Medical Laboratory Journal
Online ISSN: 2538-4449
Golestan University of Medical Sciences

Red blood cell alloantibodies in patients with thalassemia major referred to the blood transfusion center of Golestan province, Iran

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Abstract

Background: One of the major complications in thalassemia patients is alloimmunization, which occurs when the patient produces antibodies against transfused Red Blood Cells (RBCs). In the present study, the frequency of alloantibodies was investigated in patients with thalassemia major.

Methods: This cross-sectional retrospective study was performed on 99 multi-transfused patients with thalassemia major. An antibody screening test was carried out using a three-cell panel. Positive patients were followed up for antibody identification using an 11-cell panel. The information was finally analyzed using SPSS software version 16.0.

Results: Out of ninety-nine cases, 53 were female (53.53 %) and 46 were male (46.46 %). The patient's mean age was 29.22±10.46 years with an age range of 2 to 61 years. Only 5.05% (n=5) had developed alloantibodies. The most common alloantibodies were anti-D, anti-E, anti-c, and anti-K. No significant correlation was seen between the presence of alloantibody and age, sex, blood type, and spleen condition.

Conclusion: Antibody production against RBC antigens is a common problem in multi-transfused thalassemia patients. Compatibility between antigens of the Kell and Rh blood group systems in donors and recipients can be one of the useful ways to prevent alloimmunization of blood recipients and the formation of unexpected antibodies against the donor's red blood cells.

Article Type: Research Article

Article History

Received: 27 September 2023
Received in revised form: 24 December 2023
Accepted: 2 January 2024
Available online: 16 August 2025
DOI: 10.29252/mlj.19.4.27

Keywords

Alloantibody
Thalassemia
Blood transfusion

Introduction

Thalassemia is the most common genetic disorder worldwide, affecting the synthesis of one or more of the globin subunits of hemoglobin leading to ineffective erythropoiesis (1). Geographically, thalassemia is a Mediterranean disease and more prevalent in West Asian and Central Asian countries such as Turkey, Iran, Burma, it is also found in Thailand, Vietnam and Colombia. Also, it is very common in Africa, Greece, and Italy (2). It can be found in more than 60 countries with a carrier (heterozygote) population of up to 150-200 million people or 4.5% of the world population, and at least 300,000 lethally affected homozygotes are born annually (3).

In Iran, more than two million thalassemia carriers and more than twenty thousand patients with thalassemia major have been identified (4). Provinces around the Persian Gulf and the Caspian Sea with a gene frequency of more than 10% are considered the thalassemia major zones in Iran (5). Patients with this hemoglobinopathy need regular transfusions. Repeated blood transfusions can stimulate the immune system and result in the formation of anti-erythrocyte antibodies. This can cause a problem in the identification process of compatible blood in these patients and cause a delay in the process of supplying and injecting compatible blood (6). The possibility of creating antibodies after injecting a bag of blood is 1% to 1.6%; the reported worldwide alloimmunization prevalence among thalassemia patients varies from 1.13% to 40.4% (7,8). Blood transfusion is the main method of treatment for these patients. However, regular blood transfusion leads to organ damage due to iron overload and immunization to RBC antigens. In Iran, routine blood group typing of thalassemia patients identifies ABO and Rh (D) antigens only before blood transfusion for thalassemia patients. Most alloantibodies are formed against them, which causes an increase in the need for blood transfusions. On the other hand, it makes it difficult to identify compatible blood units for subsequent blood transfusions.

Several studies have shown that, in most cases, responsible antibodies are related to the subgroups of the Rh system, including E, e, C, and c as well as secondary groups such as Kell, Duffy, and Kidd (9-12).

Antigenic differences in blood group systems between the donor and recipients, as well as the immunomodulatory effect of the allogenic blood transfusion on the immune system, are effective factors in alloimmunization. Due to receiving repeated blood transfusions in thalassemia patients from different people, the preparation of compatible blood is not easily possible for these patients. Therefore, the purpose of this study was to determine the prevalence of alloimmunization in thalassemia patients who received regular transfusions and help avoid hemolytic transfusion reactions that may occur during future blood transfusions.

Methods

This descriptive, cross-sectional study investigated alloantibodies in patients with thalassemia major receiving regular blood transfusions at the Blood Transfusion Center of Golestan Province, Iran. Demographic and clinical data, including age, gender, blood group (ABO/Rh), and spleen status, were collected from patients' medical records using a standardized checklist. For laboratory analysis, five milliliters of venous blood were collected from each patient in EDTA-containing vacutainer tubes before transfusion. Samples were centrifuged one hour after collection, with serum aliquoted into two tubes for antibody screening and identification. Antibody screening was performed two hours post-sampling using three screening cells (R1R1, R2R2, rr) through standard blood bank techniques, including testing phases in saline, albumin

(22%), and indirect antiglobulin (Coombs) to detect RBC alloantibodies. Samples showing positive screening results underwent further analysis using 11-cell panels provided by the Immunohematology Department of Iran Blood Transfusion Organization, with confirmation through the standard tube method. All positive results were referred to the Immunohematology Laboratory of the Blood Transfusion Organization for validation. Statistical analysis was performed using SPSS version 16.0. Chi-square test was used to assess associations between variables at a 95% confidence interval (CI). P-values < 0.05 were considered statistically significant.

Results

Among 99 patients with thalassemia major receiving routine blood transfusions at 3-5 week intervals, 53% were female and 46% were male, with a mean age of 29.22 ± 10.46 years (Range: 2-61 years). The ABO blood group distribution showed that 37% of patients were type O, 23% type B, 25% type A, and 14% type AB, while 82% were Rh(D)-positive. Alloantibodies were detected in five patients (5.05%), with the remaining 94 (94.95%) showing no evidence of alloimmunization. Of the five alloimmunized patients, three (60%) were female and two (40%) were male. Two patients (40%) developed two alloantibodies each (Anti-c and anti-E in one case, anti-D and anti-K in the other), while the remaining three (60%) had single antibodies-two with anti-E and one with anti-D. Overall, anti-E and anti-D were the most common alloantibodies (Each 33.3%), followed by anti-c and anti-K (Each 16.7%), (Table 1 and 2). The majority of detected antibodies targeted Rh system antigens (85.7%), with Kell system antibodies accounted for the remaining 13.4%. Statistical analysis indicated no significant association (P > 0.05) between alloantibody formation and age, sex, blood type, or spleen status.

Table 1. Prevalence of alloantibodies in patients

Alloantibodies	Frequency (n)	Percentage (%)
Anti-K	1	16.7
Anti-c	1	16.7
Anti-E	2	33.3
Anti-D	2	33.3
Total	6	100

Table 2. Clinical and laboratory characteristics of alloantibody-positive thalassemia patients

Sex	Age (Year)	ABO	Rh	Splenectomy	Autoantibody	Alloantibody
Male	44	O	Pos	No	No	Anti-E and anti-c
Female	36	B	Neg	No	No	Anti-D and anti-K
Female	22	A	Neg	No	No	Anti-D
Male	34	B	Pos	No	No	Anti-E
Female	43	O	Neg	No	No	Anti-E

Pos: Positive; Neg: Negative

Discussion

Repeated blood transfusion is a life-saving intervention to improve health in patients with thalassemia major. Alloimmunization is one of the important complications of transfusion in these patients. It is the response of the immune system to foreign RBC antigens and is therefore called an alloantibody. It occurs following transfusion, pregnancy, and transplantation. In patients who are transfused regularly, such as thalassemia and sickle cell anemia patients the frequency of alloantibodies is high (13). The factors responsible for alloimmunization are complex, including at least three major reasons: the RBC antigenic differences between the blood donor and the recipient, the recipient's immune status, and the immunomodulatory effects of the allogeneic blood transfusions on the recipient's immune system (12). In thalassemia patients, the main cause of the development of RBC alloimmunization is repeated blood transfusion. This may reduce the survival of transfused RBCs and reduce the efficacy of blood transfusion, leading to increased transfusion requirements. The frequency of alloimmunization in patients with repeated transfusions varies in different studies. In thalassemia patients, the alloimmunization frequency is 4-50%, in hemato-oncological patients, 1.9-13% patients are alloimmunized, and in kidney disease patients, the alloimmunization frequency is 1.27-13.1% (14).

Several studies have evaluated the prevalence of alloantibodies in thalassemia patients. According to some studies conducted in Iran, the prevalence of alloantibodies is different in various regions, i.e., 7.4% (Tehran), 5.34% (Fars Province), 18.7% (Southwest of Iran), 2.87% (northeast of Iran), 17.9% (southeast of Iran),5% (Shiraz),1.53% (Lorestan), 40% (Mazandaran) (15-22). In the Azarkivan's study, 835 patients with thalassemia were screened for unexpected RBC alloantibodies. They reported an alloimmunization rate of 12.1% (23). In this study, 5.05% of patients with thalassemia major were alloimmunized to RBC antigens. The most common clinically significant alloantibodies detected in our study were anti-E, anti-D, anti-c in the Rh system, and anti-K in the Kell system. In another study conducted in 2016, the rate of alloimmunization in Iran was reported to be 10%. In this study, most alloantibodies were related to anti-E, anti-D, and anti-K (24). In Rostamian's study, it was shown that 13% of transfused thalassemia patients in Iran had clinically significant RBC alloantibodies, and anti-D and anti-K antibodies were the most common antibodies (25). Shaiegan's study showed that the most common alloantibodies were anti-E, anti-D, anti-E, and anti-c (26). One main 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 (27).

The prevalence of alloimmunization and the type of antibody detected are different in different regions of the country. One of the reasons for the production of alloantibodies is the antigenic difference between the donor and the recipient such as pre-transfusion testing and phenotype determination, which is mainly attributed to racial-ethnic diversity and genetic effects in response to this antigenic stimulation. The greater the antigenic similarity between the recipient and the donor, the less likely it is to stimulate the immune system. In the present study, considering the racial and ethnic diversity in the provincial population, the difference in the prevalence of alloantibody and the type of antibody in thalassemia patients is quite expected. Appropriate preventive strategies, such as RBC phenotyping before initiating transfusion and using extended RBC donor and recipient matching, especially for Rh and Kell antigen systems, could be implemented to avoid complications in these patients. According to the results of this study and other conducted studies, accurate ABO and Rh antigen typing, and knowledge of the abundance of alloantibodies can provide this possibility for treatment centers and blood product storage centers to allocate reserves for special patients with constant need to prepare blood injection, which can significantly reduce alloimmunization in future transfusions in these patients.

Conclusion

Alloimmunization is a common consequence in patients who need persistent blood transfusions (For example, thalassemia patients). Some of these antibodies are clinically significant and may compromise the safety of further blood transfusions and cause hemolytic transfusion reactions. Determination of RBCs phenotype donor and recipient before beginning chronic blood transfusion, especially Kell and Rh blood group antigens (C, c, E, and e) and careful cross-matching with thorough evaluation of the donor unit's blood group may help reduce the alloimmunization rate and delayed hemolytic transfusion reactions and enhancing the lifespan in chronic transfusion patients.

Acknowledgement

We sincerely thank the colleagues of the immunohematology department of the Central Headquarters of the Blood Transfusion Organization who helped us in this study.

Funding sources

This study did not receive any grants from funding agencies, whether public, commercial, or non-profit.

Ethical statement

The study was approved by the Ethics Committee of the Golestan University of Medical Sciences, Iran (Code: IR.GOUMS.REC.1402.375).

Conflicts of interest

The authors declare that there are no conflicts of interest.

Author contributions

All authors contributed to one or more aspects of the study.

Data availability statement

All data generated or analyzed during this study are published in this article.

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Cite this article as:

Baniaghil SS, Balochi F, Kazzak E. Red blood cell alloantibodies in patients with thalassemia major referred to the blood transfusion center of Golestan province, Iran. *Med Lab J.* 2025;19(4):27-9. http://dx.doi.org/10.29252/mlj.19.4.27