ORIGINAL RESEARCH

Study of red blood cell alloimmunization risk factors in multiply transfused thalassemia patients: role in improving thalassemia transfusion practice in Fayoum, Egypt

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BACKGROUND: b-Thalassemia is considered themost common chronic hemolytic anemia in Egypt. Alloimmunization can lead to serious clinical complications in transfusion-dependent patients. The objective of this study was to determine the frequency and types of alloantibodies. In addition, to study the risk factors that might influence alloimmunization in multiply transfused thalassemia patients in Fayoum, Egypt, with the goal that this study could help minimize some of the transfusion-associated risks in those patients.

STUDY DESIGN AND METHODS: A total of 188multiply transfused thalassemia patients attending Fayoum University Hospital were analyzed. Alloantibody identification was performed by DiaMed-ID microtyping system.

RESULTS: Alloimmunization prevalence was 7.98%.The most common alloantibody was D-related; anti-D was the most frequent alloantibody found in eight of the 188 patients (4.25 %), followed by anti-C in two patients (1.1%), anti- E in two (1.1 %), anti-c in two (1.1 %), anti-Fya in two patients (1.1%), anti-K in one (0.53 %), and an unknown antibody in one patient (0.53%). Higher rates of alloimmunization were found in female patients, in patients with b-thalassemia intermedia, in splenectomized patients, in D– patients, and in patients who started blood transfusion after 3 years of age.

CONCLUSION: The study reemphasizes the need forcost-effective strategy for thalassemia transfusion practice in developing countries. Red blood cell antigen typing before transfusion and issue of antigen-matched or antigen- negative blood can be made available to alloimmunized multiply transfused patients. Early institution of transfusion therapy after diagnosis is another mean of decreasing alloimmunization.

Thalassemia is one of the most common inherited hemoglobinopathies in the world. In Egypt, b-thalassemia is the commonest form of chronic hemolytic anemia.[1]

Lifelong and frequent red blood cell (RBC) transfusions remain the main treatment for severe cases of thalassemia.[2] Development of RBC alloantibodies and autoantibodies can complicate transfusion therapy. Some alloantibodies are hemolytic and may cause hemolytic transfusion reactions; others are clinically insignificant.[2,3] Results from a number of studies have demonstrated various frequencies of alloantibodies formation in multiply transfused patients ranging from 5% to 30 %.[4,5] Development of RBC autoantibodies can result in clinical hemolysis and in cross-matching problems.[6]

The risk of alloimmunization depends on the recipient exposure to the foreign antigen and its immunogenicity. Alloimmunization may also be influenced by the age of onset and the number of the transfusions as well as the recipient sex, age, and other underlying factors.[7] Extended RBC phenotyping and administration of antigen-negative blood for the present alloantibodies reduces posttransfusion complications and allows for long-standing successful

ABBREVIATIONS: ••• 5 •••.

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transfusion regimens to be achieved[8,9] but this process is cumbersome and costly and cannot be implemented in Egypt blood banks. In developing countries like Egypt, the RBCs used for transfusion are matched only for ABO and D blood groups.

The aim of this work was to determine the frequency of RBC alloantibodies and identify their types and study risk factors that may contribute to their production in multiply transfused b-thalassemia patients. Identifying a high-risk group will be a feasible first target, a big step forward in matching practice, and the goal of our study with the hope of changing transfusion strategy and minimizing some of the transfusion-associated risks of thalassemia patients in Fayoum University Hospital, Egypt.

MATERIALS AND METHODS

Recruitment

This study was conducted over a 1-year period from May 2013 to April 2014 on 188 multiply transfused b-thalassemia patients attending the Fayoum University Hospital. Informed consent was obtained from each subject or their legal guardians before enrollment in the study and the Fayoum University Research Ethics Committee, which is a member of Egyptian Network Research Ethics Committee, was informed of this study. Decision of transfusion was taken when the hemoglobin (Hb) level was less than 7 g/dL after exclusion of anemia caused by sepsis or viral infections and when the Hb level was more than 7 g/dL with complications like poor growth, fractures, or facial changes. The study included patients who received more than 10 units of RBCs at the time of the study, patients previously transfused with nonleukoreduced RBCs matched only for ABO and D antigens, both sexes, and patients more than 6 months of age.

Laboratory investigations

All pretransfused patients were routinely tested for ABO and D antigen (by the gel card method using a ABO-D/reverse grouping system (DiaMed).The antibody screening test was performed with a combination of three sets of commercial group O RBCs that have been typed for clinically significant antigens as well as rare antigens. These three sets are known as (Dia-Cell I, II, III; DiaMed).

Positive sera were examined using a commercial 11-cell identification panel (Diapanel, Bio-Rad). Autoantibodies were studied by incubating patient's own cell with patient's plasma at 378C for 15 minutes and then centrifuging for 10 minutes on gel card containing polyspecific antihuman globulin (anti IgG 1 C3d).

Patients with positive screen were assessed based on sex, age, history of transfusion, clinical diagnosis, and alloantibody specificity. Thalassemia patients in Egypt generally have a poor quality of life. As a result, there are only a few married female thalassemia patients in the child-bearing period in Fayoum; hence, we could not include pregnancy as a risk factor of alloimmunization in our study.

Statistical analysis

All statistical calculations were performed using computer software (SPSS Version 18, Windows 7, SPSS, Inc.). Qualitative data were statistically expressed in the form of frequency and percentages. Numerical data were statistically represented in terms of range, mean and standard deviation (6SD). Pearson chi square and t test were used for comparing categorical variables. A p value less than 0.05 was considered significant.

RESULTS

Of the 188 patients, 103 (54.78 %) were males and 85 (45.21 %) were females; their ages ranged from 2 to 45 years with a mean of age 10 years. The ABO blood groups of the 188 patients were as follows: 76 (40.42 %) patients had blood group A, 45 (23.9%) had blood group B, 42 (22.34%) had blood group O, 25 (13.3 %) had blood group AB, 167 (88.8%) were D1, and 21 (11.12%) were D–. A total of 147 (78.19%) had b-thalassemia major and 41 (21.81%) had b-thalassemia intermedia. With regard to the spleen state 66 (35.1 %) patients were splenectomized, whereas 122 (64.9 %) were not. The mean age at which transfusion started for the patients was 34.6 months. As for the frequency of blood transfusion, the mean number was 11.7 units/year.

Using antibody-screening tests and autocontrol, alloantibodies were detected in 15 (7.98%) patients. The frequency of specific alloantibodies in the study group was as follows: anti-D was found in eight (4.25%) of the study group, anti-C in two (1.1%), anti-E in two (1.1 %), anti-c in two (1.1 %), anti-Fya in two patients (1.1%), anti-K in one (0.53 %), and an unknown antibody in one patient (0.53%). The frequency of specific alloantibodies in the study group is shown in Table 1. T1

Females had a significantly higher frequency of alloimmunization (14.1%) compared to males (2.9%; $p < 0.05$). Alloimmunization was higher in patients more than 20 years of age (31.8%) compared to patients below 10 years (4.76%) and patients between 10 and 20 years old (4.91%; $p < 0.01$). Alloimmunization frequency was significantly higher in b-thalassemia intermedia patients (19.5%) compared to b-thalassemia major (4.76%; $p < 0.05$). Alloimmunization frequency was significantly higher in splenectomized patients (13.63%) compared to nonsplenectomized patients (4.9%; $p < 0.05$). Alloimmunization frequency was significantly higher in D– patients

TABLE 1. Frequency of specific alloantibodies in the study group*

Type of alloantibody	Study group (n 5 188)
Anti-D	
Anti-D	8 (4.25)
Anti-C	2 (1.1)
Anti-E	2 (1.1)
Anti-c	2 (1.1)
Anti-Duffy	
Anti-Fya	2 (1.1)
Anti-Kell	
Anti-K	1 (0.53)
Unidentified	1 (0.53)

*Data are reported as number (%).

TABLE 2. Comparison of the qualitative data regarding alloimmunization

Variable	Alloantibodies*	p value
Sex		
Male (n 5 103)	3(2.9)	<0.05
Female (n 5 85)	12(14.1)	
Age groups (years)		
<10 (105)	5 (4.76)	
10-20 (n 5 61)	3 (4.91)	<0.01
>20 (n 5 22)	7 (31.8)	
Thalassemia type		
b-Thalassemia major (n 5 147)	7 (4.76)	
b-Thalassemia intermedia (n 5 41)	8 (19.5)	<0.05
Splenectomy status		
Nonsplenectomized (n 5 122)	6(4.9)	<0.05
Splenectomized (n 5 66)	9 (13.63)	
Transfusion reactions		
Absent (n 5 138)	5 (3.62)	<0.01
Present (n 5 50)	10 (20)	
Age of onset of transfusion (years)		
<3 (n 5 154)	8(5.2)	<0.01
>3 (n 5 34)	7 (20.5)	
Duration of blood transfusion (years)		
<10 (n 5 103)	7(6.8)	>0.05
>10 (n 5 85)	8(9.4)	
Total number of RBC units transfused		
<20 (n 5 18)	1 (5.55)	
20-50 (n 5 43)	3(6.4)	
51-100 (n 5 60)	4(6.3)	>0.05
101-150 (n 5 39)	4 (10)	
>150 (n 5 28)	3 (10.7)	
ABO blood groups		
A (n 5 76)	8 (10.52)	
B (n 5 45)	4(8.5)	>0.05
AB (n 5 25)	0 (0)	
O (n 5 42)	3(6.5)	
D blood groups		
D1 (n 5 167)	7(4.2)	<0.01
D– (n 5 21)	8 (38)	

*Data are reported as number (%).

(38%) compared to D1 patients (4.2%; $p < 0.01$). Comparison of the qualitative data regarding alloimmunization is T2 shown in Table 2. Autoantibodies were not detected in any of the patients.

DISCUSSION

Only a few studies have investigated the frequency and causes of alloimmunization and autoimmunization in Egypt.[10,11] Our study is the first study to be conducted in Fayoum Governorate, a developing city in Middle Egypt; we examined the risk factors of alloimmunization in multiply transfused thalassemia patients and defined the frequency and types of alloantibodies among patients attending Fayoum University Hospital.

We demonstrated an alloimmunization incidence rate of 7.98%. Similarly, Dhawan and colleagues[12] found that the alloimmunization rate in thalassemia patients was 5.6%. Other studies by Chao and coworkers[13] and Shenoy and coworkers[14] reported that the incidences of alloimmunization were 9.4 and 9.5%, respectively. Other Egyptian studies by Hussein and colleagues,[3] Moftah and Metwalli,[15] and Saied and colleagues[16] reported higher frequencies of alloimmunization with rates of 22.8, 26.3, and 28.4% respectively. The relatively low overall rate of alloimmunization in this study and other similar studies can be attributed to the fact that the majority of blood donors, as well as recipients, are from the same ethnic group,[17] as all of our patients and blood donors were from Fayoum, Egypt. On the other hand, the Egyptian studies with higher alloimmunization rate were all conducted in Cairo, the capital that is hosting many different communities with a bigger donor pool. This indicates clearly that the frequency of alloimmunization after random multiple blood transfusions diminishes significantly if blood transfusion between donor and recipient is kept within the same ethnic group.[17] Other than the ethnic mismatching the chance of alloimmunization is expected to be higher the larger the number of donor exposures.[17,18]

The specificity of most alloantibodies detected in this study was against the D system (77.8%), which is similar to previous reports of Thakral and coworkers (61%),[19] Hmida and coworkers (59%),[20] and Dhawan and coworkers (52%).[12] The transfusion of blood matched for D could prevent alloimmunization resulting in a significant difference in the alloimmunization rates.[21,22] In our study anti-D developed in 28.6 % of all D– alloimmunized patients, followed by anti-C, anti-c, anti-E, and anti-Fya in 11.8% of alloimmunized patients. The high rate of anti-D in D– patients in our study is likely related to transfusions of units with weak D antigens, as it is not mandatory in Egypt to test apparently D– donors for weak D. Hussein and Teruya[23] demonstrated an anti-D incidence of 63.5% in Egyptian D– children with thalassemia who received units for which weak D was not tested.

In our study, alloimmunization frequency was higher in females. Similarly, Bauer and colleagues[24] reported that female sex is a risk factor for alloimmunization after RBC transfusion. Also, Sadeghian and colleagues[25] reported that clinically significant alloantibodies occurred approximately

twice as often in women compared with men. No difference in alloimmunization frequency between the two genders was found by other researchers Shenoy and colleagues,[14] Singer and colleagues,[2] Ho and colleagues,[26] Ameen and colleagues,[5] and Thompson and colleagues.[27]

In our study the rate of alloimmunizations was higher in patients more than 20 years of age compared to younger patients. The same findings were reported by Thompson and coworkers.[27] In contrast, Chaudhari,[4] Singer and coworkers,[2] and Ho and coworkers[26] reported no significant relationship between age and alloimmunization in transfusion-dependent thalassemia patients.

Our study revealed a higher percentage of positive alloantibodies among b-thalassemia intermedia compared to thalassemia major. Similar results were reported by Ahmed and coworkers,[11] in the National Research Centre in Egypt. On the other hand, Thompson and colleagues[27] found that rates of alloimmunization did not differ significantly by thalassemia phenotype.

In this study, patients who had a splenectomy had a higher alloimmunization rates than nonsplenectomized patients ($p < 0.01$). Similar findings were reported by other studies.[1-3] However, other studies have reported no difference among patients who underwent splenectomy.[28-30]

Our study supported the concept of immune tolerance developing in very young children; the alloimmunization rate in children who started transfusion at age older than 3 years was significantly higher than children who started transfusion younger than 3 years ($p < 0.01$). Similar to these results, Dhawan and coworkers[12] showed that age at first transfusion was significantly higher in alloimmunized than in nonimmunized patients. Singer and coworkers[2] reported that transfusion at ages younger than 1 to 3 years may have offered some immune tolerance and protection against alloimmunization in thalassemia patients. Early institution of transfusion therapy after diagnosis is an important mean of decreasing alloimmunization but carries the risk of more exposure to other transfusion complications. Despite advances in iron chelation and blood safety, major improvements in hemosiderosis and transfusion-acquired infections are still needed.[31]

Autoantibodies were not detected in any of our patients; this can be attributed to the fact that all thalassemia patients in Fayoum University Hospital are routinely injected with steroid therapy before each transfusion as a prophylaxis against allergy and transfusion reactions. However, the pathogenesis of RBC autoantibody formation after transfusion is not well understood.[32]

Interestingly, our study revealed an unusual frequency of ABO blood groups with a higher percentage of blood groups A (40.42%) and AB (13.3%) and a lower percentage of group O (22.34%) compared to other Egyptian studies. A study conducted in Cairo by Hussein and colleagues[3] reported ABO blood group frequencies of 34% for group A, 32% for group O, and 6.6% for group AB. The relevance of having knowledge about the blood group systems among different population is enormous and useful for obtaining genetic information, genetic counseling, and medical diagnosis as well as general and physiologic well-being of individuals in a population.[33]

In conclusion, our blood bank is relatively newly established one with limited resources, so little transfusion-related research work has been performed. In developed countries, alloimmunization against common antigens more frequently expressed on donor RBCs can be easily screened for, and using antigen-negative RBCs can prevent antibody production. In developing countries, the strategy needs to be individualized based on resources as well as blood group distributions in the various recipient and donor populations.

Our data suggest important recommendations that have to be implemented and become part of our routine practice in Fayoum University Hospital Blood Bank, including, all D– units of blood should be retested during anti-human globulin phase to rule out the weak reaction and providing blood matched only for D antigens especially for female patients and patients with potential risk factors. Initiating transfusion as early as possible by developing a screening program for newborn babies and young children attending our hospital and ensuring blood availability by building and maintaining safe sustainable voluntary donor base are very important recommendations being the major causes of delaying the start of transfusion in Fayoum, Egypt. Using a limited number of blood donors for a designated patient is also important.

ABO blood group frequencies must be studied on larger sample number to find out the real rate of blood types in this Egyptian Governorate as our study reported unusual ABO blood group rate compared to rates reported worldwide and in Egypt. Additional studies are needed on Fayoum female patients to determine the cause of increased alloimmunization rate than males, especially that there were few of females with obstetric events in our study. Recipient immune status; genetic, environmental, or inflammatory factors; or prior exposure to non-RBC antigens may have contributed.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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