Red Blood Cell Alloimmunization in Thalassemia Patients: A cross-sectional study from the South-West of Iran

Abstract

Aims*:* Despite numerous studies on the prevalence of alloimmunization, its exact rate remained uncertain in many populations. In light of the Iranian Blood Transfusion Organization's recent national program to reduce alloantibody formation, this study investigated the prevalence of alloimmunization among thalassemia patients in Kohgiluyeh and Boyer-Ahmad Province, Iran, and explored associated factors such as donor-recipient antigen homogeneity and early transfusion initiation.

Methods: A cross-sectional study was conducted on 134 beta-thalassemia major patients registered at Shahid Beheshti Hospital, Yasuj, Iran. Antibody screening was performed using both the standard tube method and the gel method in the immunohematology laboratory of Yasuj University of Medical Sciences.

Findings: Out of 134 cases, 73 (54.48%) were female and 61 (45.52%) were male, with a mean age of 19.51 ± 8.5 years. A high donor–recipient antigen homogeneity is suggested by the fact that 132 (98.5%) patients were native to Yasuj. All patients received ABO and Rh (D)-matched, leukodepleted blood transfusions at intervals ranging from 14 to 60 days (mean: 22.7 ± 6.3 days); 65.7% were splenectomized. Alloantibodies were detected in two patients (1.49% prevalence; one with anti-K and one with anti-C), both of whom had undergone splenectomy. No autoantibodies or positive direct antiglobulin test results were observed.

Conclusions: The low incidence of alloimmunization in Kohgiluyeh and Boyer-Ahmad Province may reflect high donor–recipient antigen homogeneity and the early initiation of blood transfusions. Future studies with larger cohorts and multivariate analyses are recommended to further elucidate the risk factors of alloimmunization.

Keywords:

beta-Thalassemia(https://www.ncbi.nlm.nih.gov/mesh/68017086);
blood transfusion(https://www.ncbi.nlm.nih.gov/mesh/68001803),
splenectomy(https://www.ncbi.nlm.nih.gov/mesh/?term=splenectomy)

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Introduction

Thalassemia, first described by Thomas Cooley in 1925, is a genetic disorder caused by mutations in the globin genes, resulting in impaired hemoglobin synthesis leading to defective erythropoiesis and severe anemia(1). The term "thalassemia belt" refers to regions such as the Mediterranean, SouthWestern Europe, Southeast Asia, and parts of Central Africa(2–4). In Iran, particularly in areas surrounding the Caspian Sea and the Persian Gulf, thalassemia is hyperendemic(5,6).

Regular blood transfusion remains the lifesaving treatment for thalassemia patients, although it exposes them to adverse outcomes including infections, hemosiderosis, and alloimmunization(7–9) . With each transfusion, a large number of donor-derived antigens are introduced into the recipient's circulation, potentially stimulating the immune system to produce alloantibodies against donor red blood cell (RBC) antigens absent on the recipient's RBCs—a phenomenon known as alloimmunization. Despite advances in pre-transfusion screening and extended phenotyping, alloimmunization continues to be a significant clinical challenge and it may lead to delayed hemolytic reactions and difficulty in finding compatible blood units(10). Few studies have explored alloimmunization specifically in Iran. The incidence of alloantibody formation in the thalassemia population ranges from 5.6% to 24.7% worldwide(11).

There are various studies on the rate of alloimmunization and the frequency of alloantibodies due to its importance in blood transfusion. According to a meta-analysis study, the rate of the alloimmunization is approximately 10% in Iran (95% CI: 7–13)(12). One study on patients with thalassemia major in Lorestan province, Iran, reported 1.53% of alloimmunization rate(13). In another in southern Iran, the prevalence of alloantibodies was 1.7% (14). In some studies, the alloimmunization rate in BTM patients was higher than that of in our study. In the study of Kosaryan et al., which was conducted in Sari, Iran, 40.4% of patients were identified as alloantibody positive with tube method(15). In another study by Hiradfar et al. in Ahwaz, Iran, 18.75% of patients were alloimmunized(16). In similar studies conducted in different parts of Iran, the prevalence of alloimmunization has been reported from 2.87%(Northeast) to 40.4%(North)(17). Despite these investigations, there are still regions in Iran with limited or no data on alloimmunization prevalence. Understanding these regional variations is essential for optimizing transfusion safety and guiding national blood policies. Therefore, the present study aimed to determine the prevalence of red cell alloimmunization among thalassemia patients in Kohgiluyeh and Boyer-Ahmad Province, Iran, and to evaluate potential influencing factors such as age at first transfusion and splenectomy statuse.

Materials and Methods

Study Population and Design This cross-sectional study was conducted on 134 patients with beta-thalassemia major (BTM) registered at Shahid Beheshti Hospital, Yasuj, Iran between 2020 to 2021. Given the limited number of thalassemia major patients in Yasuj (n = 134), and considering that almost all of them regularly visit the Cooley's Ward of Shahid Beheshti Hospital, Yasuj, for scheduled blood transfusions every 15 to 45 days, the study population was defined as the entire reference population. Inclusion and Exclusion Criteria: After obtaining informed consent from each patient or from the patient's legal guardian (for minors), individuals with a confirmed diagnosis of thalassemia major based on standard clinical and laboratory criteria anda regular transfusion history were included in the study. Patients were excluded if their medical records were incomplete or if they withdrew from participation during the study period.

Blood Sample Collection and Laboratory Methods

Between 2 to 5 mL of blood was collected from each patient in EDTA and clot tubes under standardized conditions. The EDTA samples were used for ABO and Rh (D) typing as well as for the direct antiglobulin test (DAT), while the clot samples were used for antibody screening. Testing was conducted using Anti-A, Anti-B, and Anti-D reagents (Iranian Blood Research & Fractionation Company, Tehran, Iran) employing the standard tube method.

Direct Antiglobulin and Antibody Screening Tests

For the DAT, one drop of a 2–5% RBC suspension was added to four test tubes, followed by three saline washes. Poly-specific AHG, anti-IgG, and anti-C3 reagents (CE – Immun Diagnostika GmbH, Germany) were added simultaneously, with one tube serving as a negative control. After agitation and centrifugation, the tubes were examined for agglutination.

For the Indirect Antiglobulin Test (IAT), two drops of patient serum were added into three separate tubes, followed by one drop each of Screening Cells I, II, and III (Biovesta, USA). After an immediate

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spin, albumin was added, and the samples were incubated at 37°C for 10 minutes. Post-washing, one drop of AHG was added to each tube; agglutination was then evaluated, and any negative reactions were rechecked using Coombs Control Cells (Biovesta, USA). Discrepant results between clinical findings and tube method results were confirmed with the gel method.

Statistical Analysis: For quantitative data assessment, data are reported as(mean, standard deviation, percentages) using SPSS version 20 and Chi-square test was used for statistical analysis of qualitative data.

Findings

A total of 134 BTM patients were enrolled, of whom 73 (54.48%) were female and 61 (45.52%) were male. The mean age was 19.51 ± 8.5 years (range: 1 to 37 years). Notably, 132 patients (98.5%) were native to Yasuj, suggesting a high degree of donor-recipient antigen homogeneity.

Blood Group and Transfusion Data The blood group distribution was: O (48.5%), B (15.7%), A (30.6%), and AB (5.2%), with 97.8% positivity for the Rh (D) antigen. All patients received ABO and Rh (D)-matched, leukodepleted blood transfusions which is a process where white blood cells (leukocytes) are removed from blood components that is typically done using filters or centrifugation to reduce the number of leukocytes to a low level, at intervals of 14–60 days (mean interval: 22.7 ± 6.3 days). Additionally, 88 patients (65.7%) underwent splenectomy. splenectomy is a surgical procedure where the spleen, an organ located in the upper left abdomen, is removed. It's often performed when the spleen is damaged, diseased, or enlarged, or when it's not functioning properly. The transfusion volume varied, with five patients receiving over 40 units annually and one patient receiving eight units within a year. The first transfusion was administered at a mean age of 15.42 ± 19.4 months (range: 1 to 108 months).

Alloantibody Screening Alloantibodies were detected in 2 patients (1.49% prevalence): one with anti-K and one with anti-C. Both of these patients had a history of splenectomy. No autoantibodies were observed using the standard tube method and all DAT results were negative.

Discussion

The prevalence of RBC alloimmunization among BTM patients in Kohgiluyeh and Boyer-Ahmad Province was found to be 1.49%, which is notably lower than reports from other regions in Iran, where the prevalence ranges from 0% to over 40% (12–17). Understanding the factors contributing to this low alloimmunization rate is essential for developing preventive strategies and optimizing transfusion protocols. Several possible explanations for this low prevalence include high donor–recipient antigen homogeneity, early transfusion initiation, leukodepleted blood transfusion, and splenectomy status(18,19).

Studies conducted in different Iranian provinces have reported varying alloimmunization rates, influenced by genetic, geographical, and transfusion-related factors(20,21).

As mentioned, a meta-analysis by Darvishi et al. estimated the overall alloimmunization rate in Iran to be approximately 10% (95% CI: 7–13) (12), considerably higher than the rate observed in our study. In Lorestan and southern Iran, alloimmunization rates were 1.53% and 1.7%, respectively(13,14), aligning closely with our findings. Studies in Sari and Ahvaz, however, reported significantly higher alloimmunization rates, 40.4% and 18.75%, respectively(15,16). The disparity may be attributed to differences in donor-recipient genetic compatibility, transfusion protocols, and immune response variability.

Globally, alloimmunization rates among transfusion-dependent patients fluctuate due to regional diversity in blood donor genetics.

In a large cohort from Saudi Arabia, the overall prevalence of RBC alloimmunization was 5.7%, with anti-K and anti-E being the most common alloantibodies(18). In India, where thalassemia patients receive frequent transfusions, 15–20% develop alloantibodies, highlighting regional differences in transfusion practices and donor selection(19). A study in the United States found that 30% of sickle cell disease (SCD) patients developed alloantibodies, largely due to racial differences between donors and recipients(20).

These findings suggest that ethnic background and antigen homogeneity between donors and recipients play a significant role in minimizing immune sensitization, particularly among BTM patients in homogeneous populations like Yasuj.

One of the strongest factors contributing to the low alloimmunization rate in our study is likely donor-recipient antigen homogeneity. Given that 98.5% of patients were native to Yasuj, their red cell antigen profiles likely closely resemble those of local blood donors, resulting in a lower likelihood of alloantibody formation. Antigen similarity between donors and recipients significantly

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reduces alloimmune responses(18,19). Studies have shown that alloimmunization rates are higher in regions with diverse ethnic backgrounds, where donor pools exhibit greater antigenic variation(20). Thus, regional transfusion centers might benefit from HLA and extended RBC antigen matching programs to further minimize alloimmunization risks.

Another possible explanation for the low prevalence of alloimmunization is the early initiation of transfusions in BTM patients. 85% of patients in this study had received their first transfusion before two years of age. Early transfusion is believed to induce immune tolerance, as the immune system is still developing and may not mount a strong alloimmune response(20,21). Studies have demonstrated that initiating transfusions early leads to lower alloimmunization rates compared to patients who begin transfusion therapy later in life.

Optimizing transfusion protocols for neonates and infants may be an effective strategy for preventing alloimmunization.

The role of splenectomy in RBC alloimmunization is controversial, with conflicting results in different studies. Some studies suggest that splenectomy decreases immune sensitization, as the spleen plays a role in filtering foreign antigens and splenectomy strongly reduces red blood cell alloimmunization(22,23). others believe that splenectomized patients are more likely to develop alloantibodies due to prolonged exposure to transfused RBCs without efficient antigen clearance(21). In our study, both alloimmunized patients had undergone splenectomy, raising the possibility that splenectomy increases alloimmunization risk. Given the low number of alloimmunized cases, more multicenter studies are needed to evaluate the impact of splenectomy on alloantibody formation.

Leukodepletion is an important strategy for reducing alloimmunization, as leukocytes contribute to immune stimulation and cytokine production(24). In our study, all patients received leukodepleted blood transfusions, which may explain the low alloimmunization rate. Previous studies have confirmed that filtration of white blood cells reduces HLA antigen exposure, lowering alloimmunization risk(25). Standardizing universal leukodepleted transfusions for BTM patients could further reduce immune sensitization.

Study Limitations

Despite its valuable findings, this study has several limitations:

1. Small sample size (n=134): While indicative, larger studies are needed to verify findings.
2. Low number of alloimmunized cases (n=2): This limits statistical comparisons.
3. Single-center study: A multicenter investigation could enhance generalizability.
4. Lack of multivariate analysis: Future studies should employ logistic regression models to identify independent risk factors.

Further research should focus on:

- Expanding patient cohorts to enhance statistical validity.
- Conducting genetic profiling of donors and recipients.
- Investigating HLA polymorphisms as potential determinants of alloimmunization risk(26).
- Comparing gel versus tube sensitivity in alloantibody detection.

Conclusion

The study reveals a low prevalence (1.49%) of RBC alloimmunization among BTM patients in Kohgiluyeh and Boyer-Ahmad Province, likely due to donor-recipient antigen homogeneity and early transfusion initiation. However, further large-scale studies with advanced statistical modeling are necessary to explore the impact of splenectomy, genetic diversity, and transfusion strategies on alloimmunization risk. Implementing standardized leukodepleted transfusion protocols and refining neonatal transfusion guidelines could significantly reduce alloimmunization burdens in transfusion-dependent patients.

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