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Frequency of Kell and Rh alloantibodies in Iranian Thalassemia Patients in Khorasan Razavi Province, Iran

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ABSTRACT

Background: Thalassemia is an inherited disease with anemia and hemolysis. Blood transfusion is a routine treatment for thalassemia patients; alloimmunization is one of the complications of blood transfusion, which is very serious for these patients, especially girls and young women.

Materials and Methods: In this cross-sectional study, 446 thalassemia patients were examined. Demographic information of patients was extracted and recorded. The phenotype of ABO, Rh, and Kell antigens (tube method) with antisera from IMMUNDIANOSTICA Company (Germany) and the frequency of alloantibodies were determined.

Results: 55.8% of the studied individuals were male, and 44.2% were female. Mean age of the studied patients was 19.94±10.63. The alloantibodies were detected in 7.5% of cell-pack receivers. The most prevalent phenotype of the ABO system was the O blood group (37.4%), and the most abundant antigen of the Rh group was 'e', which was found in 99.8% of the studied population. The most common alloantibody detected was Anti K (38.2%); concerning kell phenotype, (K^-k^+) and (K^+k^+) were found in 99.3% and 0.7% of patients, respectively. The frequency of Anti-D, Anti-C, Anti-c, and Anti-E was 23.5%, 14.7%, 2.9%, and 14.7%, respectively.

Conclusion: According to the results of this paper, finding the compatible packed cells in terms of Kell and Rh systems antigens in addition to the ABO blood group is recommended to decrease the rate of alloantibodies in thalassemia patients.

Keywords: Thalassemia; Kell blood group system; Rh system**;** Antibody

INTRODUCTION

Thalassemia is an inherited hemolytic disorder, which is happened due to a defect in the beta or alpha globin chain. No far past, lifelong blood transfusion was the best treatment for the involved patients, but stem cell transplantation is also one of the better treatments [1,2]. Blood transfusion will elevate the hemoglobin level up to a normal range, but it is associated with some side effects; these will reduce the usefulness of blood transfusion during the time and may be life threatening[3,4]. Allergic reaction, circulatory overload, transfusion-related

acute lung injury (TRALI), bacterial infection, cardiac sidrosis and alloimmunization are of transfusion complications[5].

Differences in minor blood group phenotypes between blood donors and receivers can cause alloimmunization; most alloantibodies are against the Kell, Rh, and Duffy antigen system. Red cell alloimmunization is one the most important complications of multi-transfused patients[5-7]. The produced antibodies may disturb the laboratory tests and reduce the shelf life of RBCs. Alloimmunization rate is vary (4-50%) in different thalassemia patients[8].

Due to the frequent need of thalassemia patients for Leukoreduced RBC and the problem of alloantibody formation, many studies suggested stem cell transplantation as a decisive treatment; but finding fully phenotype compatible pack cell for multi-transfused cases such as thalassemia patients will reduce the side effects and increase the efficiency[9].

Many studies have been done to determine the prevalence and identification of alloantibodies in thalassemia patients all over the world. Due to the absence of any reports on the mentioned subject in the northeast of Iran, this study was performed to report the frequency of alloantibodies in the cited group.

MTERIALS AND METHODS

This cross-sectional research was done (2018 September to 2019 February) in the Iranian Blood Transfusion Organization (IBTO) laboratory of Mashhad city. In this study, 446 thalassemia patients from Khorasan Razavi province were entered; the participants of the study were all from Iran. Of whom, 55.8% (249) were male and the rest were female. 412 patients (228 males and 184 females) were receiving 1-3 pack cells every 20 days. Thirty-four intermediate thalassemia individuals, who were included in the study, did not receive any pack cells at all. Demographic information, including age, sex, number of received pack cells, and the age of patients at the time of first transfusion were recorded. For laboratory analysis, including ABO blood grouping and Direct anti globulin (DAT) tests, 2 mL of patients' blood was collected in EDTA container tube; for antibody screening and panel tests, 4 mL was collected without any anticoagulants[10].

Autoantibody detection was performed by auto control and DAT method, and the used antihuman belonged to IMMUNDIANOSTICA Company (Germany). For detecting ABO, Kell, and Rh antigens including D, C, c, E, and e the antisera from IMMUNDIANOSTICA was used according to the kit instruction.

The antibody screening process was done by antibody screening kit of IMMUCOR (USA) Company. Samples of patients who had alloantibodies were tested by Identification Panel (IMMUCOR, USA) for detecting the alloantibodies; the results were interpreted according to the kits instruction table. Finally, the phenotype of ABO, Rh, and Kell antigens and the frequency of alloantibodies were determined.

The study was done under the supervision of the ethics committee of the Mashhad University of Medical Sciences, and the ethics code is as follows: IR.MUMS.REC.1397.299

RESULTS

The mean age of studied individuals was 19.94±10.63 years old. The frequency of ABO phenotypes and Rh antigens is shown in Table 1. The most prevalent phenotype of the ABO system was the O blood group; the most abundant antigen of the Rh group was 'e', which was found in 99.8% of the studied population.

Kell phenotype frequency was as follows: (K+k+) in 0.7% and (K_k+) in 99.3% of individuals. No (K+k_) phenotypes were reported. K antigen was found in 3 (0.7%) of patients, and k antigen was present in 100% of studied individuals.

Among Rh phenotype, R1 r was found in 269 individuals (60.3%); according to this finding, it was known as the most frequent in the studied population. Table 2 describes the frequency of different Rh phenotypes.

The result of antibody screening was positive in 31 patients, including 14 men (6.1%) and 17 women (9.2%). Finally, 36 alloantibodies were detected in 31 patients; two different types of alloantibodies were detected in 5 cases. Alloantibodies detecting in association of each other in one patient are as

follows: anti D+C, anti D+k, anti K+E, anti c+E, anti-JKa and Fya. Frequency and specificity of detected alloantibodies are cited in Table 3. Warm autoantibodies were found in four cases (0.9%); one out of four had alloantibody against D antigen in association with warm autoantibody. Another case was detected with warm autoantibody, and two alloantibodies, including anti E and K.

Table 1: Frequency of abo phenotypes and Rh antigens

RH Antigen and ABO phenotype	O	A	B	AB	D	C	E	C	E
Frequency (%)	167 (37.4)	133 (29.8)	107 (24)	39 (8.7)	407 (91.3)	403 (90.4)	109 (24.4)	419 (93.9)	445 (99.8)

Table 2: Frequency no (%) of Rh phenotypes

Antigenic pattern	CCDE	CDE	CCDEE	CDE	CDEE	CDE	CDEE	CCDE	CDE	CDE	CCDE	CDEE	CCDEE
Phenotype	R1R	R1R1	R1R2	R0R	R2R	R2R2	R1RZ	R2RZ	RZRZ	RR	R'R	R''R	R'R''
Frequency (%)	269 (60.3)	25 (5.6)	102 (22.9)	4 (0.9)	4 (0.9)	1 (0.2)	2 (0.4)	0 (0)	0 (0)	34 (7.6)	5 (1.1)	0 (0)	0 (0)

Table 3: Frequency of alloantibodies compared to age and sex parameters

Age	Number of patients	Number of patients with alloantibodies	Anti-D	Anti-C	Anti-E	Anti-K	Anti-JK[a]	Anti-Fy[a]
≤ 10	92	1 (2.9%)	0	0	0	1 (2.9%)	0	0
11-19	130	14 (41.2%)	3 (8.8%)	2 (5.8%)	3 (8.8%)	6 (17.6%)	0	0
20-28	134	11 (32.4%)	4 (11.7%)	2 (5.8%)	0	3 (8.8%)	0	1 (2.9%)
29-38	71	6 (17.6%)	1 (2.9%)	0	2 (5.8%)	3 (8.8%)	1 (2.9%)	0
39-47	11	0	0	0	0	0	0	0
48-56	6	1 (2.9%)	0	0	0	0	0	0
57+	2	1 (2.9%)	0	1 (2.9%)	0	0	0	0
Total	446	34	8 (23.5%)	5 (14.7%)	5 (14.7%)	13 (38.2%)	1 (2.9%)	1 (2.9%)
Gender								
Male	249	14 (3.1%)	2 (14.2%)	2 (14.2%)	3 (21.4%)	7 (50%)	0	0
Female	197	20 (4.4%)	6 (30%)	3 (15%)	2 (10%)	6 (30%)	1 (5%)	1 (5%)

DISCUSSION

This study was designed to evaluate the frequency and the alloantibody type among thalassemia patients in Khorasan Razavi (the north east of Iran). Three major factors, which affect alloantibody formation in recipients are antigenic homogeneity of recipient, donor, recipient immune system and recipient immune modulation due to blood transfusion[1, 11]. According to the cited facts, the exact way to decrease alloimmunization rate is selecting the similar phenotype RBC for transfusion. Avoiding alloimmunization risk is more important in young women and girls.

According to the reports from other parts of Iran, rate of red blood cell alloimmunization is as follow: 7.4% in Tehran, 5.3% in southern Iran (Shiraz) and 18.7% in southwest Iran (Ahvaz). It was 7.5% in the present study and similar to Tehran, which was lower compared to the Ahvaz. The difference between the rate of alloimmunization in our study and southwest Iran may be due to more prevalent thalassemia in southwest Iran, so the geographical difference is a reason for mismatched results [12-14,9].

Thalassemia is known as a common hemoglobinopathy in the Arab area. In the retrospective review by Riami et al. on multi-transfused thalassemia patients in Oman, the rate of alloimmunization was 9.3% in the cited population; anti-E (24%) and anti-K (24%) were the most abundant identified antibodies[15]. However, in Khorasan Razavi province Anti-K (38.2%) and anti-D (23.5%) were more frequent; it seems that this difference is due to the genetic heterozygosity between the studied groups. Failure to detect the K antigen of donated pack cells in the Khorasan area is another reason for allo-anti K prevalence in thalassemia patients. This problem is seen in the Rh system too, especially in C and E antigens. According to D antigen identification in donated pack cells, allo-anti-D was not expected to be found in the thalassemia patients; it seems that the detected allo antibodies against D in the patients are the result of false negative reports or test technical errors.

The Riami's study reported an important correlation between alloantibody formation and number of received pack cells in young patients[15]; it is very reasonable that young individuals have more effective immune system to response to the alien antigens. More need of blood products in women is the main cause of higher frequency of alloantibodies in female (9.2%) In comparison to male (6.1%).

Another study in Kerachi reported E (32.5%) and K (27.5%) alloantibody as the most current antibodies[16] whose findings are along with Riamy et al. study. It seems important that many scientists studying alloimmunization in transfused-thalassemia patients reported Rh system antigens as the most stimulator of immune system[15-19]. This fact is very applicable; alloimmunization rate can be reduced significantly by choosing completely compatible blood in terms of Rh system.

Pazgol et al. in the study of 40 beta thalassemia-major patients investigated alloantibodies just in 42.50% of studied patients. Of whom, 47.05% of alloantibody formation was against E antigen, followed by 41.2% against Kell system antigens (K and Kp^a) [12]. Race and genetic role in alloimmunization study are illustrated here. The region of pazgol and the present study is totally different, indicating the cause of variation in the alloantibody formation and frequency.

Sadeghian et al. performed a study in the similar area in 2009. Whole of detected alloantibodies were against Rh system antigens, whereas 88.88% were anti D, 33.33% anti C, and 11.11% anti E. No other antibodies related to other groups were identified[9]. Both studies were similar, but the results were various. As mentioned earlier, the most frequent detected alloantibody was anti K (38.2%). The results were different about the Rh system too. Many literatures reported higher rate of alloimmunization in splenctomized individuals[9], but it was not considered as a variable in the present paper.

CONCLUSION

Alloimmunization is an important matter; it becomes more important in young women, girls, and poly-transfused individuals such as thalassemia patients. Most of the immunohematologist researchers emphasize alloimmunization prevention which will be obtained by Rh and Kell antigens compatibility. As RBC phenotype is various in

different populations, evaluating the research in a distinct area and paying attention to the produced antibodies will help to choose suitable and fully compatible RBC for the patients in need.

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