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Red Blood Cell Immunization and Contributing Factors in 685 Thalassemia Patients

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ABSTRACT

Background: An analysis of red blood cell alloimmunization in patients with thalassemia can help to devise specific strategies to decrease the alloimmunization rate. This study explored the frequency and specificity of alloantibodies and autoantibodies against red blood cell (RBC) antigens in patients with thalassemia referring to the Iranian Blood Transfusion Organization (IBTO) Immunohematology Reference Laboratory (IRL) in Tehran.

Materials and Methods: This study first examined the laboratory records of 23,113 patients suffering from different diseases referring to IBTO's IRL for pretransfusion testing in the 2008-2015 period. ABO and Rh(D) typing and antibody screening tests were performed for all 23,113 patient records and 685 (2.97%) beta-thalassemia patients with positive pre-transfusion test results (antibody screening and/or DAT) were selected for further investigation.

Results: The antibody screening test was positive in 640 out of 685 thalassemic patients (93.4%). DAT was performed for 529 patients, 226 (33%) of which showed positive results. Meanwhile, 161 out of 685 beta-thalassemia patients (23.5%) had positive auto control test results, reflecting the possible presence of allo- and/or autoantibodies. The most common antigen-specific alloantibodies were directed against K and E RBC antigens with a frequency of 25% (Anti-K) and 11.91% (Anti-E), respectively. The development of two antibodies (double antibodies) in one patient was observed in 80 individuals (11.46%).

Conclusion: Age, gender, history of pregnancy, and splenectomy were not contributing factors to the antibody presence in the patient population under study. Extended red blood cell phenotyping should be considered as an essential procedure for expected multi-transfused thalassemia patients before blood transfusion. Considering the high frequency of anti-K and anti-E observed in this study, it is recommended that thalassemia patients in Iran are tested through phenotyping of RBC units for K and E antigens before transfusion.

Keywords: Alloimmunization; Thalassemia; Anti-K; Antibody identification (ABID); Direct antiglobulin test (DAT)

INTRODUCTION

Immunization against different antigens in transfused blood units (i.e. alloimmunization) is a blood transfusion reaction that may complicate transfusion therapy. Repeated and chronic blood transfusion induces immune responses in recipients, triggering the production of alloantibody and autoantibody against red blood cell antigens, resulting in red blood cell lysis (1). Alloantibodies are produced more frequently than autoantibodies[1].

Many studies have investigated alloantibody production and frequency in multi-transfused individuals such as thalassemia patients[2-6]. Thalassemia syndrome is a genetic hematologic disorder that causes anemia and the most common treatment procedure is chronic blood transfusion[7].

The reported worldwide alloimmunization frequency rate among thalassemia patients varies from 1.13% to 40.4%. The most common alloantibodies reported are antibodies against Rh (C, c, & E), Kell (K), Kidd (Jka & Jkb), and Duffy (Fya & Fyb) RBC antigens[8].

This study aimed to investigate the frequency of antibodies and some possible contributing factors such as age, gender, and previous pregnancy and/or abortion in beta-thalassemia patients referring to IBTO's IRL in Tehran, Iran during 2008-2015. The research subject was partly inspired by the research gap on the common frequency of RBC alloantibodies in beta-thalassemia major and intermedia patients. A complete record of each patient's antigen typing, most common red cell antibody frequencies, and transfusion history is kept in this laboratory to better manage the thalassemia patients.

MATERIALS AND METHODS

The research population consisted of 685 beta-thalassemia major and intermedia patients (out of 23,113 patients; 2.97%) with unexpected pretransfusion test results. These patients consented to participate in this study allowing for their blood samples to be collected and analyzed as problematic cases. ABO and Rh(D) typing, antibody screening and antibody identification (ABID) tests, and direct antiglobulin testing (DAT) were performed on the patients, according to our previous report (9). In brief, ABO and Rh(D) blood typing was done (Anti-A, Anti-B, & Anti-D IBRF Holding Co., Tehran, Iran) using the automated technique (Diagast 251/AV.AVINEE-5912 Loos, France). The antibody screening test was performed using commercial gel cards (MTC Invetrogel, Germany) and the standard tube method with a homemade three-cell (IBTO mini panel) kit. A homemade 11-cell antibody identification panel (IBTO 11-cell Kit, Registration No. 63882) was used to identify antibodies in sera in case of positive screening results. Direct antiglobulin test (DAT) was performed using automated technique DC-Lys EM® (Diagast 251/AV.AVINEE-59120 Loos, France) and standard tube methods. Differential DAT was performed for cases with a positive initial DAT result. The automated technique of DAT was followed by a well of antiglobulin anti-IgG, a well of antiglobulin anti-C3d, and a well of negative control.

RESULTS

Laboratory records of 685 beta-thalassemia patients (519 thalassemia major and 16 thalassemia intermedia), including 303 (44.2%) males and 382 (55.8%) females, were selected for analysis. The mean patient age (±SD) was 24.8±10.6 years (range 1-68 Y). Anti-K (25%) was the most frequent alloantibody followed by Anti-D (15.9%) and Anti-E (11.91%) in the Rh system. Anti-E was reported as the most frequent antibody by several researchers ranging from 21-50% (15, 16).

Here, 94.4% of males and 92.7% of females had positive antibody screening test results encompassing 640 out of 685 patients (93.74%), with no significant difference between male and female groups (P=0.36).

Forty patients (10.5%) in the female group declared a previous pregnancy and/or abortion in their medical history, with 92.7% of this group showing positive antibody screening test results in comparison to 87.5% of females with no previous history of pregnancy. Pregnancy had no significant effect on antibody production (P=0.23).

Moreover, 89 patients (13%) were younger than 12 years old (<12Y), 84 patients (12.3%) aged between 12 and 18 (12-18 Y) and 512 (74.7%) were older than 18 years old (>18y). Positive antibody screening result were 92.1% (<12Y), 95.2% (12-18Y), and 93.4% (>18Y) for each group, respectively. Age had no significant effect on antibody production (P=0.7).

Direct antiglobulin tests (DAT) were positive in 226 (33%) of 685 thalassemic patients (Table 1). Antibody identification (ABID) test showed positive results for 213 (94.2%) patients with positive DAT results, reflecting RBC sensitization (by auto- and/or alloantibodies). Furthermore, there were 13 patients (5.8%) with positive DAT and negative ABID results.

DAT was not performed for 156 patients (22.8%). Meanwhile, 303 (57.3%) of the thalassemia patients had negative DAT even though 284 (53.7%) of them had a positive ABID.

In general, positive auto control test results were found in 161 cases (23.5%), reflecting the possible presence of allo- and autoantibodies.

Among patients with positive DAT results, the splenectomy procedure (removal of the spleen) was performed in 302 patients (44.1%), 290 (42.3%) were not splenectomized, and there was no information available for 93 (13.6%) patients. Antibody screening results were positive among 94% of the splenectomized patients in comparison to 93.8% of non-splenectomized patients (P=0.42). The results showed that 47% of splenectomized patients developed one antibody in comparison to 59.7% of non-splenectomized patients (P<0.19), indicating that splenectomy has no significant effect on the antibody presence.

ABO/Rh(D) blood groups of the patients in this study are shown in Table 2. The ABO or Rh(D) antigen could not be determined in 36 complicated cases for various reasons such as the presence of RBC chimeras due to the transfusion of recent compatible ABO and Rh(D) type RBC units rather than type-specific RBCs (ie O blood type RBC transfused to A blood type) and the presence of strong autoantibody reactions.

Positive antibody screening results were different between Rh(D) negative and Rh(D) positive patients (A-:92.7%; A+:93%; B-:96.8%; B+:92.9%; AB-:95.2%; AB+:93.3%; O-:96%; O+:93.5%), which was statistically significant (p>0.05). The most common identified antibodies were Anti-K (25%), Anti-D (15.9%), Anti-E (11.91%), Anti Kpa (7.85%). Double antibodies were developed just in 80 patients (11.46%), mostly anti-C+E (38.8%), anti K+Kpa (17.5%), and anti E+K (16.25%). Moreover, 109 thalassemic patients (15.9%) developed Anti-D antibodies. Meanwhile, 60 (8.76%) patients showed a single antibody, 33 (4.81%) patients had both Anti-D and Anti-C antibodies, eight patients showed Anti-D and Anti-K, two patients developed Anti-D and Anti-E and six other patients developed multiple antibodies as follow: Anti-D, Anti-C, Anti-E; Anti-D, Anti-E, Anti-K; Anti-D, Anti-c, Anti-K and Anti-D,Anti-C, Anti-E and Anti-K.

Table 1: DAT positivity among the thalassemic patients

Disease\ DAT type	Polyspecific (C3d + IgG) N (%)	Monospecific AHG Only C3d N (%)	Monospecific AHG Only IgG N (%)
Thalassemia Major	86 (12.6)	79 (11.5)	15 (2.2)
Thalassemia Intermedia	21 (3)	19 (2.8)	6 (0.9)
Total	107 (15.6)	98 (14.3)	21 (3.01)
		226 (33)	

DAT: Direct immunoglobulin test, N: Total number of patients, AHG: anti-human globulin

Table 2: Blood groups frequency among the Thalassemic patients (N=685)

ABO Blood groups	Number (N)	Percent (%)
A	203	29.6
B	160	23.4
AB	52	7.6
O	251	36.6
Unresolved cases	19	2.8
Rh(D)		
Rh(D)+	519	75.8
Rh(D)-	149	21.8
Unresolved cases	17	2.5
Total	685	100

DISCUSSION

In this study, 93.4% of thalassemic patients were alloimmunized to RBC antigens. The most common alloantibodies were Anti-K, Anti-D, and Anti-E. Double antibodies were developed in 11.46% of patients. RBC sensitization happened in 94.2% of patients with both DAT and ABID positive results indicating the possible presence of both auto- and alloantibodies. According to the findings, gender, history of splenectomy, and history of pregnancy were not associated with alloimmunization. The choice of subject was partly inspired by the unavailability of information on the frequency of RBC alloantibodies and the contributing factors in beta-thalassemia major and intermedia patients referring to IBTO's IRL. A complete record of each patient's antigen typing, most common red cell antibody frequencies, and transfusion history is kept in this laboratory to better manage the thalassemia patients

Al-Riyami et al. reported no association for alloimmunization with gender and history of splenectomy, but they found a significant association between age and alloimmunization, especially in patients between 19 to 30 years old. The prevalence of alloimmunization was 9.3% with anti-E (24%) and anti-K (24%) being the most common antibodies (10).

A higher alloimmunization frequency was found in this study compared to previous reports (2, 4, 6, 8, 11-15). This higher rate in our selected patient population reflects the nature of our laboratory, which is a national immunohematology referral laboratory to resolve problematic blood samples referred from hospital blood banks throughout the country to identify antibodies and perform confirmation tests.

One reason for Anti-D alloimmunization may be the transfusion of Rh(D) incompatible blood due to human or technical errors or Rh(D) variants in serologically Rh(D) negative blood units. Transfusion of Rh(D) positive blood units to patients with weak D and partial D may also explain Anti-D immunization (17). DAT positive results reflect RBC coating by IgG or complement or both, which may cause difficulties in RBC phenotyping by serologic methods and interfere with blood unit matching (18). Patients with positive DAT may or may not have hemolysis. Some normal individuals have positive DAT without any significant RBC destruction and hemolysis. Therefore, positive DAT does not always reflect immune hemolytic anemia (19).

Thirteen patients (5.8%) had positive DAT and negative ABID results, reflecting RBC sensitization with autoantibody in patients' sera. However, some patients showed negative DAT and positive ABID results, showing the absence of cell sensitization despite the presence of antibody in their sera. Morawakage et al. reported a 2-year-old boy with β thalassemia major with C3d DAT positive and no RBC antibody results. Arinsburg argued that DAT has certain limitations in post-transfusion samples of thalassemia patients. It is said that Rh(D) typing was difficult in some complicated cases using routine serologic methods and related antisera. This may be explained by RBC coating by allo- or autoantibodies. Sometimes weak D blood grouping using serologic methods may provide wrong results and mischaracterize positive types as negative. This problem can be solved through molecular genotyping.

CONCLUSION

In summary, age, gender, and history of pregnancy or splenectomy showed no significant effect on antibody presence in this study. Extended red blood cell phenotyping should be considered as an essential procedure for expected multi-transfused thalassemia patients. Therefore, it is recommended that all thalassemia patients in Iran are transfused with ABO and Rh(D) compatible and at least E negative and K negative blood to avoid alloimmunization against these antigens.

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