IJBC 2013;6(1): 41-46

Red Blood Cell Alloimmunization in Patients with Thalassemia Major and Intermediate in Southwest Iran

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Submitted: 17-02-2013 , Accepted: 06-08-2013

Abstract

Background: Transfusion is the mainstay treatment of patients with thalassemia major and occasionally in thalassemia intermediate. Alloimmunization is an unwanted side effect of blood transfusion. The present study intended to determine the frequency of alloimmunization in patients with β- thalassemia major and thalassemia intermediate in Southwest Iran.

Patients and Methods: This was a cross-sectional study on 133 transfusion dependent β-thalassemia patients at Shafa hospital-in Southwest Iran. The method of antibody screening was the tube method. All panel test phases were done at immunohematology laboratory of Iranian Blood Transfusion Organization.

Results: There were 66 males (49.1%) and 67 females (50.9%) with the mean age of 17.5 years (SD±7.5) included in this study. The antibody screening panel test was positive in 42 patients (32.06%). Twenty five patients (18.7%) had alloantibody and 17 patients (12.7%) also had autoantibody. The predominant pattern of alloimmunization was alloantibodies against RH sub groups system in 55 percent of patients and 33% of patients had alloantibodies against Kell system. Three important factors that significantly influenced the frequency of alloimmunization were: age at the first blood transfusion, splenectomy and β- thalassemia intermediate.

Conclusion: Alloimmunization is a common complication among patients with transfusion dependent β-thalassemia in Khuzestan province, Iran. Matching the selected donors with recipients based on the extended red blood cell antigen typing may decrease the incidence of alloimmunization.

Key Words: Alloimmunization, thalassemia major, thalassemia intermediate, RH blood group, Kell blood group.

Introduction

Thalassemia is the most common genetic disorder in Iran and Southwest Iran particularly. According to the last Iranian Ministry of Health's registration report, 18500 and 1800 patients with Thalassemia major (TM) exist in Iran and Southwest Iran respectively. It has been estimated that 10 percent of these patients have Thalassemia intermediate (TI) phenotype [1,2] .

Blood transfusion is the main treatment in TM and occasionally in TI [3]. Blood transfusion is used regularly in patients with TM and regularly and/or occasionally in patients with TI [4]. The aim of treatment is to maintain pre transfusion hemoglobin equal to or more than 9.5 g/dl. Transfusion is performed to improve the anemia and to suppress the ineffective erythropoiesis [5]. Frequent transfusions prevent most serious growth, skeletal, neurological and endocrine complications

in thalassemic patients [6]. Blood transfusion is not a simple procedure like water and electrolyte infusion, but is a kind of temporary tissue transplantation and alloimmunization is a reaction resembling the tissue rejection. Blood transfusion introduces a multitude of alloantigens and living cells into the recipient's body. This procedure continues at least monthly and restimulation of recipient immune system with different antigens in repeated blood transfusions might boost the alloimmunization phenomenon [7,8]. Each component in the blood unit is able to produce antibodies. Blood antigens such as human leukocyte antigens, class I and II; granulocyte-specific antigens; platelet-specific antigens and red blood cell-specific antigens can stimulate the immune system and produce alloantibodies. The differences between blood donor and recipient, the RBC antigen potency and the recipient's immune status contribute to the alloimmunization process [9].

The first manifestation of alloimmunization is considered if the pre transfusion hemoglobin is less than the expected level for a particular patient on two occasions. It is expected to decline patient's hemoglobin as a crude estimate of one gram per week. The diagnosis of RBC alloimmunization is suspected if the hemoglobin dropping is significantly more than usual. The other causes of hemoglobin dropping namely hypersplenism, folate and vitamin B12 deficiency and infection should be ruled out. Delayed hemolytic transfusion reaction (DHTR) is a common finding of alloimmunization that occur between 24 hours to 2 weeks after transfusion. DHTR may present as fever, chills, jaundice, pain and dyspnea. Rarely, cases may be complicated with renal failure or disseminated intravascular coagulation [10]. Alloimmunization is a major problem in thalassemia management. It makes a condition in which blood transfusion may help to fuel the increasing of alloantibodies; in this case, blood transfusion not only is not a live-saving medical practice but also it may be a live-threatening procedure [11].

Because of the high numbers of TM and TI patients in Khuzestan province, Southwest Iran, and the importance of alloimmunization as a treatment failure, the present study aimed to detect the incidence rate of alloimmunization among our patients. To our knowledge this is the first study to date in Khuzestan province considering this subject.

Patients and Methods

This study was approved by the ethics committees of the Jundishapur University of Medical Sciences, Ahvaz, Iran and the Iranian blood transfusion organization. This was a cross sectional and descriptive study. Over a period of 9 months, 133 patients were selected from 420 patients (290 with thalassemia major and 130 with thalassemia intermediate).The inclusion were continuous decline of pre hemoglobin level in recent 6 months, positive direct and indirect Coombs test, history of post transfusion chill, backache, dark urine and icter. Exclusion criteria were patients with a history of hypersplenism, shortage of interval transfusion due to infection and megaloblastic anemia, positive panel test due to other diseases and positive history of intravenous gamma-globulin and corticosteroid usage during the last three months.

Amongst 420 subjects 133 patients in the age range of 10 to 25 years were randomly selected. After obtaining an informed consent from patients and their parents the participants were divided into two groups, one group included 102 TM patients and the other group comprised of 31 patients with diagnosis of TI. Diagnosis of TI was confirmed by hemoglobin electrophoresis, the date of first transfusion, pre transfusion hemoglobin level and clinical manifestations. All patients were interviewed and filled in a questionnaire consisting of demographics questions, kind of thalassemia, date of the first blood transfusion, transfusion blood type (standard, washed, leucoreduced), history of splenectomy, dyspnea, hemoglobin level, reticulocyte count, direct and indirect Coombs test, serum level of AST (aspartate aminotransferase), total and direct bilirubin, hemoglobinuria and the type of alloantibody, as well as signs and symptoms like fever, chill, jaundice and pain. From each patient 10cc of blood was obtained in pre transfusion state. The serum was drawn and analyzed to detect new antibodies against RBC antigens. The serum was mixed with saline-suspended red cells with the addition of low ionic strength saline (LISS), and incubated at 37°C for 15 minutes. A 3-cell antigen panel was used for the antibody screening procedure, and an anti-IgG reagent was used for the antiglobulin phase. The panel test which determines 18 blood groups for the following antigens "D, C, E , e, c, K, k, M, N, S, s, Fya , Fyb, Jka, Jkb, Lea, Leb and P" has been approved by

Table 1: The frequency of laboratory and clinical post transfusion findings in thalassemia patients with alloimmunization.

Post transfusion findings	Percent
Hemoglobin reduction in recent 6 months	84.7
Positive direct/indirect coombs test	78.2
Dark urine	68.7
Chill	68
Backache	58.1
Icter	27

FDA. If the screen was positive, an extended panel to identify the antibody and a direct antiglobin test (DAT) were performed. Absorption methods were employed in patients presenting with a new autoantibody. In cases of a positive DAT, further investigation using specific reagents to detect IgG, IgM, or a complement were carried out. When an antibody was detected, eluates were prepared and tested against common sample erythrocytes. In some cases polyethylene glycol was used to enhance the reactivity. The tests were carried out as follow: CBC counting was carried on by BC-3000 Plus machine Mindray, Bio-Medical, Electronics. Co. Ltd, Shenzhen – China, Hb electrophoresis was performed using D10, Bio-rad, High-Performance Liquid Chromatography, USA and finally Hb A2Column Chromatography was performed using Bio system, Aria Pharmed CO, Tehran-Iran.

All data were statistically analyzed using t test, logistic regression test and Fisher's exact test. A P-value of less than 0.05 was considered statistically significant.

Results

The mean age of patients was 17.5 (1SD±7.5). Sixty six (49.1%) were male and 67 patients (50.9%) were female. Out of these patients 102 (76.8%) had thalassemia major and 31 (23.2%) were thalassemia intermediate cases. The mean age for the first blood transfusion was 7.5 (1SD±4.7) months and 62.5 (1SD±97.1) months for thalassemia major and intermediate groups respectively. A total of 28 (21 %) of patients experienced splenectomy and there was no significant difference between the two groups of thalassemia major and intermediate.

Red cell alloimmunization was found in 25 (18.7%) of patients and 17 patients (12.7%) had auto antibodies. The prevalence of alloimmunization was 15% and 30% among thalassemia major and thalassemia intermediate patients respectively. The difference between groups was significant (P= 0.03). Table 1 shows the prevalence of positive clinical manifestations related to alloimmunization. These manifestations were not significantly different between the two groups of thalassemia major and intermediate. The received blood transfusion per year was more than 240cc/kg in both thalassemia major and intermediate groups. Out of 25 patients with alloantibodies 18 patients were found with one antibody, 5 with two antibodies and two had three antibodies. There was no significant correlation between alloimmunization rate and patients who routinely used washed packed cell. There was significant correlation between alloimmunization and the age of the start of blood transfusion (P< 0.01). The incidence rate was 12.6% and 36.1% in patients below one year and above three years respectively (Table 2). There was no significant gender and tribe difference between alloimmunization and non alloimmunization groups. The effect of splenectomy as a risk factor for alloimmunization was statistically significant (P=0.03).The leukocyte depleted filters did not significantly influence the alloimmunization rate. The alloantibodies percent pattern was as follow: 55% against RH subtypes, 33% against Kell and 12% against the other groups (Table 3). Five out of 66 patients (7.6%) among females and 4 out of 55 patients (7.3%) among males had the evidence of alloimmunization. The mean age of patients with alloimmunization was 9.6 years (range 3.7-20). Four patients (44.4%) with alloimmunization were more than 3 years old at the time of first blood transfusion. The mean age at the first blood transfusion in patients with alloimmunization and without alloimmunization were 2.8 and 1.7 years

Table 2: Correlation between the prevalence of alloimmunization and the age of the first blood transfusion.

The age of the first blood transfusion (years)	Alloimmunization percent
<1	12.36
<2	16.33
<3	33.33
>3	38.1

respectively (P=0.1). Direct or indirect antiglobulin tests were positive in 5 patients (62.5%). The blood alloantibodies by a panel of antibodies using standardized blood bank methods were detected in 4 patients, which were of anti-K and anti-D types. They had received regular blood transfusions during periods ranging from 1 to 25.5 years.

Discussion

Blood transfusion is widely used in treatment of blood diseases such as TM, TI, sickle cell anemia, aplastic anemia and so on. RBC alloimmunization is a known complication of blood transfusions and it is an obstacle in future treatment. Alloimmunization against blood groups occurs following transfusion, pregnancy and transplantation. In patients who are transfused regularly such as thalassemia and sickle cell anemia patients the frequency of alloimmunization is high.

Blood selection based on the limited red blood cell antigens matching (ABO, RH) may increase the incidence of alloimmunization in TM and TI patients but extensive blood matching may decrease this phenomenon therefore the varying incidence of alloimmunization in different populations may be related to the strategy of blood banking in selection of limited or extensive blood matching policy. In our institution blood matching is limited to ABO and Rh (D antigen). The rate of alloimmunization depending on the blood bank policy varies from 4.97% to 37% in different countries [12]. In the present study the alloimmunization rate was found to be 18.7 and 12.7 percent in TM and TI patients respectively. The rate of alloimmunization in the other parts of Iran has been reported as following: 7.4% (Tehran), 5.34% (Fars province) and 2.87% (Northeast Iran) [13]. Furthermore, the alloimmunization frequency in other countries is 30% (Kuwait), 19.5% (Egypt), 7.4% (Hong Kong), 5% (Italy), 4.97% (India), and 3.7% (Greece) [13,14-16]. By comparing these frequencies with Southwest Iran, the prevalence of alloimmunization in our population was more than other studies from Iran and some other countries, and is similar to the frequency in Egypt. Moreover the high frequency in Southwest Iran may be due to (I) population heterogeneity, (II) limited blood matching between donor and recipient. The most pattern of alloimmunization in the present study was alloantibodies against RH and Kell blood groups. In RH system the antibody was mostly against E and D antigens. These results are in concordance with Ameen et al. and Sirchia et al. and Bhatti et al. reports [17-19]. Among our patients the alloimmunization rate against D antigen was nearly 20%; which is lower than Sadeghian et al. study but is higher than Karimi et al. and Natukunda et al. study [13, 20, 21].

One reason for the high frequency of anti D among thalassemia patients in South and North Iran is an error in differentiation of D and Du. One of the most important reasons for alloimmunization is the transfusion of some red blood cells with rhesus D incompatible with thalassemia patients due to false negative results in weak D typing of blood donors. Transfusion of weak D blood donor to D negative patient may stimulate the immune system. Approximately one percent of D-positive blood donors are typed as weak D (historically known as DU. The determination of weak D positive RBCs in D negative blood group and antibody screening for Rh (D, C and E) antigens as the most common alloantibodies are essential in control plans in blood banking laboratory. Ameen et al. have reported Anti-K in 72% of patients and anti-E in 45.6% and in our study Anti K, anti-E and anti D were found in 33%, 24% and 20.62 of patients respectively [17]. In another study, Karimi et al. detected high prevalence of antibodies against Rh system (47.7%) [20].

In a study in Hong Kong splenectomy

Table 3: Distribution of alloantibodies.

Blood Groups	Percent
RH system	55
D	20.62
E	24
C	10.31
Kell system	33
Kidd system	7
Duffy system	3
Rare blood system	2

showed no prominent effect in the frequency of alloimmunization rate; however Singer et al., have reported a significant effect of splenectomy in the incidence of alloimmunization [16]. Similarly our findings indicate splenectomy as a risk factor for alloimmunization. One of the most important roles of spleen is filtration, this role is removed in patients with splenectomy; therefore this may confirm the effect of splenectomy on increasing the rate of alloimmunization.

It has been revealed that there is no significant correlation between washed packed cell and alloimmunization. Moreover using washed packed cell isn't an adequate method for blood transfusion, and had been used prior to pre storage filtration and/or bedside filtration.

In the present study, similar to a study by Dutcher et al. alloimmunization rate was related to the age of first transfusion [22]. This implies that the age of the first blood transfusion is a risk factor for alloimmunization. Transfusion at early age may offer some protection against red cell alloimmunization because of immune tolerance in young children. In some centers, thalassemia patients receive full blood matched transfusions in order to prevent alloimmunization. There is no consensus among different institutions about this strategy and this method increases the costs dramatically [23-25].

Conclusion

Alloimmunization is a common complication among patients with transfusion dependent β-thalassemia in Khuzestan province, Iran. Matching the selected donors with recipients based on the extended red blood cell antigens typing may decrease the incidence of alloimmunization. The This diverse population within Khuzestan province, the limited blood matching, the age at the first blood transfusion and splenectomy were the common risk factors for alloimmunization in this study.

Acknowledgment

The authors are grateful to the thalassemia patients, the personnel of the Thalassemia and Hemoglobinopathy Center and the Iranian Blood Transfusion Organization for their collaboration. This work was supported in part by research funds from Thalassemia and Hemoglobinopathy Center, Jundishapur University of Medical Sciences, Ahvaz, Iran and Iranian Blood Transfusion Organization. This study was the fellowship thesis of Dr. Hiradfar.

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