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# Frequency and Risk Factors of Red Blood Cell Alloimmunization in Thalassemia Major Patients in Markazi province

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**Abstract**

Background: Thalassemia is one of the most common genetic disorders throughout the world. Blood transfusion plays an important role in the treatment of thalassemia but it leads to numerous complications such as iron overload and alloimmunization. This study evaluated the frequency and risk factors associated with alloimmunization in thalassemia major patients living in Markazi province, Iran.

**Materials and Methods:** In this descriptive study, 48 thalassemia major patients who underwent blood transfusion at Amirkabir hospital were included. Patients' demographic data were recorded using a questionnaire. In order to perform alloimmunization screening and autoantibody assessment, patients were referred to Tehran Blood Transfusion Organization Laboratory.

**Results:** The current study was performed on 48 patients with thalassemia major,. The mean age of patients was 12.5 ± 8.3 years. Among patients 26 (54.16%) were male and 22 (45.83%) were female, 13 patients (27.08%) had alloantibodies. Among 48 patients, 19 (39.58%) had undergone splenectomy. The patients' age of the first blood transfusion ranged from 1 month to 14 months and the mean age of the first blood transfusion was 9.5 ± 7.08 months. The blood transfusion intervals in patients were from 21 days to 40 days and the blood volume received at each transfusion session was 10-15 cc/kg of the body weight. In the current study, the data analysis indicated no significant correlation between alloantibodies and RH phenotype (P=0.43), patients' gender (P=0.9), or blood groups (P=0.4); whereas, a significant correlation was found between alloantibodies and splenectomy (P=0.02) as an increase in the prevalence of alloantibodies was reported in splenectomised patients.

**Conclusion:** No significant difference was found between the patients with and without alloantibodies in terms of the prevalence of Rh phenotype, gender, and blood groups. However, there was a significant difference between the patients with and without alloantibodies in terms of splenectomy.

**Key words:** Allo-immunization, Risk factors, Thalassemia major

## Introduction

Thalassemia is one of the most common genetic disorders throughout the world, and depending on the defect in one or more Hemoglobin chains. Thalassemia shows different phenotypes and genotypes (1). The thalassemia belt that includes Mediterranean countries ranging from Iran and India to the Southeast Asia, contains the greatest number of patients (2, 3). Blood transfusion plays an important role in the treatment of thalassemia by which the hemoglobin level is maintained from 9 to 11.5 gr/dl. The purpose of blood transfusion is to help patients grow appropriately; and to reduce the symptoms of anemia and the appearance change(4, 5). Frequent blood transfusion in thalassemia patients leads to numerous complications such as iron overload and alloimmunization (6, 7). Since blood transfusion is performed based on the main



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blood groups (ABO), frequent blood transfusion produces antibodies against red blood cells or alloimmunization due to the incompatibility in the blood subgroups(8). Alloimmunization is one of the major problems in treating patients with hypertransfusion and leads to serious problems such as increasing the need for blood transfusion, causing delayed hemolysis, shortening blood transfusion intervals, and prompting the occurrence of anemia symptoms, especially fatigue and jaundice in patients with Thalassemia (9).
According to previous studies, the prevalence of alloimmunization is different from 4 to 50 percent depending on the type of the test and individuals' race, (10). Factors predisposing alloimmunization include the age, the splenectomy history, the first blood transfusion age, the racial difference between the recipient and the donor, immunity levels in the recipient, and immunosuppressive factors in the recipient(11). Due to the controversies over the prevalence and importance of this problem in treating thalassemia, this study evaluated the frequency and risk factors of alloimmunization in thalassemia major patients living in Markazi province in 2017.

## Materials and Methods

In this descriptive study, 48 patients diagnosed with thalassemia major undergoing blood transfusion at Amir Kabir hospital in 2017 participated. Informed written consent was obtained from each patient. Patients with hepatitis or AIDS were excluded. This research was approved by the Ethics Committee at Arak University of Medical Sciences (IR.ARAJMU.REC.1394.285).
Demographic data, including age, sex, race, blood transfusion record, blood transfusion intervals, injected blood volume, first blood transfusion age, blood groups (ABO), and splenectomy history were collected using the patients' records or conducting interviews and the data were recorded using a questionnaire. In order to perform alloimmunization screening and autoantibody assessment, patients were referred to Tehran Blood Transfusion Organization Laboratory and a 5cc blood sample was obtained from each patient. The screening test was performed using gel method. The tests were performed at least two weeks after blood transfusion. An antibody screening test was performed using a 3-CELL panel, and an antibody identification test was conducted on positive screening patients using the 11-CELL panel provided by the Iranian Blood Transfusion Organization. Auto-control testing was performed for the autoantibody assessment.

**Statistical analysis**

Statistical tests: The data were reported as the mean ± standard deviation (the significant level of $P < 0.05$ was used to compare the variables).

## Results

The current study was performed on 48 patients with thalassemia major and thalassemia intermediate who referred to Amirkabir Hospital of Arak, Iran. The mean age of patients was 12.50 ± 8.30 years (Table I). Based on the results, 26 patients (54.16%) were male and 22 others (45.83%) were female, 13 patients (27.08%) had alloantibodies, 6 patients (12.5%) had autoantibodies, and 7 others (14.58%) had alloantibodies and autoantibodies, concurrently (Figure 1). The results indicated that from among 48 patients, 20 patients (41.66%) had the blood type A, 10 patients (20.83%) had the blood type B, 5 cases (10.41%) had the blood type AB, 13 patients (27.8%) had the blood type O; 44 patients (91.66%) were Rh positive and 4 others (8.33%) were Rh negative. A total of 19 patients (39.58%) were A+, 1 patient (2.08%) was A-, 10 patients (18.6%) were B+, 4 patients (5.1%) were AB+, 1 patient


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(1.7%) was AB-, 11 patients (20.83%) were O+, and 2 patients (4.16%) were O- (Figure 2). There was no patient with the blood group $B^-$. Based on the results derived from 48 patients, 19 patients (39.58%) underwent splenectomy (Table II). The age of the first blood transfusion also ranged from 1 month to 14 months after the birth, and the mean age of the first blood transfusion was 9.5 ± 7.08 months. The blood transfusion intervals among patients were from 21 days to 40 days with the mean interval of 27.76 ± 4.24 days. The blood volume received at each transfusion session was 10-15 cc/kg of the body weight, and the mean blood intake at each transfusion session was 10.67 ± 1.72 cc/kg of the body weight (Table II). The on comparison between patients with and without alloantibody using a statistical analysis showed no significant difference between the groups in terms of the prevalence of Rh phenotype (P = 0.43) (Table III), the gender (P = 0.9) (Table IV), and blood groups (P = 0.4)(Table VI). However, in terms of splenectomy the difference between two groups was significant (P = 0.02) (Table V). Based on the results, 26 patients (54.16%) were male and 22 others (45.83%) were female, 13 patients (27.08%) had alloantibodies, 6 patients (12.5%) had autoantibodies, and also 7 others (14.58%) had alloantibodies and autoantibodies concurrently. The results indicated that from among 48 patients, 20 patients (41.66%) had the blood type A, 10 patients (20.83%) the blood type B, 5 cases (10.41%) the blood type AB, 13 patients (27.8%) had the blood type O; 44 patients (91.66%) were RH positive, and 4 others (8.33%) were RH negative.

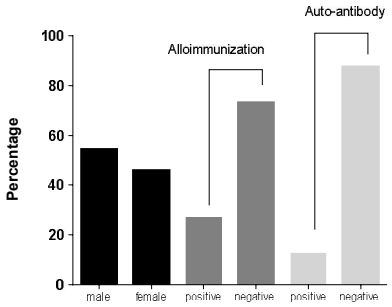


Figure 1. The patients' distribution in terms of their sex, alloimmunization, and auto-antibody

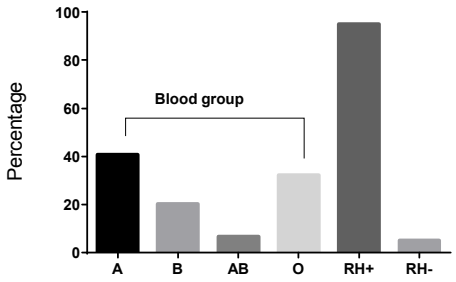


Figure2.The patients' distribution in terms of their blood groups and RH

*Table I. The diffusion index of age, the age of the first blood transfusion, the received blood volume, and the blood transfusion intervals*

| | Number | Maximum | minimum | Mean | SD |
|---|---|---|---|---|---|
| Age(Year) | 48 | 18.00 | 7.00 | 12.50 | 8.30 |
| First blood transfusion age(Month) | 48 | 14.00 | 1.00 | 9.50 | 7.08 |
| Received blood volume(CC/ Kg) | 48 | 15.00 | 10.00 | 10.67 | 1.72 |
| Blood transfusion interval (Day) | 48 | 40 | 21.00 | 27.76 | 4.24 |



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*Table II. Splenectomy frequency distribution in patients*

| | Percentage | Frequency |
|---|---|---|
| Undergone splenectomy | 39.58 | 19 |
| No splenectomy | 60.42 | 29 |
| Total | 100 | 48 |

*Table III. The comparison of Rh phenotype prevalence in patients with and without alloantibody*

| | Number | RH+ | RH- | p-value |
|---|---|---|---|---|
| Patients with alloantibody | 13 | 12 | 1 | 0.3 |
| Without alloantibody | 35 | 33 | 2 | 0.36 |

*Table IV. The gender comparison in patients with and without alloantibody*

| | Number | Female | male | p-value |
|---|---|---|---|---|
| Patients with alloantibody | 13 | 7 | 6 | 0.9 |
| Patients without antibody | 35 | 15 | 20 | 0.8 |

*Table V. The splenectomy prevalence in patients with and without alloantibody*

| | Number | Undergone splenectomy | Normal spleen | P value |
|---|---|---|---|---|
| Patients with alloantibody | 13 | 8 | 5 | 0.02 |
| Patients without alloantibody | 35 | 10 | 25 | |

*Table VI. The blood groups comparison in patients with and without alloantibody*

| | With alloantibody | Without alloantibody | p-value |
|---|---|---|---|
| A+ | 7 | 12 | |
| A- | 1 | 0 | |
| B+ | 2 | 8 | |
| B- | 0 | 0 | 0.5 |
| AB+ | 0 | 4 | |
| AB- | 0 | 1 | |
| O+ | 2 | 9 | |
| O- | 1 | 1 | |



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## Discussion

Alloimmunization is an immune response against the antigens of red blood cells that often occurs following any blood product transfusion (9). The development of alloantibodies varies from 20 to 38 percent depending on the case group and the sensitivity of the methods used for the diagnosis (12-14). Based on the results of the current study, from among 48 patients with thalassemia major and intermediate, 13 patients (27.08%) suffered from alloantibodies that is quite predictable in terms of the prevalence of alloantibodies in Iran and throughout the world. In previous studies on this subject and in line with this study, the prevalence of antibodies amounted to 30% in Kuwait (15), 4.97% in India (9), 17% in Italy (16) 3.7% in Greece (13), and the prevalence of alloantibodies in Asian patients amounted to 38% (11). According to some studies conducted in Iran, the prevalence of alloantibodies is different in various regions, i.e. 2.87% in the northeast of Iran (10), 5.5% in Sistan and Baluchestan Province (17), and 5% in Shiraz (18). In general, a relatively high prevalence rate has been reported in the mentioned studies that might be due to the selection of serious forms of thalassemia major, and also the prevalence of thalassemia in various parts of Iran might be resulted from alloimmunization in the relevant regions. For instance, the prevalence of thalassemia in the central part of Iran is low but it is high in the regions adjacent to the Caspian Sea, the Persian Gulf, and the Oman Sea, probably affecting the prevalence of alloantibodies (19). In previous studies, alloantibodies have been reported to be mainly present against the Kell and Rh systems, yet in the current study, most alloantibodies were against the kell and C systems (10). Most of autoantibodies and alloantibodies in this study were non-hemolytic, being consistent with the results of researches throughout the world. Comparing the results of other research around the world and the similarity of research with those observed in the current study, it can be concluded that the occurrence of the antibody production and blood reaction happen are mostly observed in patients who receive frequent blood transfusions(12). In the current study, the autoantibodies prevalence was reported to be 12.5 %, being within the accepted range. Based on the results of the current study and also other similar studies, it was indicated that alloantibodies happen to be positive more commonly in Iranian patients when compared to other countries. One possible reason for such a finding may be that red blood cell (RBC) phenotype was not checked in our patients at the moment of diagnosis and this may increase the possibility of alloimmunization following blood transfusion. Other explanations may either be the difference in antibody detecting techniques or differences in patients' genetics which might play a role in expressing and developing alloantbodies.

The recipient's age and the immune potency may be effective in the immune response (20). In the currenthist study, Alloimmunized patients had the mean age of 13.5 (± 8.60) years and no correlation was found between the age and prevalence of alloimmunization as confirmed by similar past studies. In this study, six patients from among the patients with alloimmunization experienced the first transfusion case when aged 6 to 11 months old, being in contrast to the studies that suggest transfusion under one-year-old leads to the induction of the immune tolerance (9, 13, 15, 21). The current study demonstrated no significant correlation between the prevalence of alloantibodies and the first blood transfusion age, and this finding had been confirmed by the past studies. In the current study, there was no significant correlation between the prevalence of alloantibodies and patients' gender. In the same vein, there was no significant correlation between gender and alloimmunization in previous studies.



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There was also no significant correlation between the prevalence of alloantibody and blood groups as well as Rh. In the past studies, blood group O+ was prevalent in alloimmunized patients(9). In this study, there was a significant correlation between the prevalence of alloimmunization and splenectomy. Contradictory results have been reported about the correlation between alloimmunization and splenectomy in various studies. In a study carried out by Singer (2000), the correlation between splenectomy and alloimmunization was reported to be significant, where an increase in splenectomy was reported to be associated with an increase in the prevalence of alloimmunization (11). In another study conducted in Sistan and Baluchestan, Iran, in 2013, no significant correlation was found between splenectomy and the prevalence of alloimmunization (17). There was no specific correlation between the number of received blood units and the intervals of blood transfusions with alloimmunization in this investigation.

## Conclusion

The frequency of alloantibodies in the patients was 27.08%, and there was no significant difference between the patients with and without alloantibodies in terms of the prevalence of Rh phenotype, gender, and blood groups. However, there was a significant difference between the patients with and without alloantibodies in terms of splenectomy. This study had some limitations, including the scarcity of the number of samples and the lack of proper collaboration on the part of the patients. Hence, further studies are suggested to be done at other clinical centers with vaster statistical societies and fewer restrictions.

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## Conflict of interest

Authors declared no conflict of interst.

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