# Prevalence of Alloantibodies in Thalassemia Patients and Its Relationship With Age, Gender and Blood Group

**Leili Koochakzadeh[1], Mohammad Kajiyazdi[2], Fahimeh Khoshhal[3], Alireza Hashemi[4], Mehdi Khabazkhoob[5]**

[1] *Department of Pediatrics, Children's Medical Center Hospital, Tehran University of Medical Sciences, Tehran, Iran*

[2] *Department of Pediatric Hematology and Oncology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

[3] *Department of Pediatrics, School of Medicine, Dezful University of Medical Sciences, Dezful, Iran*

[4] *Noor Ophthalmology Research Center, Noor Eye Hospital, Tehran, Iran*

[5] *Department of Basic Sciences, School of Nursing and Midwifery, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

***Received: 12 Apr. 2022; Accepted: 18 Dec. 2022***

**Abstract-** To determine the prevalence of alloantibodies in patients with thalassemia and its relationship with age, sex, and blood group. A cross-sectional study was conducted on thalassemia patients requiring a blood transfusion presenting to Children's Medical Center and Bahmari Hospital in 2021. All patients who received blood transfusions in the first year of life were included in the study, and patients with sickle cell anemia and thalassemia intermedia were excluded. Blood samples were collected, and the level of alloantibodies was measured. One hundred and ninety-five patients were evaluated in this study, of whom 100 (51.3%) were male. The mean age of the subjects was 21.37±8.57 years (range: 1-42 years). The prevalence of alloantibody positivity was 16.41% (11.17-21.65) in all subjects, 19% (11.18-26.82) in males, and 13.68% (6.65-20.72) in females (*P*=0.318). The mean age of alloantibody positive and negative subjects was 18.94±9.7 and 21.85±8.29 years, respectively (*P*=0.079). The prevalence of alloantibody positivity was 21.88% (6.73-37.02) in Rh+ and 15.34% (9.75-20.93) in Rh- patients (*P*=0.364). The prevalence of alloantibody positivity was 13.46% (3.87-23.06) in blood type A, 26.67% (1.32-52.02) in blood type AB, 14.29% (3.25-25.32) in blood type B, and 17.44% (9.26-25.63) in blood type O, indicating no significant difference in this regard (*P*=0.625). The results showed that thalassemia patients were at risk for alloantibody positivity. Age, sex, and blood type had no significant association with the prevalence of alloantibody positivity. Considering the serious complications of these antibodies, these patients should be continuously screened for the presence of alloantibodies.



*Acta Med Iran* 2023;61(1):52-56.

**Keywords:** Alloantibodies; Thalassemia; Blood group

## Introduction

Thalassemia is the most common major blood disorder worldwide (1). It is an inherited hematologic disorder caused by defects in the synthesis of one or more of the hemoglobin chains. Beta thalassemia has received more attention from researchers due to its worse prognosis (2).

The highest and lowest prevalence of beta thalassemia is reported in the Eastern Mediterranean region and Americas, respectively (3). Moreover, it affects approximately 4.2 out of every 10,000 people throughout the world (3). Considering the defects in the hemoglobin chains in these patients, red blood cells have a shorter lifespan and are destroyed rapidly. Thalassemia is associated with serious complications, including tissue hypoxemia resulting from anemia, growth disorder, hepatosplenomegaly, cardiomegaly, thinning and brittleness of bones, and finally death, which occur if no treatment is provided (4). Regular blood transfusions correct that above complications. However, in the process of blood transfusion, large amounts of RBC

**Corresponding Author:** M. Kajiyazdi
Department of Pediatric Hematology and Oncology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
Tel: +98 2122705270, E-mail address: Mkajiyazdi50@gmail.com

antigens enter the body, which leads to the production of alloantibodies through stimulating the immune system. El-Beshlawy *et al*., (5) reported an incidence of 18% for alloantibodies in thalassemia patients; however, prevalence as high as 50% have reported in thalassemia and sickle-cell anemia patients. Variations in the incidence of alloantibodies post transfusion depend on several factors. Studies have shown that a high percentage of alloantibodies are IgG antibodies that facilitate the removal of RBCs by the reticuloendothelial system through sensitizing transfused RBCs.

Previous studies suggest that with an elevation in the levels of these antibodies, the need for blood transfusion increases, which results in a high demand for compatible blood types, iron overload, and endocrine abnormalities like diabetes, pituitary disorders, thyroid problems, etc. (6). These patients may also suffer from dilated cardiomyopathy, liver fibrosis, and different infections .

Although several studies have been conducted in this regard, it is necessary to investigate its associated factors in different ethnicities and countries due to the birth of more than 600,000 infants with thalassemia across the world that require treatment and regular blood transfusion (7). The present study was conducted to evaluate the prevalence of alloantibodies in thalassemia patients and its relationship with age, sex, and blood type in an Iranian population.

## Materials and Methods

A cross-sectional study was conducted in 2021. The study population was all thalassemia patients presenting to Children's Medical Center and Bahrami Hospital that required blood transfusions. All patients who received blood transfusions in the first year of life were included in the study, and patients with sickle cell anemia and thalassemia intermedia were excluded.

After obtaining informed consent from the patients or their legal guardians, about 5 ml blood was collected from each patient and collected in EDTA containing tubes. If the subject had a history of pregnancy or blood transfusion, the blood sample was maintained for 72 hours and the specimens with a hemolytic appearance were excluded.

If agglutination (1 to 2+) is observed in the AHG stage after the addition of sensitized red blood cells, the absence of agglutination can be reported as negative.

After adding two drops of the patient's serum or plasma to 12×75 test tubes, one drop of standard RBC (I, II, III) was added to determine unexpected antibodies and the samples were centrifuged for a certain time. Then, two drops of albumin 22% or LISS solution was added according to the manufacturer's instructions. The specimens were incubated at 37˚ C for 10-15 min in case of adding LISS and 15-30 min in case of adding albumin and then centrifuged.

To control the results, one drop of sensitive RBC IgG control cell was added to negative tubes.

The following conditions were considered as a positive result:

1- Presence of agglutination/hemolysis in the immediate spin after incubation at 37˚ C.
2- Presence of agglutination/hemolysis after adding AHG.
3- 3-An Anti D serum control was used for daily control. If the reaction strength of the diluted control serum in albumin (6% w/v) with the Rh(D) positive RBC was greater than 2+, the serum control is passed (good, OK).

The results were considered invalid, and the experiment was repeated if there was no agglutination after adding sensitive RBC.

After determining alloantibody positive samples, its prevalence and 95% confidence interval were calculated. The prevalence of alloantibodies is presented according to age, sex, blood type, and Rh. Logistic regression analysis was done to evaluate the correlation of these factors with the prevalence of alloantibodies.

**Ethical issues**

Informed consent was obtained from all participants or their parents. The principles of the Helsinki Declaration were followed in all stages of this study. The protocol of the study was approved by the Ethics Committee of Iran Tehran University of medical sciences.

## Results

In this study, 195 patients were evaluated of whom 100 (51.3%) were male. The mean age of the subjects was 21.37±8.57 years (range: 1-42 years).

Regarding the Rh factor, 163 patients (83.6%) were Rh+; moreover, blood type A, AB, B, and O were found in 26.7%, 7.7%, 21.5%, and 44.1% of the patients, respectively.

Table 1 shows the prevalence of alloantibody positivity in thalassemia patients according to gender, age, Rh and blood groups.

**Table 1. The prevalence of alloantibody positivity in thalassemia patients according gender, age, Rh and blood groups**

| | | %(95%CI) |
|---|---|---|
| | Total | 16.41(11.17-21.65) |
| **Gender** | Female | 13.68(6.69-20.68) |
| | Male | 19.00(11.22-26.78) |
| **Age** | <8 | 27.27(0.5-55.05) |
| | 20-Aug | 18.92(9.88-27.96) |
| | >20 | 13.64(7.15-20.12) |
| **Rh** | Negative | 21.88(7.23-36.52) |
| | Positive | 15.34(9.75-20.92) |
| **Blood groups** | A | 13.46(4.04-22.89) |
| | AB | 26.67(3.36-49.98) |
| | B | 14.29(3.51-25.06) |
| | O | 17.44(9.32-25.56) |

The prevalence of alloantibody positivity was 16.41% (11.17-21.65) in all subjects, 19% (11.18-26.82) in males, and 13.68% (6.65-20.72) in females. The odds of alloantibody positivity were 1.48 (0.68-3.19) times higher in males compared to females (*P*=0.318). The mean age of alloantibody positive and negative subjects was 18.94±9.7 and 21.85±8.29 years, respectively (*P*=0.079). The prevalence of alloantibody positivity was 21.88 (6.73-37.02) in Rh+ and 15.34 (9.75-20.93) in Rh- patients, indicating no significant correlation between the Rh status and the prevalence of alloantibodies (*P*=0.364).

The prevalence of alloantibody positivity was 13.46% (3.87-23.06) in blood type A, 26.67% (1.32-52.02) in blood type AB, 14.29% (3.25-25.32) in blood type B, and 17.44% (9.26-25.63) in blood type O, indicating no significant difference in the prevalence of alloantibodies between different blood types (*P*=0.625).

## Discussion

Several studies have evaluated the prevalence of alloantibodies in thalassemia patients (8-11). However, the prevalence of alloantibodies is of utmost importance in these patients due to its complications. A summary of the results of other studies is presented in Table 2.

According to Table 2, the prevalence of alloantibodies in thalassemia patients varies significantly from 1.53% in a study by Kiani *et al*., (10) to 40% in a study by Kosaryan *et al.,* (11) A review of the literature suggests a moderate prevalence of alloantibodies in the present study, although the high prevalence of alloantibodies in other studies indicates meticulous control and monitoring of thalassemia patients. It seems that several factors affect this diversity, for example, attention should be paid to the genetics and ethnicity of the participants of different studies. Moreover, the age range of the patients and hematologic testing centers are also effective factors in this regard. Furthermore, the serological methods used to detect these antibodies may also affect their positivity, which may vary in different studies. For example, in the study by Kiani *et al.,* (10) that reported the lowest prevalence, the age range of the patients was relatively low and the children received blood transfusions from the age of three years, while the patients were above 20 years and started to receive blood transfusions from the age of two years in the study by Kosaryan *et al.,* (11) Jeengar *et al.,* (12) showed a higher mean age at first blood transfusion in alloimmunized patients. Some previous studies found that continuous exposure to this antigen before three years of age may cause tolerance and lack of an immune response. Considering the high mean age of the subjects in the present study and the fact that they started to receive blood transfusions from one year of age, a high prevalence of alloantibodies was expected. It seems that the use of more accurate methods, study time, and better blood storage equipment during recent years have led to a lower prevalence of alloantibodies in the present study compared to previous studies.

The results showed no correlation between the prevalence of alloantibodies and age. The results from previous studies indicated an increase in the prevalence of alloantibodies with age due to repeated blood transfusions. However, a few studies, like a study by Afshari *et al.,* found no correlation between age and the prevalence of alloantibodies. As mentioned earlier, the role of age is more related to age at first blood transfusion session.

The results showed no significant intergender difference in the prevalence of alloantibodies. El Beshlawy *et al.,* (5) reported a prevalence of 19.1% and

17% for alloantibody positivity in male and female patients, respectively. Several other studies also found no relationship between sex and the prevalence of alloantibodies (12-16). Although studies have generally reported a relationship between sex and prevalence of alloantibodies, some reported a higher prevalence in male and some other in female patients (17,18).

According to the results, the ABO blood type and Rh factor had no significant effect on the prevalence of alloantibodies, which was consistent with the results of a study by Eghbali *et al.*, (19) in an Iranian population. Raouf Abdulqader *et al.*, (20) conducted a study in an Iraqi population and found similar results. This finding has been reported in several studies, and it seems that blood type has no significant role in the prevalence of alloantibodies.

The prevalence of alloantibody positivity was moderate. Considering the serious complications of these antibodies, these patients should be continuously screened for the presence of alloantibodies. Age, sex, and blood type had no significant association with the prevalence of alloantibody positivity.

**Table 2. Summary of other studies**

| Authors | Place | Age (mean) | Simple size | Prevalence (%) |
|---|---|---|---|---|
| **Fawwaz Al-Joudi (8)** | Malaysia | 39.12±16.5 | 5719 | 13.1 |
| **Yu-Hua Chao (21)** | Chinese | 19.20±6.7 | 64 | 9.4 |
| **Alexis A. Thompson (15)** | North America | 28.50±9.5 | 697 | 21 |
| **Azza Mohamed Ahmed (22)** | Egypt | 9.29±3.98 | 389 | 11.3 |
| **Azza S. El Danasoury (23)** | Egypt | 12.00±6.1 | 235 | 19.5 |
| **Mohammad Hadi Sadeghian (24)** | Iran | 14.42±7.59 | 313 | 2.87 |
| **Nrages Obeidi (25)** | Iran | 17.70±8.20 | 90 | 10 |
| **M. KARIMI (26)** | Iran | 14.4 | 711 | 5.3 |
| **A. Azarkeivan (27)** | Iran | 22.6±9.27 | 441 | 11.3 |
| **Keikhaei B (28)** | Iran | 17.5±7.5 | 133 | 18.7 |
| **Mirzaeian Amin (9)** | Iran | 13.8 | 385 | 17.9 |
| **Mehrnoush Kosaryan (11)** | Iran | 22.5±7 | 218 | 40.4 |
| **Singer ST (13)** | America | 15 | 64 | 22 |
| **Noor Haslina MN (29)** | Malesia | - | 46 | 8/6 |
| **Khalid H (30)** | Pakistan | - | 75 | 22/7 |
| **Kiani A (10)** | Iran | 13±6 | 65 | 1.53 |
| **Ho HK (31)** | Hong Kong | 18 | 68 | 7.4 |
| **Ameen R (32)** | Kuwait | - | 190 | 30 |
| **Spanos T (33)** | Greece | 11.5 | 1200 | 15.7 |

## References


1. Li CK. New trend in the epidemiology of thalassaemia. Best Pract Res Clin Obstet Gynaecol 2017;39:16-26.
2. Jaing TH, Chang TY, Chen SH, Lin CW, Wen YC, Chiu CC. Molecular genetics of β-thalassemia. Medicine (Baltimore) 2021;100:e27522.
3. De Sanctis V, Kattamis C, Canatan D, Soliman AT, Elsedfy H, Karimi M, et al. β-Thalassemia Distribution in the Old World: an Ancient Disease Seen from a Historical Standpoint. Mediterr J Hematol Infect Dis 2017;9:e2017018.
4. Abu-Shaheen A, Heena H, Nofal A, Abdelmoety DA, Almatary A, Alsheef M, et al. Epidemiology of Thalassemia in Gulf Cooperation Council Countries: A Systematic Review. Biomed Res Int 2020;2020:1509501.
5. El-Beshlawy A, Salama AA, El-Masry MR, El Husseiny NM, Abdelhameed AM. A study of red blood cell alloimmunization and autoimmunization among 200 multitransfused Egyptian β thalassemia patients. Sci Rep 2020;10:21079.
6. Tangvarasittichai S. Impact of alloimmunization on transfusion- dependent patients. Ann Adv Chem 2017;1:70-82.
7. Huang H, Xu L, Chen M, Lin N, Xue H, Chen L, et al. Molecular characterization of thalassemia and hemoglobinopathy in Southeastern China. Sci Rep 2019;9:3493.
8. Al-Joudi F, Ali AB, Ramli MB, Ahmed S, Ismail M. Prevalence and specificities of red cell alloantibodies among blood recipients in the Malaysian state of Kelantan. Asian J Transfuse Sci 2011;5:42-5.
9. Amin M, Gholamhossein T, Majid N, Marziyeh H, Narges S, Akbar D. Prevalence of alloimmunization against RBC antigens in thalassemia major patients in Southeast of Iran. J Blood Disorders Transf 2013;4:2.
10. Kiani AA, Abdi J, Shirkhani Y, M Kashi. Prevalence of alloimmunization against RBC antigens in thalassemia major patients of Lorestan province in 2004. Blood (J Iran Blood Transfus Organ Res Cent) 2006;3:265-71.

11. Kosaryan M, Mahdavi MR, Roshan P, Hojjati MT. Prevalence of alloimmunisation in patients with beta thalassemia major. Blood Transfus 2012;10:396-7.
12. Jeengar RK, Upadhyaya A, Agarwal N, Mehta A. Red cell alloimmunization in repeatedly transfused children with beta thalassemia major. Int J Contemp Pediatr 2017;4:5.
13. Singer ST, Wu V, Mignacca R, Kuypers FA, Morel P, Vichinsky EP. Alloimmunization and erythrocyte autoimmunization in transfusion-dependent thalassemia patients of predominantly Asian descent. Blood 2000;96:3369-73.
14. Al-Mousawi MM, Al-Allawi NA, Alnaqshabandi R. Predictors of Red Cell Alloimmunization in Kurdish Multi Transfused Patients with Hemoglobinopathies in Iraq. Hemoglobin 2015;39:423-6.
15. Thompson AA, Cunningham MJ, Singer ST, Neufeld EJ, Vichinsky E, Yamashita R, et al. Red cell alloimmunization in a diverse population of transfused patients with thalassemia. Br J Haematol 2011;153:121-8.
16. Sahu A, Parida P, Mahapatra S, Sahoo BB. Detection of red cell alloantibodies in thalassemia patients. Int J Contemp Pediatr 2020;7:5.
17. Hussein E, Desooky N, Rihan A, Kamal A. Predictors of Red Cell Alloimmunization in Multitransfused Egyptian Patients With β-Thalassemia. Arch Pathol Lab Med 2014;138:684-8.
18. Abdelrazik AM, Elshafie SM, El Said MN, Ezzat Ahmed GM, Al-Gamil AK, El Nahhas MG, et al. Study of red blood cell alloimmunization risk factors in multiply transfused thalassemia patients: role in improving thalassemia transfusion practice in Fayoum, Egypt. Transfusion 2016;56:2303-7.
19. Eghbali A, Rahimi-Afzal R, Mehrabi S, Sanatkar A, Mousavi Hasanzadeh M. Frequency and Risk Factors of Red Blood Cell Alloimmunization in Thalassemia Major Patients in Markazi province. Iran J Ped Hematol Oncol 2019;9:25-31.
20. Abdulqader AM, Mohammed AI, Mohammed NI. Red Cell Alloimmunization and Autoimmunization in Multi-Transfused Thalassemia Patients in Sulaymaniyah Province-Iraq. Korean J Clin Lab Sci 202;52:98-104.
21. Chao YH, Wu KH, Lu JJ, Shih MC, Peng CT, Chang CW. Red blood cell alloimmunisation among Chinese patients with β-thalassemia major in Taiwan. Blood Transfus 2013;11:71-4.
22. Ahmed AM, Hasan NS, Ragab SH, Habib SA, Emara NA, Aly AA. Red cell alloimmunization and autoantibodies in Egyptian transfusion-dependent thalassemia patients. Arch Med Sci 2010;6:592-8.
23. el-Danasoury AS, Eissa DG, Abdo RM, Elalfy MS. Red blood cell alloimmunization in transfusion-dependent Egyptian patients with thalassemia in a limited donor exposure program. Transfusion 2012;52:43-7.
24. Sadeghian MH, Keramati MR, Badiei Z, Ravarian M, Ayatollahi H, Rafatpanah H, et al. Alloimmunization among transfusion-dependent thalassemia patients. Asian J Transfus Sci 2009;3:95-8.
25. Obeidi N, Mankhian AR, Hatami G, Emami H. Antibody screening in patients with thalassemia major. Lab Med 2011;42:618-21.
26. Karimi M, Nikrooz P, Kashef S, Jamalian N, Davatolhagh Z. RBC alloimmunization in blood transfusion-dependent beta-thalassemia patients in southern Iran. Int J Lab Hematol 2007;29:321-6.
27. Azarkeivan A, Ahmadi MH, Gharehbaghian A, Zolfaghari S, Nasizadeh S, Maghsudlu M, et al. Antibody screening and identification by gel method in thalassemic patients. Sci J Iran Blood Transfus Organ 2008;5:99-108.
28. Keikhaei B, Far AH, Abolghasemi H, Mousakhani H, Ghanavat M, Moghadam M, et al. red blood cell alloimmunization in patients with thalassemia major and intermediate in southwest Iran. Iran J Blood Cancer 2013;6:41-6.
29. Noor Haslina MN, Ariffin N, Illuni Hayati I, Rosline H. Red cell immunization in multiply transfused Malay thalassemic patients. Southeast Asian J Trop Med Public Health 2006;37:1015-20.
30. Khalid H, Muhammad Y, Nadeem I, Lubna N, Hassan Z. Red cell alloimmunization in repeatedly transfused thalassemia major patients; 2004.
31. Ho HK, Ha SY, Lam CK, Chan GC, Lee TL, Chiang AK, et al. Alloimmunization in Hong Kong southern Chinese transfusion-dependent thalassemia patients. Blood 2001;97:3999-4000.
32. Ameen R, Al- Shemmari S, Al- Humood S, Chowdhury RI, Al- Eyaadi O, Al- Bashir A. RBC alloimmunization and autoimmunization among transfusion- dependent Arab thalassemia patients. Transfusion 2003;43:1604-10.
33. Spanos T, Karageorga M, Ladis V, Peristeri J, Hatziliami A, Kattamis C. Red cell alloantibodies in patients with thalassemia. Vox Sang 1990;58:50-5.