Prevalence of Anti-Erythrocyte Alloantibodies and Relevant Factors among the Patients with Thalassemia Major in Kermanshah, Iran

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Abstract

Background

The major treatment for thalassemia is a transfusion. The present study aimed to determine the frequency of erythrocyte alloantibodies and the relevant factors among the patients with thalassemia major in Kermanshah city, Iran.

Materials and Methods: This cross-sectional study was performed on 218 thalassemia major patients with 2-48 years old, referred to thalassemia boarding clinic at the Dr. Mohammad Kermanshahi Hospital in Kermanshah city, Iran. Blood samples from thalassemia patients (5 ml) were examined in terms of the presence of erythrocyte antibodies (using the screening antibody test), and additional information including age, blood Rh, spleen status was extracted from the patients' profile. The information was finally analyzed using SPSS software version 20.0.

Results: A total of 74 patients (33.9%) had alloantibody, and 144 (67.1%) lacked antibody. The mean age of the subjects was 21.85±8.083 years. The oldest and youngest subjects were 48 and 2 years old, respectively. The sample population included 114 men (52.3%) and 104 women (47.7%). 35.8% of the patients underwent splenectomy. There was no significant relationship between blood Rh, spleen status, and incidence of alloantibodies (all P> 0.05).

Conclusion

The high prevalence of Alloimmunization in the present study requires a more detailed examination of donated blood for compatibility of main and sub blood groups.

Key Words: Alloantibody, Iran, Thalassemia, Prevalence.

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1- INTRODUCTION

Thalassemia is the most common inherited anemia worldwide, which is caused by a defect in the production of hemoglobin molecule chains. Alpha and beta-thalassemia is the most common genetic defect in thalassemia syndrome. Three main forms of beta-thalassemia, according to the synthesis rate of beta chains, include thalassemia major, intermedia, and minor. The most severe form of the disease is β-thalassemia major (1, 2). Approximately 100,000 children around the world are born with severe forms of the disease annually. In Iran, there are over 2 million thalassemia carriers, and more than twenty thousand thalassemia major patients (3, 4).

Continuous blood transfusions are the only way to rescue the lives of patients with thalassemia major since these patients lack sufficient power to produce healthy and active erythrocytes. On the other hand, the unhealthy blood cells produced by patients are rapidly destroyed, and there is no alternative except continuous blood transfusions once within 3-4 weeks, which causes different RBC antigens to enter the patient's body. Consequently, the immune system of patients without these antigens may be stimulated, and antibodies are produced against them.

These antibodies are called alloantibodies, and alloantibody-producing patients are called Alloimmunized. The increasing prevalence of Alloimmunization against erythrocytes can cause several blood transfusion issues, including Hemolytic Transfusion Reaction (HTR), as well as shortening the useful life of injected erythrocytes (5, 6). Various statistics on the prevalence of alloantibodies have been reported worldwide. The prevalence of this phenomenon in Sari, Lorestan, Ahvaz, the United States, and Taiwan are 6.5%, 8.6%, 1.5%, 40.4%, and 9.4%, respectively (7-11).

Since only ABO and Rh system groups are often controlled in the selection of transfused blood, the majority of alloantibodies are formed against subgroups and cause incompatibility in laboratory cross-matching or clinical incompatibility. Several studies have shown that, in most cases, responsible antibodies are related to the subgroups of the Rh system, including E, c, C, and secondary groups such as Kell, Duffy, and Kidd (12-14). There are two procedures for the detection of these alloantibodies, including screening through the representative O^+ cell, and the proximity of the serum to the panel cells (a cell set having almost common antigens with a specific phenotype), in the case which the previous step is positive (5).

Compatible blood supply is not easily available for Alloimmunized patients, and this doubles the importance of paying attention to this issue and preventing Alloimmunization. Therefore, the present study intended to screen and identify clinically important unexpected antibodies following blood transfusions; therefore, the immunization process, incompatibility, and several issues for these patients can be prevented by discovering common antibodies and not injecting the relevant antigen.

2- MATERIALS AND METHODS

2-1. Study Design

The present study is cross-sectional. The sample population included 218 thalassemia patients with 2-48 years old, which have a medical profile in Mohammad Kermanshahi Hospital, Kermanshah city, Iran. Data collection consisted of two stages. In the first stage, the checklist was completed according to the variables (age, gender, blood group, blood Rh and spleen status) via the recorded data in the thalassemia patients' profile in Mohammad Kermanshahi Hospital. In the second stage, 5 ml of each

patient's blood sample was taken before blood transfusion and sent to the Kermanshah Blood Transfusion Organization laboratory to check for the presence of alloantibodies. In the laboratory, the serum was isolated from a blood sample and placed next to a 2-5% suspension of screening cells at room temperature (37°C), and in the incubator (Screening cells are a mixture of red blood cells with blood group O and contain almost all blood antigens).

After 20 minutes of incubation, all tubes were centrifuged for one minute (1000 rpm). Then agglutination was examined microscopically and macroscopically. No agglutination, Anti-Human Globulin (AHG), was used to check for defective antibodies or low antibody titer. In the case of spotting the agglutination in any of the above stages, antibody screening was considered positive. In the absence of agglutination, the check cells (Red blood cells sensitized with Immunoglobulin G) were used to ensure the accuracy of the results and AHG activity. In the case of a positive response to check cells, the test result was confirmed.

2-2. Inclusion and exclusion criteria

Thalassemia patients with a minimum of ten times transfusion were included in the study. Accordingly, patients with a history of receiving less than ten-time transfusion were excluded from the study.

2-3. Data analysis

Frequency and percentage indices were used to report categorical variables. For age, mean and Standard Deviation (SD) were applied. To determine the association between spleen status and patient's blood Rh with a prevalence of alloantibodies, the Chi-square test was used. The analyses were performed using the SPSS software version 20.0. The significance level was set at 0.05.

2-4. Ethical consideration

Human rights were respected based on the Helsinki Declaration 1975, as revised in 1983. The ethical committee of Kermanshah University of Medical Sciences approved the study (KUMS.REC.1394.492). The informed consent was taken from the patients, their parents, and first-degree relatives. The authors completely observed ethical issues (including plagiarism, data fabrication, and double publication).

3- RESULTS

The present study was performed on 218 thalassemia patients in Mohammad Kermanshahi Hospital. The mean age of the subjects was 21.85±8.083 years. The oldest was 48 years old, and the youngest subject was two years old. The sample population included 114 men (52.3%) 104 women (47.7%). Eighty-four subjects (38.5%) had blood type O, 14 subjects (6.4%) had blood type AB, 40 subjects (18.3%) had blood type B, and 80 subjects (36.7%) had blood type A.

Besides, 205 (94%) subjects had Rh-positive, and 13 (6%) subjects had Rh-negative. 35.8% of the patients underwent splenectomy (**Table-1**). The mean age of starting blood transfusion was 22.43±52.811 months. A total of 74 patients (33.9%) had alloantibody, and 144 (67.1%) lacked antibody (**Table.2, Figure.1**).

Table-1: Frequency distribution of relevant variables in the subjects.

Variables		Frequency (%)
Gender	Male	114 (52.3)
	Female	104(47.4)
RH	Positive	205(94.0)
	Negative	13(6.0)
Blood Type	O	84(38.5)
	AB	14(6.4)
	B	40(18.3)
	A	80(36.7)
Spleen Status	Yes	133(61)
	No	78(35.8)
	Unspecified	7(3.2)
Place of Residence	Kermanshah	118(54.1)
	Counties	85(39)
	Other cities	6(2.8)
	Unspecified	9(4.1)
Age, year: Mean (Standard deviation) [Min-Max]		21.85 (8.083) [2-48]

Table-2: Frequency distribution of alloantibody in the subjects.

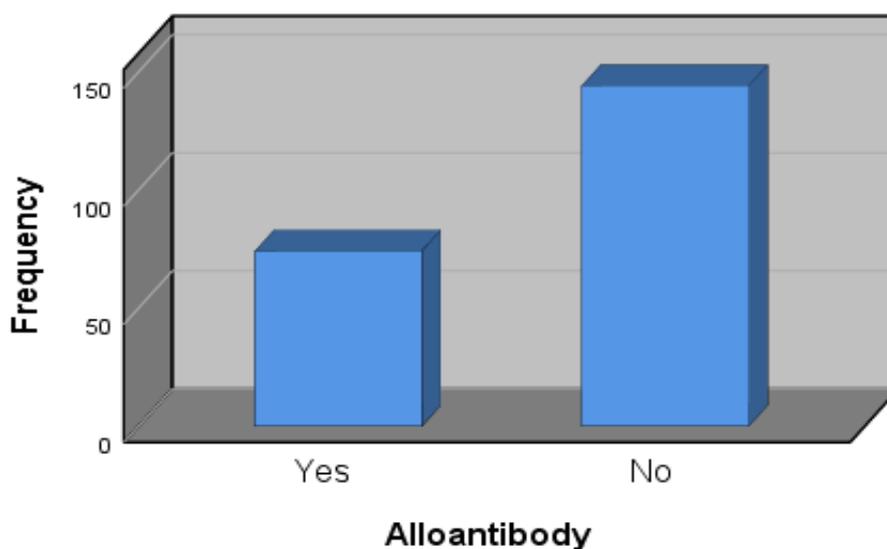| Variables		Frequency (%)
Alloantibody	Yes	74(33.9)
	No	144(67.1)

Fig.1: Frequency distribution of alloantibody in the subjects.

According to the following table, there was no significant relationship between spleen status and patient's blood Rh with the prevalence of alloantibodies (**Table-3**).

Table-3: Frequency distribution of spleen status in terms of prevalence of alloantibodies

Variables		Alloantibody Frequency (%)		P-value
		Yes	No	
Spleen Status	Yes	49(84.5)	9(15.5)	0.127
	No	25(96.2)	1(3.8)	
Rh	Yes	49(84.5)	9(15.5)	0.127
	No	25(96.2)	1(3.8)	

4- DISCUSSION

Alloimmunization is the response of the immune system to foreign erythrocyte antigens and is expressed as an antibody and therefore called an alloantibody. Most clinically important alloantibodies are immunoglobulin G genus and cause cell removal in the reticuloendothelial system through the sensitization mechanism of injected cells. Alloimmunization can develop against the Rh system or other clinically important subgroups, and this phenomenon is observed among subjects with blood and the usage of its products regularly and frequently, such as hemoglobinopathy patients. When alloantibodies are produced, the transfusion turns into an inevitable and complex issue. Some of the issues and intricacies include an increase in the need for blood transfusions, a reduction in the possibility of compatible blood transfusions, increasing iron overload, and related problems such as diabetes and blood-related contagious infections (15). According to the results, 33.9% of the major thalassemia patients had alloantibodies. This is consistent with the results of the study conducted by Aygun et al. They reviewed the medical records of the patients with sickle cell anemia and thalassemia in a hospital from 1989-1999. They found that although all of these patients received compatible blood in terms of primary blood types and Rh group, alloantibodies were observed in 29% of children and 47% of adults (16). In a study performed by Kosaryan et al., alloantibodies were observed among 47% of patients with thalassemia. There was no significant relationship between alloantibodies and the time of blood transfusion, frequency, and duration of blood transfusions (7). In another study on pregnant patients and individuals with accidental blood transfusion, the presence of alloantibodies was 1-3%, and in patients with recurrent transfusions was 70% (17). Hassan et al. reported a 22.7% prevalence of alloantibodies in 75 patients with thalassemia (18). In a study by Gader et al., the prevalence of alloantibodies was reported to be 71% in 68 patients with recurrent transfusions, and the prevalence of sickle cell anemia among them was 22.06% (6). Yu-Hua et al. (2011) conducted a study in Taiwan on 64 major thalassemia patients and observed that Alloimmunization was among six (9.4%) patients. Among the identified antibodies, Anti-E was observed in four patients, Anti-C in one, and Anti-Mia in one (11). In the study carried out by Thompson et al. in the US in 2010 on 697 thalassemia patients, alloantibody was positive in 115 patients (16.5%) (10); another point extensively reported in various studies is the role of the spleen condition and its relationship with alloantibodies. The role of the spleen in the

Alloimmunization seems to be dual and contradictory. A body of researchers believe that splenectomy leads to the discharge of the collection/removal center of unknown antigens, and immune complexes can no longer be cleaned, and this increases the escalating prevalence of alloantibodies in the serum of patients (18-20). In contrast, another group of researchers not only have avoided such relationship between spleen status and prevalence of alloantibodies (21, 22), but also believe that splenectomy reduces the need for blood transfusions because other transfused erythroid are no longer removed from the bloodstream, and this group suggests this issue as a solution at the early stage of blood intake (23-26).

However, no significant relationship was observed between the presence/absence of spleen and antibody production in the present study. However, if this assumption is accurate, it can be expected that with the reduction of blood intake, less volume of foreign antigens enters the body, and we may observe a direct relationship between splenectomy in the first months of blood transfusion and reduction or absence of the prevalence of alloantibodies. Other factors involved in the production and prevalence of alloantibodies include the age of onset of blood transfusion, the time interval between receptions, the patient's age, and the number of injections.

At first glance, one might expect that with increasing age and frequency of injections, we should observe an increase in the prevalence of alloantibodies due to increased antigen input and sufficient time to stimulate immunity as well as secondary responses. However, the evidence is not available to corroborate this (21, 24). Tahanezhad et al. conducted a study on 70 patients in Ahvaz city and found that six patients had alloantibodies while 25.7% of the patients had undergone splenectomy, and there was not a significant relationship between the presence of alloantibodies and age, sex, spleen status and the onset of first blood bag intake (9). In Lorestan, in a similar study, the prevalence of Alloimmunization was 1.53%, and the onset of blood transfusion was in all patients under three years old, and 17% of patients underwent splenectomy (8). In this study, there was no direct relationship between blood intake and sex with the number of alloantibodies.

4-1. Study Limitations

Since the most common types of antibodies (anti kell, anti-Rh) are known in references and similar studies, the type of antibody was not examined in our study, which is one of the limitations of our study.

5- CONCLUSION

The high prevalence of Alloimmunization in thalassemia patients in Kermanshah is a sign of incompatibility between donated blood and the recipient in some cases. Therefore, to reduce the incidence of Alloimmunization, it is recommended to perform an accurate evaluation of the donated blood in terms of compatibility of the main and sub blood groups with the recipient and also to examine the recipient's red blood cell phenotype before the first blood transfusion.

6- AUTHORS' CONTRIBUTION

MG and MT designed the study, observed accuracy and validity of the study and supervised the project. MR did the statistical analysis of the data and interpreted it. BF collected the data and follow the study. MG and MT wrote the paper. All authors edited and revised the final manuscript and accepted its publication.

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