influencing on alloimmunization among multiple- transfused thalassemia major patients. Methodology: ABO blood grouping,Rh (D) typesand Phenotyping done by the electromagnetic technology using Qwalys 3 Diagast. Antibody screening was done by using 3-cell panel followed by11- cell panel of Biorad Corporation. Results: 10 patients developed alloantibodies against RBC Antigen. Among total alloimmunizedpatients, 7.35%were female and 4.27% were male. Majority of alloantibodies were directed against antigen in the Rh and Kell system. i. e. Anti c, Anti E and Anti K. Frequency of Alloantibody positivism is maximum in AB positive patients. From extended Antigen typing of voluntary donors, we can see the frequency of D, C and e Antigens are more than frequency of c, E and K Antigens. Conclusion: Frequency of red cell alloimmunizationwas 5.40% in this study. Alloantibodies found were mainly against Rh blood group systemand Kell system. Red cell alloantibody formation was not influenced by age at first transfusion, number of blood transfusion, splenectomy and leuckodepleted blood transfusion. In our study alloimmunized patients did not revealed any evidence of haemolytic transfusion reaction. The frequency of Antibody positivity depends on immunogenicity of Antigen. Females and group AB patients are showing more frequency of alloimmunization. Routine pretransfusion matching of blood, other than ABO and RhD antigen is not recommended because of low rate of red cell alloimmunization and high cost associated with such testing. [Shah S NJIRM 2016; 7(1):41-46]

Key Words: Alloantibody, Thalassemia, Immunogenicity

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Introduction: The thalassemia syndromes are a heterogeneous group of inherited disorder s caused by genetic lesions leading to decreased synthesis of one or more of the globin subunits.[1] The globin chains that are produced in relative excess can damage the red cells or their precursors. As a result, there is an overall deficit of haemoglobin tetramers in the red blood cells (RBC) and the mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) are reduced. [2] Thalassemia is considered the most common genetic disorderworldwide. It occurs with a particularly high frequency in a broad belt extending from the Mediterranean basin through the Middle East (Iran), India and Southeast Asia.

Lifelong red blood cell transfusion has remained the main treatment of severe thalassemia [3.] The recommended treatment for beta thalassemia major involves regular blood transfusions, usually administered every 2 to 5 weeks[4]. Repeated blood transfusion can stimulate the patient's immune system and results in the formation of antierytrocyte antibodies usually IgG class [3,5-7]. Although autoantibodies appear with less frequency, but they can result in clinical haemolysis and complication of blood cross matching. Alloimmunization against red blood cell will increase the need for blood transfusions in patients with thalessemia. Some alloantibodies arehaemolytic and may cause haemolytic transfusion reactions and limit the availability of further safe transfusion but others are clinically insignificant [8,9]. Patients may require immunosuppressive drugs, a spleenectomy , or alternative treatments. [10-12]

In guidelines for chronic transfusions in patients with thalassemia, antigen phenotyping before the first blood transfusion, laboratory tests including CBC, cross match and RBC antibody screening are recommended.

The reported frequency of antibody formation is highly variable in different parts of the world ranging from 2.37% to 3 [13].

The purpose of this study was to determine the frequency of RBC alloantibodies, the type of these antibodies and factors influencing on alloimmunization among multiple- transfused thalassemia major patients. Despite the recognition of autoantibodies as transfusion

associated risk, little is known about the extent and cause of these phenomena among thalassemia patients or the appropriate prevention methods. Approaches for prevention or treatment of alloimmunization are under debate. They range from provision of RBCs matched for all the major antigens associated with clinically significant antibodies to blood matched only for antibodies that have already been made. Reason for controversy as to the best approach lay in the fact that many antibodies are not harmful, and expensive prevention methods may therefore benefit only some patients. [14-16] In addition, donor feasibility and appropriate transfusion strategy should use.

Factors that predispose to alloimmunization are complex and involve at least 3 main contributing elements, antigenic difference between the donor's and the recipient's red cells, recipient's immune status and the immunomodulatory effect of the allogenic blood transfusions on the recipient's immune system. [17,18,19]

Material and Methods: This descriptive study was performed on 117 male and 68 female patients with thalassemia major who had received regular transfusion in Civil Hospital Ahmedabad, Gujarat, India from 1 January 2013 to 31 December 2013 between 0 to 12 years of age. .

At the first, the patient's age, sex, age on first blood transfusion, ABO and Rh blood group, spleen presence, age at spleenectomy done, were recorded in questionnaires. The transfusion records of all patients were examined for the presence of alloimmunization, time interval from the start of transfusion, antibody specificity, and isotope. Exposure to nonleukoreduced blood also recorded. After receiving a written consent from each patient, before transfusion, 10 ml of venous blood was collected in two separate tubes, K2 EDTA added tube for auto control and foreword grouping and without anticoagulant tube for antibody screening antibody identification and reverse grouping.

ABO typing.(Cell & Serum grouping) ABO blood grouping and Rh(D) typing ,blood grouping was done by fully automated blood grouping & matching system Qwalys 3 Diagast using electromagnetic technology.

Initially antibody screening was done by using 3-cell panel of Biorad corporation (Biotest Cell- P3), according to standard blood bank methods [17,19-21] Two part serum and one part RBCs were mixed and evaluated in three phases (RT,37 C and coombs phases) by using of LISS prepared in Biorad (LISS MB L2). All data was entered in predefined tables. Negative results were confirmed by adding check cells of Biorad (Coomb Cell –E). Dia cell I contains the following antigens:

D, C, e, C^w, k, Kp^b, Fy^b, JK^a, JK^b, Fy^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.

In case of a positive screen, antibody identification were performed in the same phases as Ab screening , by using 11- cell 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_1, M, N, S, s, Lu^a, Lu^b, Xg^a).
[Note: The antigens of different blood group systems on red cells differ with different lot number].

Finally according to presented antigram pattern of each panel, type of specific antibody against each antigen was determined. An auto control was also put simultaneously to determine the presence of autoantibody.

From January 2013 to August 2013 total 1700Voluntary Donors were screened for Antigen typing. For Phenotyping of Blood Donors A 1.0-2.0 ml blood sample was drawn from the antecubital vein of each Donor in a tube containing ethylene diamine tetra acetic acid (EDTA). Rh Antigens were tested by the electromagnetic technology using Qwalys 3 Diagast a fully automated blood grouping and matching system [22] . All samples that showed a negative agglutination with anti D in microplate (Duolys) were tested again in the AHG phase with monoclonal antisera (IgG&IgM) by standard tube technique for the presence of Du [(23)] . As a quality control, both Rh control and Coomb's control cell were used to ensure a highly diagnostic sensitivity and specificity, regarding the Rh (D) detection.

Results:
From 185 total patients with thalassemia major, 117 patients (63.24%) were males and 68 patients (36.75%) were females between 0 to 12 years of age. 10 patients of 185 patients developed alloantibodies against RBC Antigen. That is 5.40%. Among total

alloimmunizedpatients, 5 patients were female (7.35%) and 5 patients were male (4.27%). Majority of alloantibodies were directed against antigen in the Rh and Kell system. The frequency of Anti-c and Anti-E is more i.e. 4 cases of each were found. Anti K is present in 2 cases.Figure 1.

Distribution of Alloantibody positive patients according to no. of transfusion is shown in Tabke-1. From 185 patients 26 patients were undergone spleenectomy. Among Alloantibody positive patients 3 patients were undergone spleenectomy. Females are more commonly affected then male. Alloantibodies are more frequent in 7-12 years then 0-6 years of age.Table-2. Frequency of Alloantibody positivism is maximum in AB positive patients. Table-3. Extended Antigen typing of voluntary donors coming in blood donation camp organized by our department were done during January 2013 to August 2013. Total 1700 Donors were screened. From Figure 2 we can see the frequency of D,C and e Antigens are more than frequency of c, E and K Antigens.

Table 1: Distribution of patients with alloantibodies according to number of transfusion

No of transfusion	No of patients	No. of patients with alloantibodies
5-20	89	4
21-35	53	3
36-50	25	3
51-65	16	-
66-80	02	-
> 80	-	-

Table 2: Laboratory and clinical finding in the thalassemia patients with alloantibody

Antibody	Sex	Age (years)	Splenectomy	Duration of transfusion (years)	Blood Group	
					ABO	Rh
Anti-c	F	6	Done	5 Year	O	Positive
Anti-c	F	12	Done	11Year	O	Positive
Anti-c	M	8	Not done	7Year	AB	Positive
Anti-c	M	4	Not done	3Year	B	Positive
Anti-K	F	3	Not done	2 Year	A	Positive
Anti-K	M	12	Not done	12 Year	AB	Positive
Anti-E	F	7	Done	7 Year	A	Positive
Anti-E	M	1	Not done	2Month	B	Positive
Anti-E	F	11	Not done	10 Year	O	Positive
Anti-E	M	8	Not done	7 Year	AB	Positive

Table 3: Frequency of ABO and Rh blood group in thalessemic patients

	A	B	AB	O	Rh	
					Positive	Negative
Patients without alloantibody	38	61	19	57	174	1
Patients with alloantibody	2 (5.26 %)	2 (3.27%)	3 (15.78 %)	3 (5.26 %)	10 (0.57 %)	0

Figure 1: Frequency of alloantibodies in transfusion dependent thalassemia Patient

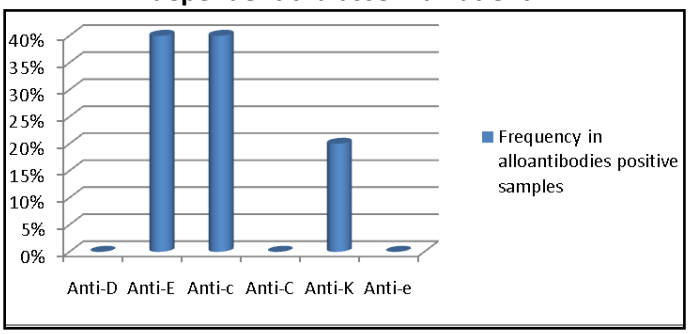
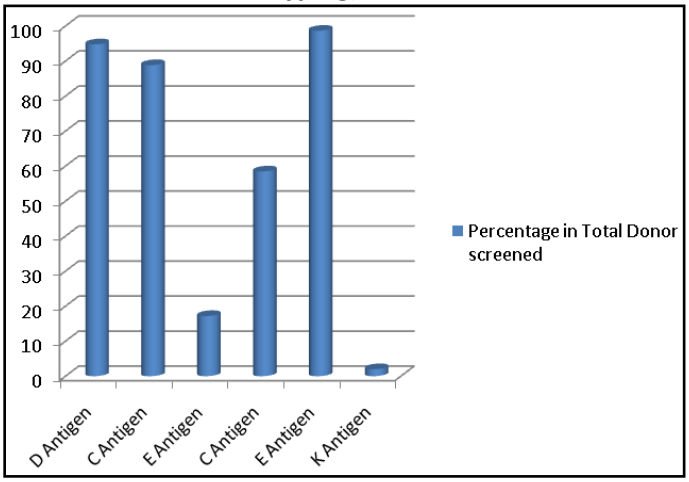


Figure 2: Frequency Distribution of Rh Antigens (Rh Phenotyping) of Donors



Discussion: Thalassemia was first reported in the literature in 1925, when Cooley and Lee described a form of severe anemia, occurring in children and associated with bone changes and spleenomegaly . Although bone marrow transplantation is the only cure, regular blood transfusion is available treatment for these patients. [24] Early and regular blood transfusion therapy in patients with thalessemia decreases the complications of severe anemia and prolongs survival. In the long term, however, the beneficial effect of

transfusions is limited by complications such as chronic viral infactions, hemosiderosis and alloimmunization against RBC. [25]

The present study was conducted to detect frequency of red cell alloimmunization and factors influencing on alloimmunizationin transfusion dependent thalassemia patients in Gujarat. Out of 185 multitransfusedthalassemic patients,10 patients developed red cell alloantibodies.

Frequency of red cell alloimmunizationwas 5.40% in this study. The most common alloantibody found were Anti-c and Anti- E Present in 4 cases (40%) Anti K is present in 2 cases (20%). These alloantibodies were mainly against Rh blood group system.

This study also revealed that red cell alloantibody formation was not influenced by age at first transfusion, number of blood transfusion and splenectomy. As we have taken only paediatric patients we do not found patient with more than 80 transfusions.

Our study revealed that 10 patients have been alloimmunized, but most of our patients did not revealed any evidence of haemolytic transfusion reaction. The majority of detected alloantibody were non haemolytic. Therefore, the recommended preventive measures would be beneficial for only a few patients who would develop these problems .[19]

Our results indicated that the frequency of alloimmunization in Gujarat is 5.40 %. Similar studies are also done at different centres. The frequency of alloimmunization in Iran was 2.8% in 313 patients, in 30% of 190 thalassemia patients in Kuwait, 4.97% of 161 in Indian patients, 5% of 1435 Italian patients and also 3.7% of 1200 thalassemia patients in Greece. [5, 17, 26-28]The prevalence of alloimmunuzation in previous studies is ranging from 2.8% to 30%. The prevalence of alloimmunuzation in our study is near lower side.

From Figure 2 we can see that frequency of Antigen E, Antigen c and Antigen K is lesser then Antigen D, Antigen C and Antigen e in Donors. But as per our study the frequency of Alloantibodies Anti E, Anti c and Anti K is more than Anti D, Anti C and Anti e. Because the Immunogenicity of common Rh antigens are in this order D>c>E>C>e.[29] D is highly immunogenic but because our centre reconfirm the D antigen of all RBC units labelled Rh-negative by testing red cells from an integral attached segment in the AHG phase with monoclonal antisera (IgG&IgM) by standard tube technique for the presence of Du [14]. We never miss a single weak D /du unit. So we do not find any case of Anti D positive sample. Then we found four cases of Anti c and of Anti E as Antigen E and Antigen c are more immunogenic after Antigen D. Excluding ABO, K is rated second only to D in immunogenicity. When K neg people are transfused with a unit of K pos. blood the probability of their developing anti-K may be as high as 10 percent. We found 2 cases of Anti K.[30] So we can explain that the frequency of Antibody positivity in transfused patient is not directly proportional to frequency of antigen in the donor but it depends on immunogenicity of Antigen.

Our department has started leuckodepleted blood for thalessemia patients from April 2013. The role of leukodepletion in preventing Red Cell alloimmunization has been described in several studies showing that patients receiving leukodepleted blood appeared to have a lower red cell alloimmunization rate, suggesting that it is the removal of leucocytes that reduces immune activation due to allogenic transfusion [9]. However, various studies have suggested that apoptosis and loss of viability of residual white cells in leukodepleted blood that have been stored before being transfused may lead to the release of immunostimulatory white cell antigens and soluble biologic mediators, resulting in sensitization of the recipients [31-35] This may explain why we continued to see alloimmunization in our patients even after we started using leukodepleted units. And It is very short time interval for us to assess the effect of leuckodepleted blood on frequency of alloimmunization in thalassemia patient.

To prevent alloimmunization against red cell antigens the recommendation is to provide antigen matched red cell s to all transfusion dependent thalassemic patients. It is true that providing antigen matched blood will effectively prevent alloimmunization; however, the cost effectiveness to establish such programs for chronically transfused patients is debatable.

It is also difficult to establish and maintain a personalized donor pool for each patient. In the existing setup it is felt that there is no pressing need for routine pre-transfusion matching of blood other than ABO and Rh “D” antigens. Low rate of red cell alloimmunization and high cost associated with such testing also testify this contention.

Conclusion: Red cell alloimmunization is an important development in patient with transfusion dependent thalassemia. Red cell alloantibody formation was not influenced by age at first transfusion, number of blood transfusions and spleenectomy. Females and group AB patients are showing more frequency of alloimmunization.

The frequency of Antibody positivity in transfused patient is not directly proportional to frequency of antigen in the donor but it depends on immunogenicity of Antigen.

Effect of leuckodepleted blood is still debatable and It is very short time interval for us to assess the effect of leuckodepleted blood on frequency of alloimmunization in thalassemia patient.

Routine pretransfusion matching of blood, other than ABO and RhD antigen is not recommended because of low rate of red cell alloimmunization and high cost associated with such testing.

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