Original Article

Zahedan Journal of Research in Medical Sciences

Journal homepage: www.zjrms.ir

Prevalence of Alloimmunization against RBC Antigens in Thalassemia Major Patients

Amin Mirzaeian,[1] Gholamhossein Tamaddon,*[2] Majid Naderi,[3] Marziyeh Hosseinpour,[4] Narges Sargolzaie[5]

1. Bsc of Laboratory Sciences, Cellular and Molecular Research Center, Shahr-e-Kord University of Medical Sciences, Shahr-e-kord, Iran
2. PhD Student of Hematology, Department of laboratory Sciences, Faculty of Paramedical Sciences, Shiraz University of Medical Sciences, Shiraz, Iran
3. Pediatrics Hematologist & Oncologist Ali Ebn-e Abitaleb Hospital Research Center For Children And Adolescents Health [RCCAH], Zahedan University Of Medical Sciences, Zahedan, Iran
4. Bsc of Laboratory Sciences, Medical School Zahedan University of Medical Science , Zahedan, Iran
5. Assistant Professor of Community Medicine, Community Medicine Department Zahedan University of Medical Science, Zahedan, Iran

Article information

Article history:
Received: 6 Nov 2011
Accepted: 20 Feb 2012
Available online: 25 Apr 2013
ZJRMS 2013; 15(7): 55-58

Keywords:
Thalassemia major
Alloantibody
Cell panel

*Corresponding author at:
Department of Hematology,
School of Medical Sciences,
Tarbiat Modares University,
Tehran, Iran.
E-mail:
tamadon@sums.ac.ir

Abstract

***Background*:** Regular blood transfusions to treat the patients with thalassemia major generate antibodies acting against red blood cells antigens. This immune response is called *alloimmunity*. This study was conducted with the purpose of determining the prevalence of alloantibodies and autoantibody, identifying the type of causative antigen, and recognizing the factors affecting alloimmunization among the patients with thalassemia major receiving blood.

***Materials and Methods*:** In this cross-sectional study, 385 patients with thalassemia major participated. After recording their demographic information, serum specimens taken from the patients were screened using pooled cells obtained from Biorad Company. The positive cases were examined to identify antibodies using panel cells obtained from Iranian Blood Transfusion Organization. In this study, SPSS 16 was employed for performing statistical analysis.

***Results*:** Of the 385 patients, 69 subjects (17.9%) comprising 221 men and 164 women had alloantibody. In 57 cases, the antibody type was exactly identified. In 21 patients (5.5%) the existence of autoantibody was determined. The mean ages of the participants were within 14.3±7.5 and 13.3±7.9 years old for male and female groups, respectively. 28 patients had splenectomy and age at the onset of blood transfusion ranged from a month after birth to nine years.

***Conclusion*:** In these patients, the most significant blood group systems acted by alloantibodies were Rh and Kell. Since the results of this study show 17.9% incidence of alloimmunization in these patients, it is recommended to carry out injected blood compatibility test (cross-match) after antibody screening.

Introduction

Thalassemias are form of inherited genetic diseases caused by decreased synthesis of one or more globulin chains. They are classified on the basis of the type of globulin involved [1, 2].

The most severe situation in thalassemia is determined in the first year of life by severe anemia and growth retardation [1]. Regular blood transfusion to the patients is the only way of survival [3]. Following regular blood transfusion, the immunity system of thalassemic patient produces antibodies, mostly of the IgG class, in exposure to donated red blood cells. These immune antibodies are of alloantibodies and/or autoantibodies. Of the most important problems in this group of patients is the produced antibody. Since following blood transfusion and thereafter destruction of red blood cells by produced antibodies, increased number of hemoglobin in patients would be inefficient and so more blood transfusion is required [2-4]. As per the chronic blood transfusion guideline for thalassemic patients, antigen phenotyping, running antibody screening and compatibility test has been recommended, but is not routinely performed in Iran [5-7]. The incidence of alloantibody production has been reported between 2.37 to 37% in different parts of the world [7]. There are more than two million thalassemia carriers and over twenty thousand patients with thalassemia major in Iran [3].

Thalassemia is a common disease in Sistan and Balouchestan province due to ethnic and tribal marriages. Since limited information is available about the incidence of immunization in such patients, this study was conducted with the purpose of determining the prevalence of alloantibodies and autoantibody, identifying the type of causative antigen, and recognizing the factors affecting alloimmunization among the patients with thalassemia major, going to Aliasghar Hospital in Zahedan and receiving blood, in 2010.

Materials and Methods

This is a cross-sectional study conducted on 385 major thalassemic patients going to Aliasghar Hospital in

Zahedan and receiving blood, in 2010. For the purpose of this research, convenience and non-probability sampling method was employed.

First, a questionnaire was completed to record demographic information including age, sex, age at the onset of blood transfusion, blood group, splenectomy and its exact time, and ethnicity. The patients with HIV, HBV, and HCV history and those with any signs of infection in the last two weeks prior to the study were removed.

First informed consent was obtained from the patients participating in this program. After that and prior to blood transfusion, about 10cc venous blood was injected into two separate tubes, a K2 EDTA anticoagulant tube for automatic control of blood count and a clot activator tube for serum preparation, in order to look for alloantibodies in the patient' serum specimens, which then were stored in a -20°C freezer and examined daily.

First, antibody screening was performed using pooled cells obtained from Biorad Company (Biotestcell-P3), according to the standard procedures and blood bank manual. [9, 10] In the screening stage, two volumes of patient's serum and one volume of cell were mixed and evaluated in three phases (room temperature, 37°C, and antihuman globulin or Coombs' phase) using low ionic strength solution obtained from Biorad Company (LISS MBL2). All results were reordered in the prepared tables. Negative specimens were controlled or checked using Coombscell-E obtained from Biorad Company.

All patients with positive specimen in the screening stage were selected for antibody identification. Then, similar to the screening stage, they were examined in three phases using cell panels prepared in eleven different vials by the Iranian Blood Transfusion Organization (Panel cell 11IP 11C42, 11IP 11 11C41). Next, antibody type specific to each antigen was determined. In order to determine autoantibody in the antibody identification stage, an autocontrol tube was also used and the results were recorded.

The results were statically analyzed using SPSS-16, and *t*-test and χ^2 tests. The *p*-values less than 0.05 were considered significant.

Results

Of the 385 patients under study, 221 subjects were male (57%) and 164 cases were female (43%) with average age of 14.3±7.5 and 13.3±7.9, respectively. The patients were within the age range of 1-45 years old with mean of 13.8±7.7 (Table 1). Ethnically, 308, 70, and 7 subjects were, in turn, Baluch, Sistanian, and none-Balouch or Sistanian. They were received blood from the Blood Transfusion Organization of Zahedan.

Of the patients, 155 (40.3%), 132 (34.3%), 80 (20.8%), and 18 (4.7%) were with O, B, A, and AB blood types, respectively. Moreover, 356 (92.5%) and 26 patients (7.5%) were, in turn, with positive and negative Rh (Table 2).

Their age at the onset of blood transfusion ranged from 1 month to 9 years with the mean of 12.3±15.5 years old (Fig. 1). In addition, 28 patients (7.3%) had splenectomy history.

Table 1. Age distribution in studied patients

Age (year)	N(%)	Allommunized patients (%)
<5	67(17.4)	11(15.9)
6-10	77(20)	11(15.9)
11-15	70(18.2)	18(26.1)
16-20	77(20)	16(23.2)
>20	94(24.4)	13(18.8)
Total	385(100)	69(100)

Table 2. Prevalence of alloimmunization based on ABO blood type and Rh among 69 patients with alloantibody

		ABO				Total
		A	B	AB	O	
Rh +	N (%)	9(13)	26(37.7)	0(0)	31(44.9)	66(95.7)
Rh-	N (%)	0(0)	1(1.4)	1(1.4)	1(1.4)	3(4.3)
Total	N (%)	9(13)	27(39.1)	1(1.4)	32(46.4)	69(100)

Table 3. Incidence of alloantibodies and autoantibody

System	Alloantibody	Number	Frequency
	Anti-C	6	1.6
	Anti-c	5	1.3
Rh	Anti-E	10	2.6
	Anti-e	3	0.8
	Anti-C^w	5	1.3
	Anti-K	4	1
Kell	Anti-k	1	0.3
	Anti-Kp^a	3	0.8
Duffy	Anti-Fy^b	3	0.8
Kidd	Anti-Jk^a	3	0.5
	Anti-Jk^b	1	0.3
Lewis	Anti-Le^b	3	0.8
MNS	Anti-s	3	0.8
Lutheran	Anti-Lu^a	6	1.6
Sex Linked	Anti-Xg^a	2	0.5
Undetermined		12	3.1
Total		69	17.9
Autoantibody		21	5.5

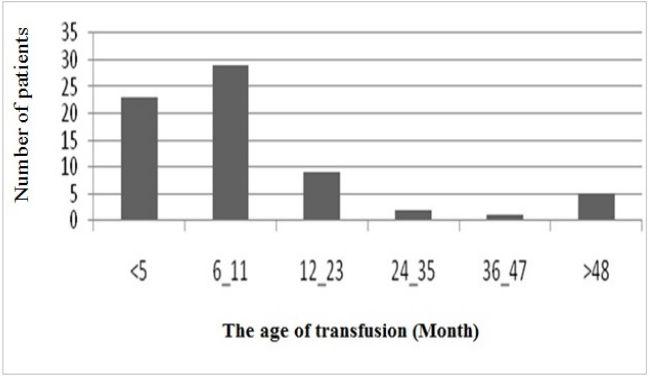

Figure 1. Frequency of alloimmunization among 69 patients with Alloantibody according to the age of transfusion

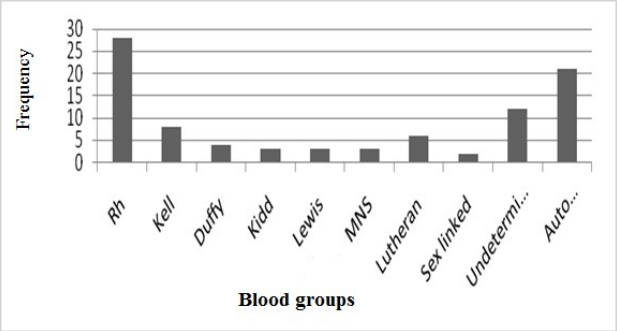

Figure 2. Distribution of obtained alloantibody according to of blood group antibodies

Sixty nine patients (17.9%) had alloantibody among which causative antibody was identified in 57 cases. The majority of alloantibodies against antigens were Rh and Kell systems. (Fig. 2) The most common alloantibody was Anti-E (in 10 patients). Type and incidence of antibodies are presented in table 3. Additionally, the existence of autoantibody was determined in 21 patients (5.5%).

Discussion

Among the complex factors contributing to alloimmunization, three of them are especially important: antigenic pattern differences between donor and recipient's red blood cells, recipient's immune status, and immunomodulatory effect of allogeneic blood on the immune system of the recipient [4].

Previous studies have reported 5-30% alloimmunization in Thalassemic patients [11-13]. In a survey done by Eshghi et al. no alloantibody was observed in the patients going to Aliasghar Hospital in Zahedan [10]. However, they found 69 cases (17.5%) who became alloimmunized, among which the antibody type was exactly identified in 57 cases.

The immunity status of the receptors is a significant factor in developing alloimmunization [14]. Usually, following blood transfusion in splenectomized patients, a lymphocytosis would be observed [2, 8, 15]. Increase in the count is related to B cells, and CD4:CD8 ratio will not change much generally [16]. Due to the spleen role in the retention of the foreign antigen, it is obvious that alloantibody rate in the patients with no splenectomy history is higher, while, autoimmunization rate is higher in splenectomized patients [2, 8, 15]. In this study, 28 patients were splenectomized among them 4 cases (5.8%) had alloantibody and 3 cases (14.3%) had autoantibody. Statistically there was no significant difference between the incidence of alloimmunization and splenectomy history.

The role of white blood cells reduction in the blood drawn for this group of patients (Leuko reduct RBC) in preventing alloimmunization and autoimmunization has been determined. Following storing blood bags at 1-6°C, apoptosis is induced in white blood cells [17, 18]. Release of immune-simulating antigens from the remaining white blood cells and soluble mediators are of the important factors in developing autoimmunity and autoantibody in this group of patients [18, 19].

Age and immune status of patients are important in immune responses [2]. In this study, the majority of alloimmunized patients were 11 to 15 years old (26.1%) followed by 16-20 (23.2%), over 20 (18.8%), less than 5 (15.9%), and 6-11 (15.9%) years old. This was difference not statistically significant. No significant correlation between age and alloimmunization has reported in a number of other studies [7, 9, 20].

In our study, there were some patients with alloantibody showing no hemlolytic transfusion reaction. Therefore, regular examination of immune status of such patients is recommended.

Although, higher rate of alloimmunization is reported in the patients who have received more units of blood, [20, 22] but there is no recognized correlation between the number of unit transfused and alloimmunization in thalassemic patents [23].

In our study, the age at the onset of blood transfusion in 258 patients (67%) was less than 1 year old and in 26 out of 69 alloimmunized patients (42%) was 6 to 11 years old. These findings were inconsistent with other studies. Regarding these studies, blood transfusion at a young age (less than one year old) may result in immune tolerance and protection against alloimmunization [2, 5, 7, 13]. However, no statistical correlation was seen between the incidence of alloimmunization and the age at the onset of blood transfusion. In addition, there was no statistically significant difference in the mean age at the onset of blood transfusion between alloimmunized and non-alloimmunized groups (12.4±16.7 versus 12.7±16).

Since the majority of generated alloantibodies and autoantibodies are non-hemolytic, so accurate identification of immunized patients seems necessary, in order to reduce blood transfusion complications [15]. However, 69 alloimmunized cases were identified in this study, but a few number of them had alloantibody-related clinical symptoms.

Among the participants, 14.7% of females and 24% of males had alloimmunization. This difference was statistically significant. Men were 1.76 times more likely at risk of alloimmunization than women (OR=1.76).

Based on χ^2 test, there was no statistically significant difference between the percentages of alloimmunization incidence with respect to ABO and Rh blood groups. However, this difference is not statistically significant, but it may be important clinically. The lowest and highest incidences were seen, in turn, in AB (1 patient) and O (32 patients) blood groups. Moreover, less alloimmunization rate was seen in those of negative Rh than positive Rh (4.3% versus 95.7%). However, the incidence rate of alloimmunization was higher in Sistanians than in Baluches and other ethnicities, but this difference is not statistically significant. The results of this study support the idea of the highest amount of antibody is generated against Rh and Kell systems [2].

Since in this center all patients were received ABO and Rh(D) compatible blood types, it is recommended to focus on matching antigens of other blood systems, especially Rh and Kell, in the crossmatch test to prevent alloimmunization in such patients [4].

Acknowledgements

This article is result of the research project No. 2230-90. It is financially sponsored by the Research Deputy and technically helped with getting access to the facilities of Department of Cellular and Molecular Research Center of Medical Sciences University of Zahedan. Special thanks to the Technical Deputy and the Laboratory of Immunohematology of Iranian Blood Transfusion Organization that provide the researchers with panel cells. All patients participating in this study are deeply appreciated.

Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

Funding/Support

Shiraz Medical University.

References

1. Campell AGM, McIntosh N. Forfar & Arnells textbook of pediatrics. 5th ed. New York: Churchill Lvingstone; 1998: 45-53.
2. Ameen R, Al-Shemmari S, Al-Humood S, et al. RBC alloimmunization and autoimmunizaqtion among transfusion dependent Arab thalasamia patients. Transfusion 2003; 43(11): 1604-10.
3. Kiani A, Abdi J, Shirkhani Y, and Kashi M. [Prevalence of alloimmunization against RBC antigens in thalassemia major patients of Lorestan province in 1383] Persian. Sci J Iran Blood Transfus Organ 2006; 3(3): 265-271.
4. Mahboudi F, Zeinali S, Merat A, et al. The molecular basis of thalassemia mutation in Fars province, Iran. Iran J Med Sci 1996; 21(3-4): 104.
5. Spanos T, Karageorga M, Ladis V, et al. Red cell alloantibodies in patients with thalassemia. Vox Sang 1990; 58(1): 50-5.
6. Bhatti FA, Salamat N, Nadeem A, et al. Red cell immunization in beta thalassemia major. J Coll Physicians Surg Pak 2004; 14(11): 657-60.
7. Singer ST, Wu V, Mignacca R, et al. Alloimmunization and erythrocyte autoimmunization in transfusion-dependent thalassemia patients of predominantly asian descent. Blood 2000; 96(10): 3369-73.
8. Gupta R, Singh B, Rusia U and Goyal S. Red cell alloimmunization in routinely transfused patients of beta thalassemia major. IJBTI 2010; 1(1): 1-4.
9. Hok-Kung H, Shah-Yin H, Chun-Kit L, et al. Alloimmmunization in Hong Kong southern Chinese transfusion dependent thalassemia patients. Blood 2001; 97(12): 3999-4000.
10. Eshghi P, Sanei-Moghaddam E, Mir-Masoudi M. [Evaluation of alloimmunization in major B. thalassemic patients in Zahedan in 2001] Persian. J Mazandaran Univ Med Sci 2003; 13(40): 36-42.
11. Shamshain BS, Arzanian MT, Shamshiri AR, et al. Frequency of Red Cell Alloimmunization in Patients with beta major thalassemia in an Iranian referral hospital. Iran J Pediatr 2008; 18(2): 149-153.
12. Sirchia G, Zanella A, Parravicini A, et al. Red cell alloantibodies in thalassemia major. Results of an Italian cooperative study. Transfusion 1985; 25(2): 110-2.
13. Michail-Merianou V, Pamphili-Panousopoulou L, Piperi-Lowes L, et al. Alloimmunization to red cell antigens in thalassemia: Comparative study of usual versus better-match transfusion programmes. Vox Sang 1987; 52(1-2): 95-8.
14. Sadeghian MH, Keramati MR, Badiei Z, et al. Alloimmunization among transfusion dependent thalassemia patients. Asian J Transfus Sci 2009; 3(2): 95-8.
15. Hassan Kh, Younus M, Ikram N, et al. Red cell alloimmunization in repeatedly transfused thalassemia major patients. Int J Pathology 2004; 2(1):16-19.
16. Singer ST, Wu V, Mignacca R, et al. Alloimmunization and erythrocyte autoimmunization in transfusion dependent thalassemia patients of predominantly asian descent. Blood 2000; 96(10): 3369-73.
17. Mincheff M. Changes in donor leukocytes during blood storage. Implications on post-transfusion immunomodulation and transfusion-associated GVHD. Vox Sang 1998; 74 Suppl 2: 189-200.
18. Frabetti F, Musiani D, Marini M, et al. White cell apoptosis in packed red cells. Transfusion 1998; 38(11-12): 1082-9.
19. Ghio M, Contini P, Mazzei C, et al. Soluble HLA class I, HLA class II, and Fas ligand in blood components: A possible key to explain the immunomodulatory effects of allogeneic blood transfusions. Blood 1999; 93(5): 1770-7.
20. Fluit CR, Kunst VA, Drenthe-Schonk AM. Incidence of red cell antibodies after multiple blood transfusion. Transfusion 1990; 30(6): 532-5.
21. Rosse WF, Gallagher D, Kinney TR, et al. Transfusion and alloimmunization in sickle cell disease. The cooperative study of sickle cell disease. Blood 1990; 76(7): 1431-7.
22. Vichinsky EP, Earles A, Johnson RA, et al. Alloimmunization in sickle cell anemia and transfusion of racially unmatched blood. N Engl J Med 1990; 322(23): 1617-21.
23. Wang LY, Liang DC, Liu HC, et al. Alloimmunization among patients with transfusion dependent thalassemia in Taiwan. Transfus Med 2006; 16(3): 200-3.

Please cite this article as: Mirzaeian A, Tamaddon Gh, Naderi M, Hosseinpour M, Sargolzaie N. Prevalence of alloimmunization against RBC antigens in thalassemia major patients. Zahedan J Res Med Sci (ZJRMS) 2013; 15(7): 55-58.