

ORIGINAL ARTICLE

RED CELL ALLOIMMUNIZATION IN MULTITRANSFUSED THALASSAEMIA MAJOR PATIENTS

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Background: Lifelong transfusions are life savers for thalassaemia patients but are associated with many complications. Alloimmunization is a major problem for blood banks. Antigens of foreign red blood cells induce the formation of antibodies in patients suffering from thalassaemia. The purpose of this study was to examine the frequency of red cell alloantibodies and to express the type of these antibodies in thalassaemia patients. **Methods:** Patients that have received multiple transfusions were included in this study. Those with the positive Coombs test (DAT) results were excluded from the study and remaining patients were screened for antibodies. A panel of known blood group antigens was used for the patients who had a positive antibody screening test because they had alloantibodies in their serum. First, three cell panel was applied. If the screen was positive then eleven cell panels was used to identify the specific antibody. Both the cell panels were applied at room-temperature, liss (low ionic strength saline) and coombs phase. **Results:** Three hundred & two patients were selected out of which 65.6% (n=198) were males and 34.4% (n=104) females. Patient's age ranged from 1.5 years to 26 years \pm 5.40 years. All of the patients were given regular red cell transfusion at 2-4 weeks interval. They were given non leukodepleted transfusions. It is not the practice in any thalassaemia Centre in Pakistan to give phenotypically matched blood for Kell, Kidd, Duffy or any other minor group antigens to patients on regular blood transfusion. Alloimmunization was positive in 12 (4.0%) of the 302 patients studied. Male were 66.67% (n=8) and female were 33.33% (n=4). Samples of these positive patients were further tested to determine specificity of alloantibodies. Anti C^w was most common, detected in 4 out of 12 (1.3%) patients. Anti K, k, S and Lu^a were detected in 2 out of 12 (0.7%) each. **Conclusion:** Thalassaemia major patients on regular blood transfusions can develop red cell alloantibodies. Detailed pre-transfusion screening would add towards better management of these patients.

Keywords: Transfusion; Thalassaemia major; Alloantibodies; Antigens

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INTRODUCTION

Thalassaemia is the most common inherited disease of humans and the most communal single gene haemoglobin disorder in the world. The carrier rate estimated by world bank in 2006 was 7%.¹ Thalassaemia is a genetic disorder with an important drop in the rate of formation of some or all globin chains.² The surplus globin chain is accountable for the futile erythropoiesis and shortened survival of red blood cell (RBC).³ Hereditary Disease Program of W.H.O. reported that the carriers of haemoglobin disorders are estimated to be 269 million worldwide.⁴

Beta thalassaemia is an inherited anaemia due to errors in the synthesis of beta-globin chain. They cause severe anaemia in homozygous and compound heterozygous state; comprising the entity Thalassaemia major that requires lifelong transfusions.⁵ Beta thalassaemia intermedia is less severe than beta thalassaemia major. It may require episodic blood transfusions.⁶

Beta Thalassaemia is also the most common hereditary disease in Pakistan. It is a common problem

in Balochistan, Sindh and KPK. Carrier rates are 1.4-9.6%. A study in KPK and northern Punjab revealed prevalence of 3.2% in Punjabis and 7.9 % in Pathans respectively. Overall prevalence rate is 5.4% of the population and in future nearly 36,000 children would be suffering from thalassaemia.⁵ Thalassaemia is classified according to its clinical severity into major, intermediate and minor.

Thalassaemia major a severe transfusion-dependent disorder. Thalassaemia intermedia clinically presents with anaemia and splenomegaly but often requires transfusion. And thalassaemia minor which is a symptomless carrier state with several hundreds of haemoglobinopathies, though the Thalassaemia Alpha, Beta and the sickling disorders contribute to the vast majority.⁶ The purpose of this study was to examine the frequency of red cell alloantibodies and to express the type of these antibodies in thalassaemia patients

MATERIAL AND METHODS

This cross-sectional study was done for 6 months at haematology laboratory/pathology department of Ayub

medical college Abbottabad. While analytical procedure along with compilation of data was done in the Institute of Basic Medical Sciences (IBMS), Khyber Medical University Peshawar.

A total of 307 patients were selected. Direct antiglobulin test (DAT) was performed. Five among them were DAT positive so they were eliminated from the study. The rest of the 302 thalassaemia patients were included in the study. All of them had more than 5 transfusions. 66.67% (n=201) of these patients were registered in thalassaemia centre of Ayub Hospital Complex Abbottabad and the rest of 33.34% (n=101) patients at KBDO (Kids Blood Diseases Organization) Mansehra. A verbal and written consent was taken from patients and their parents.

Clinical data was recorded in a Performa, with reference to: age; gender; age at first transfusion; frequency of transfusions; total number of transfusions given and any increase in the requirement of transfusion. 5 cc of blood sample was taken from patient's cubital vein after cleaning the area with an antiseptic. One cc of blood was kept in ethylene diamine tetra acetate containing tube, and 4 cc in plain tube. Direct coombs test was performed on the blood mixed with EDTA. Serum was extracted from non-ethylated sample and kept at -20 C. Samples with positive DAT were excluded from the study while rest of the samples were tested for alloantibodies using three cell panel and eleven cell panel by DiaMed.

Indirect coombs test (IAT) was done to identify the incidence of alloantibodies using micro typing system reagent (DiaMed-ID, DiaMed) RBCs comprising of 3% suspension of recognized human group O red cell. Two drops of reagent cells were mixed with 1 drop serum, incubated at 37°C for 10 minutes, and then centrifuged for 10 minutes before recording the results.

RESULTS

A total 302 patients were finally included in this study. 198 patients (65.6%) were male and 34.4% were female. All of them were diagnosed patients of thalassaemia major. Most of the patients were selected from Abbottonians Medical Association, Thalassaemia Centre Abbottabad rest were from kids' blood diseases organization (KBDO) Mansehra. All the patients belonged to surrounding areas of Abbottabad, Haripur, Havelian and Mansehra. Frequency of transfusion varies from 2 to 4 weeks. All of them were given non-leukodepleted transfusions.

Patients with positive red cell alloantibody had a mean age of 14.1years. Range was from 1.5 years to 24 year and male to female ratio was 2:1. Total life time transfusions given to these patients ranged from 7 units to 521 units with a mean of 88.59. Alloimmunization was positive in 12 (4.0%) of the 302 patients studied.

Among 190 males, 8 were positive for alloantibodies while 4 females are positive in 100. Male to female ratio of alloimmunization is 2:1. As more males are included in this study.

Two patients of group A were positive for antibodies. While 6 patients were group B rest two were from group O. Specificity of alloantibodies was determined by analysing the samples from these alloantibody positive patients. Anti C^w was more prevalent, detected in 4 out of 12 (1.3%) patients. Anti K, k, S and Lu^a were detected in 2 of 12 (0.7%) each. Percentages of different alloantibodies are presented in table-2 and 3. Most frequent alloantibody detected is C^w of RH group 1.3% (n= 4). While Kell (K and k), MNS (S) and Lutheran (Lu^a) are 0.7% (n=2) each. A pie chart describes proportion different alloantibodies.

Table-1: ABO blood groups and RH type of patients

ABO Blood Groups	Frequency	Percent	RH Positive	RH Negative
A	98	32.5	94	4
B	84	27.8	78	6
AB	30	9.9	28	2
O	90	29.8	90	0
Total	302	100.0		

Table-2: Frequency of alloantibody detected in thalassaemia major patients

Antibody	Frequency	Percent	Cumulative Percent
Present	12	4.0	4.0
Absent	290	96.0	100.0
Total	302	100.0	

Table-3: Frequency and specificity of alloantibodies in alloimmunized patients with thalassaemia major

Types of Antibodies	Number of Patients	Percentage
RH C ^w	4	1.3
Kell K	2	0.7
Kell k	2	0.7
MNS S	2	0.7
Lutheran Lu ^a	2	0.7

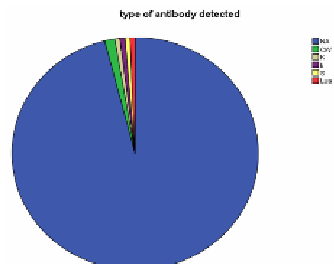


Figure-1: Different types of alloantibodies and their proportion

DISCUSSION

Multitransfused thalassaemia patients come across a number of different donors for their transfusion requirements. Alloimmunization is due to initiation of immune system response in recipients because of minor blood group antigens. As they have exposure to many antigens which make them susceptible to alloimmunization. Red cell alloimmunization depends on genetic and acquired factors related to patients, dose of antigen and immunogenicity.⁷ Many other factors like demographics, genetic makeup, immune competence, number of transfusion given, time and frequency of screening, and sensitivity of the method are also responsible. Much less data is available on this problem of multi-transfused patients.

This study was done in Hazara region to find the frequency of alloimmunization. It covered surrounding areas of Abbottabad, Havelian and Haripur. Primarily 307 patients were selected, 5 of them were DAT positive and were eliminated from the study. Our results showed that 4.0% of patients became alloimmunized due to regular or intermittent transfusion. Different frequencies of allo immunization have been reported by various studies worldwide. In comparison to our data, a number of these reports had a high proportion of alloimmunization. Low rates have also been reported.

Regarding age at first blood transfusion in our study alloimmunized patients were more than 3 months of age at that time. Eldest patient was 5 years old. Comparing the eldest and youngest, the age of first blood transfusion in our study was not found to be a risk factor for alloimmunization. Likewise, in Malaysian studies by Noor Haslina no association was seen between alloantibodies and age at start of transfusion ($p=0.5$, $p=0.8$ respectively).⁸

These results are in accordance to another survey from our area. It showed incidence of irregular red cell alloantibodies as 4.97%. Majority being from the Rh system, and one case of anti-K, anti-Jsb and anti-Jka respectively.⁽⁵⁾ Our study revealed red cell alloantibodies of anti-C^w, anti-K, anti-k, anti-S, anti-Lu^a. They are mainly from Rhesus, Kell, MNS and Lutheran system.

The perceived frequency of red cell alloimmunization is comparatively less in our study compared to the reported incidence in the literature.⁸⁻¹¹ Red cell alloimmunization in multi transfused beta thalassaemia major patients from multiple populations was reported with a number of frequencies such as 30% in Kuwaiti⁹, 22.6% in

Greek¹⁰ 9% in Malaysian⁸ and 5.2% in Italian populations¹¹. Overall frequency ranges were between 5–30%. Low alloimmunization rate in this study suggests that there is homogeneity of red cell antigens in recipients and blood donors in the Pakistani community.¹²

CONCLUSION

Repeated red cell transfusions augment the chances of development of red cell alloantibodies in thalassaemia major patients.

Allo-immunization should always be suspected if a patient is not able to maintain haemoglobin at a preferred level despite regular transfusions. Detailed screening for antibodies against the minor red cell antigens will help in better care of these patients. Transfusion of blood negative for the antigen against which an antibody has been found in the patient's serum should be the protocol. This will not only reduce transfusion requirement in these patients but also reduce morbidity.

Regular consultancy, iron chelation and care for allo and autoantibodies along with continuous training of technicians and health personals is necessary. Further studies are also required for proper management of complications.

AUTHORS' CONTRIBUTIONS

SM has done data collection and performed the tests, NF supervised the procedure, RI designed the paper, MI conceived the idea. UF has done handling of statistical data. MA has done formation of tables.

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