



ORIGINAL ARTICLE

Red Cell Alloimmunization and Autoimmunization in Multi-Transfused Thalassemia Patients in Sulaymaniyah Province-Iraq

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ABSTRACT

Thalassemias are considered important health issues throughout Iraq, involving its Kurdistan region. This disorder, particularly its major form, needs lifelong regular transfusions. But this form of medical care is associated with various complications including red cell alloimmunization and autoimmunization. This study determined the frequency and associations of alloimmunization among multi-transfused patients with β -thalassemia major. The subjects were 204 patients who were registered at a thalassemia care center in Sulaymaniyah-Iraqi Kurdistan. The patients' records were analyzed, their red cells were phenotyped for ABO/RhD antigens using the gel card method, and irregular antibody screening/identification was performed using the standard tube method. Alloantibodies were detected in 5.8% of the patients, while DAT was positive in 4% of the patients, which indicated autoantibodies. The identified alloantibodies were anti-E (2.4%), anti-C (1.4%), anti-e (1%), and anti-K (1%). A patient's age at the start of transfusion (>2 years) ($P=0.042$) and a positive history of transfusion reactions ($P=0.003$) were correlated with a significantly higher rate of alloantibody formation. From the results of our study, we conclude that measures to decrease the development of alloantibodies may incorporate matching for Rhesus and Kell systems and early induction of blood transfusions.

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INTRODUCTION

Thalassemias are one of the most prevalent hereditary anemias characterized by a partial or complete defect in the synthesis of α or β globin chain [1]. They comprise a major health issue in many countries [2]. Iraq, a country located in the eastern Mediterranean region is afflicted with thalassemia and other hemoglobinopathies with around 15,000 symptomatic cases registered at different thalassemia care centers throughout the country [3]. Anemia is a

constant feature of the thalassemia syndromes, caused by ineffective erythropoiesis and hemolysis, both are due to unbalanced globin chain synthesis [4]. Treatment of β -thalassemia major includes lifelong blood transfusions commonly administered every 2 to 5 weeks, aiming to sustain the pre-transfusion hemoglobin level above 9.0~10.5 g/dL [5]. In addition to general complications of blood transfusion, one of the major consequences of repeated transfusion in thalassemia patients is the development of alloantibodies and in some cases autoantibodies against red blood cells (RBC) antigens [6]. RBC antigens alloimmunization is an immune response usually provoked by transfusion of RBCs and pregnancy. Some alloantibodies are hemolytic leading to hemolytic transfusion reactions, others are

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clinically insignificant [7, 8]. Many studies have demonstrated diverse frequencies of alloantibodies in multiply transfused patients with thalassemias [9-12]. The mechanism of development of alloimmunization is complex and implies many contributing factors including recipient exposure to foreign donor antigen, RBC antigenic immunogenicity, recipient immune status, age at onset of transfusion, and the number of transfusions [7, 13]. Also, splenectomy may affect the alloimmunization rate. The absence of spleen may hasten the immune response to the infused unfamiliar antigens which are not effectively filtered [4, 14]. Autoantibodies against own erythrocytes, although less commonly encountered in thalassemias, may lead to clinical hemolysis and troubles in cross-matching of blood [15-17]. Extended RBC phenotyping and preparing antigen-negative blood for the detected alloantibodies decreases post-transfusion complications and permits for long-standing favorable transfusion regimens to be attained but this process is burdensome and cannot be carried out routinely in our blood banks. Although the frequency of the most common RBC antigens in our region was determined in a study by Hisham et al [18] no researches were performed on the extent of alloimmunization and autoimmunization among thalassemia patients in our northeastern province. The current study aimed to find out the frequency of RBC alloantibodies and determine their types, since measures to reduce alloimmunization rates may include extended matching for antigens that are more prone to alloimmunization. Besides, we evaluated risk factors that may attribute to the formation of alloantibodies in repeatedly transfused thalassemia patients. Identifying a high-risk group will be an important step forward in matching practice in our province.

MATERIALS AND METHODS

A total of 204 patients with β -thalassemia major registered in the local thalassemia center in

Sulaymaniyah, Iraqi Kurdistan, and receiving regular blood transfusions were enrolled in this study. The study was performed following approval by the local institutional ethical committee (approval No 55/5.2019). Informed consent was collected from enrollees. The diagnosis of β -thalassemia major was based on clinical data, blood counts, standard hemoglobin analysis mostly with high performance liquid chromatography (HPLC) or capillary electrophoresis, and molecular studies as appropriate. All patients transfused with plasma-depleted blood matched for ABO and Rhesus D (RhD) antigens. The patients received leuco-depleted blood during transfusion using a bed-side filter. Clinical and transfusion data of the enrolled patients were collected from the medical recording system belonging to the local thalassemia center including age, sex, ABO and RhD grouping, ethnicity, age at first transfusion, the total number of transfused RBC units, frequency of blood transfusions per year, transfusion-related side effects especially history signifying alloantibody-dependent reactions, and splenectomy status.

Blood samples in EDTA and plain tubes were collected from the patients for ABO/RhD blood grouping and antibody screening/identification, respectively according to the standard protocols [19]. Although, all registered thalassemia patients had ABO and RhD phenotyped in their records, ABO and RhD blood grouping were repeated by the gel card method using the ABO-D/reverse grouping system (Bio-Rad Laboratories, DiaMed GmbH, Cressier, Switzerland). The antibody-screening test was carried out by indirect antihuman globulin test-tube method (IAT) using the patient's serum and in-house screening-cell prepared by mixing three fresh group O RBCs withdrawn from three normal subjects. Thus, we developed screening cells obtained from regional ethnic groups so that clinically significant alloantibodies against RBC antigens in the local population could be detected. An auto control, utilizing the patient's cell and serum, was tested side by side with each screen to rule out the

existence of autoantibodies. In patients with a positive screen (except samples with positive auto control which were excluded from identification test), the antibody-identification process was done by tube method using an extended commercial 11-cell identification panel (ID-DiaPanel, Bio-Rad Laboratories, DiaMed GmbH, Cressier, Switzerland). The reactions were graded from negative to 4+ as indicated by the manufacturer. Any tube test that was negative in the AHG phase was controlled by adding in-house prepared sensitized control cells (which are RhD-positive RBCs coated with anti-D). The specificity of detected antibodies was attained when the reaction pattern of the tested sample against panel cells was interpreted by the identification tables supplied by the manufacturer. Direct antihuman globulin test (DAT) was performed for the samples with positive auto control using polyspecific AHG reagent in standard tube method, to find out that a positive auto control is surely the result of antibody-mediated agglutination and not simply the result of non-immunologic clumping. Figure 1 shows the sequence of the procedures used in this study.

Statistical package IBM SPSS version 25 (IBM Corp., Armonk, NY, USA) was used to analyze the data. Continuous data are presented as means \pm SD and ranges. The Chi-squared test was used to compare differences in the categorical data between alloantibodies-positive and alloantibodies-negative patients. An independent t-test was used for differences in continuous data. All *P*-values <0.05 were regarded as statistically significant.

RESULTS

The current study involved 204 Iraqi Kurdish β -thalassemia major patients, 106 (52%) were males and 98 (48%) were females, with their ages ranging from 2~27 years and a mean age of 11.2 (\pm 4.8). The ABO blood groups of the included patients were in the following order: A, O, B and AB, composing 44%, 32%,

22%, and 2%, respectively. The RhD antigen was positive in 97.5% of the enrollees while it was negative in 2.5%. The mean age at the start of transfusion for all patients was 11 (\pm 17) months, with 186/204 (91%) receiving their first unit of blood before the age of 2 years. Besides, the rate of alloimmunization was 16.7% (3/18) in patients transfused at \geq 2 years of age while it was only 4.8% (9/186) in patients transfused earlier than 2 years of age as shown in Figure 2. The mean total number of transfused RBC units among all patients was 198 (\pm 199) units and ranged from 14 to 790. Concerning the frequency of blood transfusion, the mean number in all patients was 15 (\pm 4) units/year. Almost half of all patients 101/204 (49.5%) had a positive history of transfusion reactions while splenectomy was observed in only 47/204 (23%). Among the alloimmunized patients who developed transfusion reactions 45% (5/11) had delayed hemolytic transfusion reaction (DHTR) manifested as history of jaundice and dropping of hemoglobin while 55% (6/11) developed allergic reactions manifested as itching and rash. Further, among the non-alloimmunized patients who developed transfusion reactions 67% (60/90) developed allergic reactions, 28% (25/90) had febrile non hemolytic transfusion reaction (FNHTR) manifested as fever and chills, and 5% (5/90) developed DHTR.

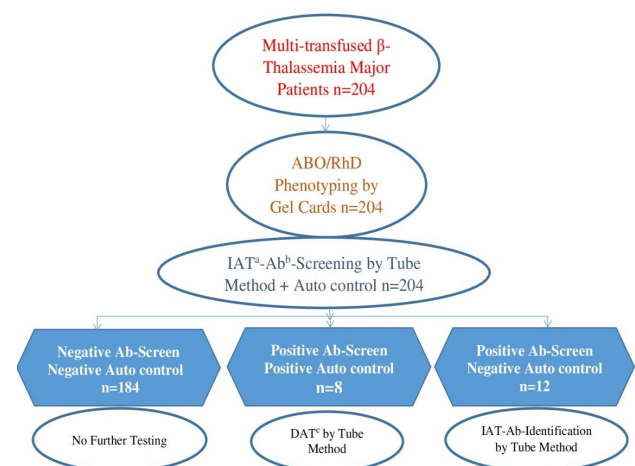


Figure 1. Flow chart demonstrating the sequence of the procedures followed in this study. ^aIAT, indirect antihuman globulin test; ^bAb, antibody; ^cDAT, direct antihuman globulin test.

Utilizing antibody-screening test and auto control, alloantibodies were detected in 12 (5.8%) of the 204 patients while autoantibodies were found in 8 (4%) of the patients. The frequency of specific alloantibodies in the study group as defined by antibody identification test was as follows: anti-E was detected in five (2.4%) of

the patients, anti-C in three (1.4%), anti-e in two (1%), and anti-K in two (1%) of the patients (Table 1).

The associations of distinct parameters in thalassemia patients with and without alloantibodies are shown in Table 2. In general, the patient's age, sex, ABO/RhD blood groups, frequency of blood transfusion per year, the total number of transfused blood units, and spleen status did not show statistically significant differences between alloantibody positive and allo-

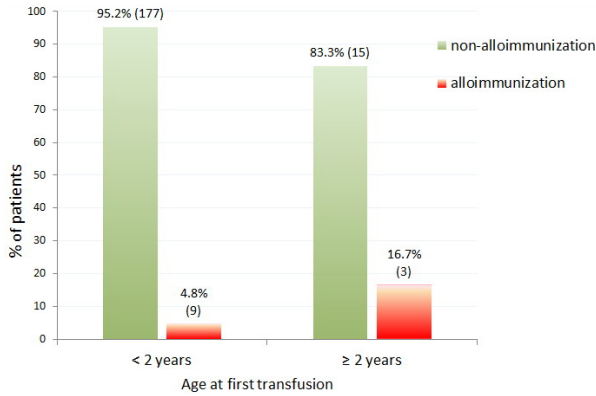


Figure 2. Demonstrate the relation of age at first transfusion and alloantibody formation.

Table 1. Alloantibodies identified among the studied multi-transfused patients with β-thalassemia major

Presence or absence of alloantibodies	N	%
Negative alloantibody	192	94.2
Positive alloantibody	12	5.8
Anti-E	5	2.4
Anti-C	3	1.4
Anti-e	2	1
Anti-K (Kell)	2	1
Total study group	204	100

Table 2. Relation between alloantibody development and several variables in the studied multi-transfused patients with β-thalassemia major

Variables	Non-alloimmunized	Alloimmunized	P-value
Number of patients	192	12	-
Age/years			0.243
Mean ±SD (range)	11 ±5 (2~27)	12 ±3 (9~16)	
Sex			0.293
Male	98 (51)	8 (66.7)	
Female	94 (49)	4 (33.3)	
ABO blood groups			0.920
A	84 (44)	6 (50)	
B	42 (22)	2 (17)	
O	62 (32)	4 (33)	
AB	4 (2)	0 (0)	
RhD blood group			0.174
Positive	188 (98)	11 (92)	
Negative	4 (2)	1 (8)	
Age at first transfusion			0.042
<2 years	177 (92.2)	9 (75)	
≥2 years	15 (7.8)	3 (25)	
Frequency of blood transfusion/Years			0.893
<12 units/year	14 (7.3)	1 (8.3)	
≥12 units/year	178 (92.7)	11 (91.7)	
Total number of transfused RBC units			0.878
Mean ±SD (range)	197 ±204 (14~790)	206 ±73 (108~384)	
History of transfusion reaction			0.003
Positive	90 (47)	11 (91.7)	
Negative	102 (53)	1 (8.3)	
Splenectomy			0.114
Yes	42 (22)	5 (41.6)	
No	150 (78)	7 (58.4)	

antibody negative patients. However, we observed a significantly greater alloimmunization in thalassemia patients who started transfusion beyond the age of 2 years in comparison to those who began it earlier ($P=0.042$). Additionally, a history of transfusion reaction was observed more frequently in patients with positive alloantibodies (91.7%) compared to patients without alloantibodies (47%) ($P=0.003$).

DISCUSSION

This study demonstrated that the overall frequency of alloimmunization was 5.8%. The worldwide reported frequency of alloantibody formation in β -thalassemia varies substantially with a range of 4.0~37% [20]. The relatively low frequency of alloimmunization detected in this study could be partially due to the low mean age of our enrolled patients, and most importantly due to the reality that most of the blood donors, as well as our recipients, are from similar ethnic groups because most of our patients and donors were Kurdish Muslims. This finding is in accordance to a study performed in another province in Kurdistan region of Iraq, Dohuk that demonstrated a frequency of 3% within the most common ethnic group (Kurdish Muslims) while observing a remarkably higher frequencies 10.4% and 21.4% in different ethnic groups (Kurdish Yazidis and Syrians emigrants), respectively [21].

In the present study, all the alloantibodies were formed against antigens in the Rhesus and Kell systems. This is widely consistent with other reports worldwide where immunization to the Rh system and the K antigen considered the most common [20-22]. Anti-E antibody was the most frequent, followed by anti-C, anti-e, and anti-K which coincide with other previous studies that showed that these are the most common alloantibodies in multi transfused thalassemia patients [4, 6-11, 15, 21]. Furthermore, the rate of autoantibodies observed in the current study was 4%, in agreement with other studies reporting a range of 0.47%~45% [6, 7, 10, 11, 15, 16, 23, 24]. Yet, other studies deny the detection of

autoantibodies [21, 25]. Although the formation of autoantibodies against red cell antigens in association with RBC alloimmunization has been recorded in previous studies [12, 15, 16] leading to autoimmune hemolytic anemia (AIHA) and difficulty in cross-matching, we did not address the pathogenesis, associations, and consequences of autoantibodies in the current study.

In the current study, no significant association with gender was observed, similar to other studies [7, 10, 21, 22, 26, 27]. However, gender was implicated in the development of alloantibodies by various studies, although some identified males and others identified females as being more subjected to develop antibodies [8, 25]. Likewise, no relation between alloantibody formation and each of age, ABO, and RhD blood groups was observed in our study consistent with previous reports [21, 24, 27] while in disagreement with others [25, 26]. Various studies confirmed that RBC alloimmunization is more likely to occur in patients who were more frequently transfused with a higher total number of units received [25-27], while others did not affirm such an association [21, 24]. Our results are similar to the later reports. Further, no significant difference in the rate of alloantibodies between splenectomized and non-splenectomized patients was observed in this study. This was in agreement with earlier studies [11, 21, 24] but contrary to other reports where higher rates were detected with splenectomy [7, 8, 22]. It was hypothesized that the missing role of the spleen in filtering the foreign, damaged, and conformationally changed RBCs after splenectomy may further augment the immune response to the infused antigens. On the other hand, in the current study, we found that the development of alloantibodies was significantly lower in thalassemic patients who begun transfusion therapy much earlier (≤ 2 years) in comparison to those who commenced later in life. This observation was shared by earlier reports [21, 25]. This finding elucidates that an immature immune system may cause an acquired immune tolerance to foreign antigens decreasing the

risk of alloantibody formation [10, 21]. As expected, we observed that most of the alloimmunized patients (91.7%) had a positive history of transfusion reactions compared to non-alloimmunized patients and is observed by other studies [21, 28].

In conclusion, the rate of alloimmunization and autoimmunization among the studied patients with β -thalassemia major is generally low. It is still possible to further decrease the rate by performing extended matching to involve the Rh and K antigens. Further, the introduction of transfusions at an early age may be an additional beneficial approach.

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Conflict of interest: None

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