FREQUENCY OF RED CELL ALLOANTIBODIES AND AUTOANTIBODIES IN THALASSEMIA MAJOR CHILDREN

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ABSTRACT

Introduction: Beta Thalassemia is an inherited haemoglobin disorder characterized by reduced synthesis of beta – globin chains.[1] The most severe forms of β-thalassemia major present within the first year of life with severe anaemia and failure to thrive.[2] The haemoglobinopathies are major genetic problems in Pakistan. The purpose of this study was to determine the frequency of red cell alloantibodies and auto-antibodies in thalassemia major children receiving regular blood transfusions. It was a cross sectional survey. It was performed at The Children Hospital and Institute of Child Health, Lahore in Dec. 2009 – May 2010.

Subjects and Methods: 130 diagnosed patients of beta thalassaemia major from Children Hospital Lahore were selected. Blood grouping and direct antiglobulin test was performed in these patients. Alloantibody screening and identification tests were done using the 3 cell panel followed by 11 cell panel of Diamed.

Results: Among the 130 patients 8.5% (n = 11) patients were diagnosed to have alloantibodies. Autoantibodies were detected in 2.3% (n = 3).

Conclusion: Presence of red cell antibodies and red cell autoantibodies are frequent findings in transfused thalassaemic patients and should not be overlooked in patients with thalassaemia major receiving regular blood transfusion. Regular screening for red cell alloantibodies and autoantibodies would add towards the better management of these patients.

Key Words: Beta Thalassaemia Major, Multiple blood transfusions, Alloimmunization.

INTRODUCTION

Beta Thalassemia is an inherited haemoglobin disorder characterized by reduced synthesis of beta –globin chains.[1] The most severe forms of β-thalassemia major present within the first year of life with severe anaemia and failure to thrive.[2] The haemoglobinopathies are major genetic problems in Pakistan. About 5 percent of the Pakistani population carries β-thalassaemia and about 5250 infants with β-thalassemia major are born annually.[3] Regular blood transfusion every 3 to 4 weeks remains the treatment for most of the thalassaemia major patients[4]. Regular blood transfusion is associated with a number of risks and complications including iron overload, transmission of infectious agents, haemolytic transfusion reactions and alloimmunisation etc.[5] There is a high probability that the donor will have red cell antigens not present in the recipient and it will result in alloimmunization. Similarly autoantibodies formed in these patients can complicate transfusions[6]. Alloimmunization is an immune response generated in an individual by a foreign antigen from a different individual of the same species.[7] This type of sensitization results in difficulty in obtaining compatible blood, transfusion reactions, occasionally haemolysis and life – threatening events.[8] This study is designed to determine frequency of red cell alloantibodies and autoantibodies so that serious hazards because of immunization may be avoided by screening these patients for alloantibodies and autoantibodies.

MATERIALS AND METHODS

A total 130 cases of Beta Thalassaemia Major, diagnosed on High Performance Liquid Chromatography of all ages and both genders at Children Hospital Lahore were included in the study from outdoor patient department. An informed consent was obtained in all the cases from the parents of children and their demographic profile obtained.

A 5 – 10 ml venous blood was collected. Blood grou-ping and direct antiglobulin test was performed on all samples. A poly – specific coombs reagent was used. Blood was allowed to clot, serum was separated and stored in labeled test tubes. Red cell alloantibodies were detected using standard blood bank methods (saline, albumin / LISS and coombs phases). A_3- cell antigen panel from Diamed was used to detect the anti-

body by indirect anti-globulin test (IAT). Antibody identification was performed in antibody screening positive samples using 11 cell panel. A proper quality control of test was monitored.

All data collected was entered and analyzed through statistical package SPSS version 17. Results of antibody screen and identification testing were analyzed on SPSS. The frequencies of blood groups, alloantibodies and autoantibodies in selected patients were determined. Qualitative variables in this study such as gender, blood group, presence or absence of alloantibody and autoantibodies were expressed in terms of frequencies and percentages and presented as frequency distribution tables. Numerical variables like age were presented as means with standard deviation.

RESULTS

A total of 130 children were enrolled to determine the frequency of alloimmunization and autoantibodies in these patients presenting with Thalassemia major. All of them were receiving regular blood transfusion. 58.5% (n = 76) patients were males and 41.5% (n = 54) were females (Fig. 1).

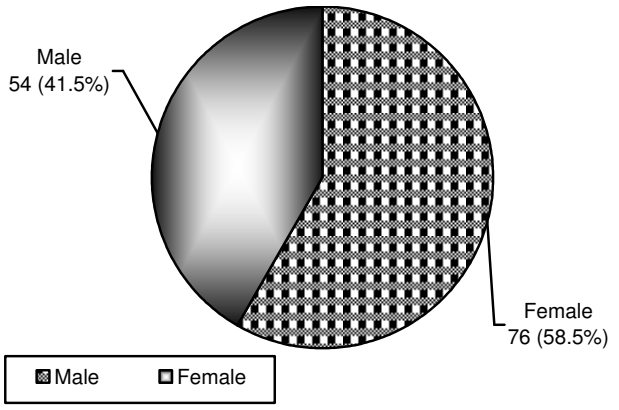


Fig. 1: *Gender Distribution.*

Table 1: *Age distribution of the patients (n = 130).*

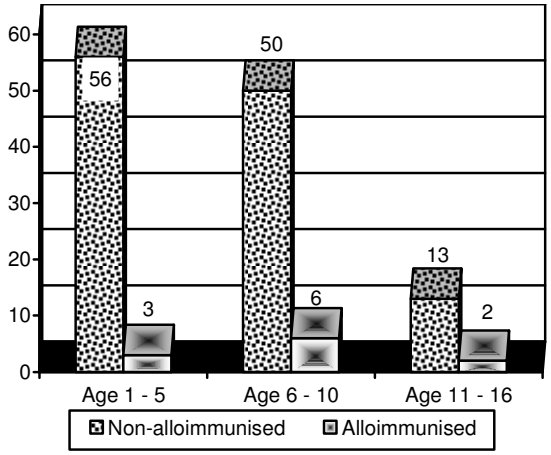
Age of the Patients	*No. of Patients*	*% age*
1 – 5	59	45.38
6 – 10	56	43.1
11 – 16	15	11.5
Total	130	99.98
Mean and S.D : 6.28 ± 3.18		

The patients were divided in 3 groups on the basis of age. First group from 1 – 5 years comprising of 45.38% (n = 59), 2nd group from 6 – 10 years of 43.1% (n = 56) patients and 3rd group of 11 – 16 years having 11.5% (n = 15) patients. Mean age was 6.28 ± 3.18 (age range 1 – 16 years) (Table 1).

Red cell alloantibodies were found in 8.5% (n = 11) patients. Among them 54.5% (n = 6) were males and 45.4% (n = 5) were females (Table 2). Auto-antibodies were detected in 3 patients (2.3%) with increased hemolysis and difficulty in finding compatible blood in two patients. The mean age of patients who developed red cell alloantibody was 8.8 ± 3.79. Age ranges from 2 – 16 years. 3 patients were in group 1 (age 1 – 5), 6 in group 2 (age 6 – 10) and 2 in group 3 (age 11 – 16),

Table 2: *Frequency of red cell Alloantibodies and auto-antibodies in thalassaemia major (n = 130).*

Immunisation	*No. of Patients*	*% age*
Alloantibodies	11	8.5
Autoantibodies	3	2.3



Graph 1: *Alloimmunization in different age groups. Alloimmunized patients age Mean & S.D: 8.8 ± 3.79 years.*

Table 3: *Frequency of ABO and RH Blood groups in thalassaemia patients.*

Blood Group	*Non-alloimmunized*	*Alloimmunized*
A	21%	16%
B	32%	27%
O	42%	54%
AB	5%	2%
Rh positive	82%	24%
Rh negative	18%	76%

(Graph 1).

Blood grouping of all patients included in this study were performed. Frequencies of different blood groups in both alloimmunized and non alloimmunized groups were calculated. Blood group O was the commonest in both groups followed by group B. Rh negative group is seen in76% of alloimmunized patients and Rh positive group is predominant in 82% of non alloimmunized patients (Table 3).

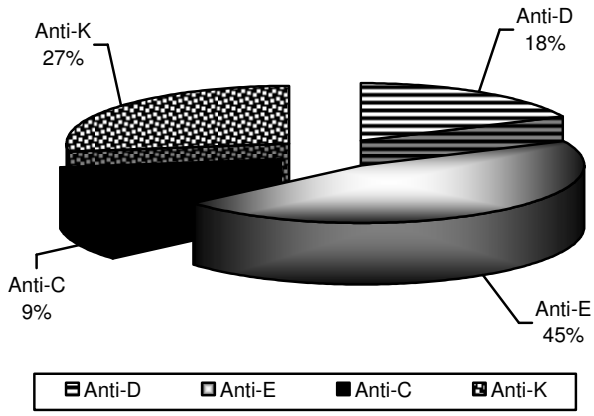


Fig. 2: *Frequency of Alloantibodies identified in thalassemia major patients (n = 11).*

Anti D Antibodies were detected in 2 patients , one male and one female, anti E in 5 patients, 3 male and 2 females, anti c in 1 female and anti K in 2 male and 1 female patients were identified. Anti Rh antibodies are 72% of total alloantibodies and Kell group antibodies comprise 28%.Higher frequency of alloimmunization was seen in Rh negative patients. Male to female ratio of alloimmunized patients was 1.2:1.Transfusion interval in immunized patients was 5 to 20 days Mean, SD; 12 ± 4.79 and in non-immunized patients interval was 7 days to 5 weeks Mean, SD; 20 ± 10.2.

DISCUSSION

This study was conducted to find out the frequency of red cell alloantibodies and autoantibodies in thalassaemic children in our hospital so that the need for pretransfusion screening in these patients can be evaluated. The rates of immunization have been variably reported across the world. According to our study, the frequency of development of red cell alloimmunization in thalassaemic patients receiving multiple blood transfusions in our set up is 8.5% and autoantibodies 2.3%. Our results are in agreement with studies conducted at Agha Khan University Karachi by Bilwani, et al, who revealed a rate of alloimmunization of 9.2% in 97 patients of thalassaemia major. Fifty three patients were males and 44 females. Mean age was 10.6 years. Mean age of patients who developed red cell alloimmunization was 11.9 years.[9]

Our data contrast with the study conducted by Khalid, et al, which showed higher rate of alloimmunization in patients of Beta thalassaemia major. Alloantibody was detected in 17 (22.7%) patients out of total 75 patients.[10] However another study by Bhatti, et al at AFIT Rawalpindi, reported the low rate of RBC alloimmunization i.e. 4.97% in 161 patients which mainly belonged to Rh system and Kell system and autoantibodies in 1.87% patients with increased hemolysis.[11]

Sadeghian et al studied a total of 313 thalassemia patients in the northeast of Iran. They identified 12 alloantibodies in 9 patients (2.87%) that all were against Rhesus (Rh) blood group antigens (D, C and E). Higher frequency of alloimmunization was observed in female, Rh negative and splenectomized patients.[12]

In India, Chaudhari reported six (18.8%) out of 32 patients of thalassemia major with alloimmunization. All alloimmunized subjects were recipient of more than 20 units of transfusion. Total seven clinically significant alloantibodies were identified. Anti E and anti c were commonest antibodies in four (12.5%) patients.[13]

Thompson reported on 697 thalassemic patients who had received transfusions. Allo- and auto-antibodies were reported in 115 (16.5%) and 34 (4.9%) subjects, respectively. Alloantibodies occurred in 19 of 91 (21%) splenectomized subjects who started transfusion after 1990, and only 18 of 233 (7.7%) nonsplenectomized subjects ($P < 0{\cdot}001$). Data from this study demonstrate that RBC antibodies continue to develop in chronically transfused thalassaemia patients at a high rate. Splenectomy preceded the development of antibodies in most cases. Increased rates of RBC sensitization among splenectomized patients is concerning and deserves further study.[8]

Pahuja et al, in India studied the frequency of alloimmunization among 211 multitransfused thalassemics of Indian origin. All the patients have been receiving blood matched for ABO and Rh(D) antigens only. The frequency of alloimmunization was 3.79%. The alloantibodies identified were anti-E, anti-K, anti-D, anti-Kp(a), anti-C(w), anti-c and anti-Jk(a). In the present study, no significant association was observed between splenectomy and the development of alloantibodies as well as between ages at initiation of transfusion.[14]

Gupta conducted a study on 116 thalassemics receiving regular transfusion. Red cell alloantibodies were found in 11 patient's i.e 9.48%. Mean age of the patients was 14 years (1.5 to 27 years).the interval between consecutive transfusions varied from 18 to 30 days. The common alloantibodies found were anti–E and anti–K. None of the 8 out of 116 patients, who underwent splenectomy showed any antibody development.[5]

Noor et al, studied 58 multiply transfused thalassemia patients. Red cell alloantibodies were found in 5 of 58 patients (8.6%) Layla AM, et al, in Bahrain found

out that out of 76 thalassemic patients, nine (11.8%) had developed allimmunization.[15,16]

In our study most of the patients were between the ages of 6 – 10 years. Five patients had history of mild hemolytic transfusion reactions. Higher frequency of alloimmunization was observed in Rh negative patients. Two patients with autoantibodis presented with increased hemolysis and difficulty in cross – matching. Least incompatible issued to them and immunosupression started. There is no doubt that alloimmunization is a significant clinical problem in multiple transfused patients, and many strategies were suggested to reduce its development, thereby avoiding such complication as the difficulty of obtaining compatible RBCs and also preventing the occurrence of delayed haemolytic transfusion reactions, which usually occur in a small percentage of such patients. Controversy exists as to whether limited versus extended red cell phenotyping for ABO and D in addition to other minor blood types in transfusion-dependant patients should be done before the patients receive their first matched RBC transfusion. The weight of published evidence leans heavily on limited phenotyping (ABO, D, C, E and Kell) other than being costly and time consuming, extended phenotyping.[17] Due to high cost, a study from Brazil recommended the use of extended matched transfusions only to patients who have already developed one or more RBC alloantibodies.[18] In our institute we are following a similar protocol; i.e. routine ABO and D typing for all transfusion – dependant patients and reserving further phenotyping only when a patient becomes alloimmunized. In Pakistan antibody screening is not included with cross – match before transfusion. Therefore we should weigh benefits against cost of doing antibody screening at the start of blood transfusion and it is recommended that pre-transfusion antibody screening on patients samples needs to be initiated in Pakistan to ensure safe transfusion practice so that antigen negative blood can be provided.

It is ***concluded*** that red cell alloantibodies and autoantibodies should not be overlooked in patients with thalassemia major receiving regular blood transfusions. It should always be considered if the patient repeatedly suffers from haemolytic transfusion reaction or not being able to maintain haemoglobin at a desired level inspite of regular transfusions. Guidelines should be considered for local blood banks with limited technical facilities and donor resources and where phenotypically matched blood cannot always be made available.

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