Transfusion and Apheresis Science xxx (xxxx) xxx

Contents lists available at ScienceDirect

Transfusion and Apheresis Science

journal homepage: www.elsevier.com/locate/transci

Prognostic Value Evaluation of HLA-DRB1*07:01, *10, *12, *13:01 Alleles for Alloimmunization in Transfusion-Dependent Thalassemia

Fatemeh Mezginejad [a], Gholam Reza Anani Sarab [b], Kamran Atarodi [a], Arezoo Oodi [a], Azita Azarkeivan [a,*]

[a] Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran.
[b] Cellular & Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran.

ARTICLE INFO

Keywords:
Thalassemia
Alloimmunization
HLA-DRB1

ABSTRACT

Background: Transfusion is a lifesaving treatment for lots of patients. However, in chronic blood recipients such as thalassemia patients, there are high concerns about alloantibody production that affects the quality of their life. Therefore, research on risk factors of alloimmunization has been started and followed. This study aimed at the determination of correlation probability between some HLADRB1 alleles and alloimmunization in Iranian thalassemia patients.

Materials and methods: The present study was conducted on 60 alloimmunized and 60 non-alloimmunized transfusion-dependent thalassemia patients. Antibody screening and identification tests were carried out using the tube method, and HLA-DRB1 genotyping was done using a single specific primer-polymerase chain reaction (PCRSSP).

Results: The results of antibody screening showed that the most prevalent alloantibodies were Anti-K (46.7 %), and followed by Anti-E (32.5 %), Anti-C (15.8 %), Anti-D (13.3 %), Anti-e (8 %), and Anti-c (5.8 %), respectively. DRB1*07:01 was detected more in non-responder patients (28.3 %). However, data analysis showed that there is no significant relationship between DRB1*07:01, *10, *13:01 frequency among responder and non-responder groups ($p = 0{\cdot}195$, 0.648, 0.402, respectively). There was not any significant correlation between HLA-DRB1*10 and *13:01 allele and alloimmunization. There was a significant association between HLA-DRB1*12 and alloimmunization ($p < 0{\cdot}05$, OR = 2.071, CI: 1.716–2.501).

Conclusion: The findings of this study represented that there is a significant relationship between HLADRB1*12 and Kell and E alloantibodies. Although more developed studies on other HLA alleles are demanded, these findings can be valuable in determining important alloimmunization risk factors to better management of transfusion complications.

1. Introduction

Thalassemia caused by a decrease or lack of hemoglobin synthesis is the most common inherited disorder of hemoglobin around the world [1]. Repeated blood transfusion is a vital treatment in transfusion-dependent thalassemia (TDT) patients [2,3]. Despite the development in compatibility transfusion tests, world statistics express that 4–50 % of TDT patients produce alloantibodies against blood group antigens and it remains a challenging issue in patient management that affects the patient's subsequent transfusions and limits their opportunities for transfusion [4,5]. Moreover, hemolytic reactions caused by alloantibodies against blood group antigens except the ABO group have been reported as the second and third leading causes of death due to blood transfusion [2,3,6]. In addition to the risk of patient mortality, it was demonstrated that alloantibodies are associated with a high risk of hepatomegaly, splenomegaly, and renal failure [6]. In this regard, human studies are needed to detect and manage the factors involved in the reaction to foreign antigens. Some variables influence alloantibody development, including differences between donor and recipient antigens, differences in human leukocyte antigen (HLA), cytokines polymorphisms, cellular and humoral immune systems, and recipient health conditions [1,7,8]. Although strategies have been devised to reduce the

* Corresponding author at: Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Thalassemia Clinic, IBTO bldg. Hemmat Exp Way, Next to Milad Tower, Po Box 14665-1157, Tehran, Iran.
E-mail address: azazarkeivan@yahoo.com (A. Azarkeivan).

https://doi.org/10.1016/j.transci.2021.103271
Received 26 July 2021; Received in revised form 28 August 2021; Accepted 5 September 2021
Available online 6 September 2021

risk of alloantibody production, finding compatible blood for patients is a major concern in blood transfusion [4]. Since HLA alleles are the highly important genetic factors that have been proven to be associated with alloimmunization risk [9–14], researches have been started and revealed that thalassemia patients are no exception to this rule. As in recent years, there have been numerous worldwide reports of an association between HLA-DRB1 group-specific alleles (GSA) and the chance of alloantibodies production in thalassemia patients [3,12,15]. For instance, previous studies have shown the association between production of C alloantibody with HLA-DRB1*12 and HLA-DRB1*13 GSA, E alloantibody with HLA-DRB1*11 and HLA-DRB1*09 alleles in TDT patients [11,16]. Additional above, HLA-DRB1*15 GSA dispose patients to alloantibody and autoantibody formation [10,13]. However, the high genetic heterogenicity of HLA-DRB1 GSA among different populations worldwide is not ignorable. Despite studies on the association between HLA and alloantibody development in Iran, it is still more demanded to investigate the association of more HLA-DRB1 GSA with alloimmunization in TDT patients. Hence, the aim of this study was to investigate the relationship between some unstudied HLA-DRB1 GSA including HLA-DRB1 *07:01, HLA-DRB1*10, HLA-DRB1*12, HLA-DRB1*13:01 with the production of alloantibodies in Iranian TDT patients to more complete obtained data in this area by providing information such as the frequency of these alleles in thalassemia patients in the Responder and non-Responder groups and discovering the probable relationship between HLA alleles and alloimmunization.

2. Materials and methods

2.1. Study design

The present study was conducted on 120 TDT patients divided into 2 groups including 60 responders (presences of alloantibodies in TDT patients) and 60 non-responder (TDT patients without any history of alloantibodies) patients. Studied patients were selected from TDT patients who were referred to the Zafar Adult Thalassemia Clinic. ABO, Rh D matching and cross match before transfusion had been performed for all TDT patients in the transfusion center. All patients received more than 20 leukoreduced blood transfusions at the time of testing. Patients with hepatitis C or sickle cell anemia were not included in this study. This study was approved by the Medical Ethics Committee of Transfusion Medicine Ethics Committee (IR.TMI.REC.REC.1399.024). Written informed consent was obtained from all volunteered participants. There were no restrictions for age, gender, and ethnicity (but patients should have Iranian nationality).

2.2. Antibody screening

To alloantibody screening and detection in this study, the patients' plasma was used. So, for this purpose 5 mL blood was collected from patients' ethylenediaminetetraacetic acid (EDTA) tubes. So that the Screening 3 Tube method was performed using three classes of screening cells (types I, II, and III- IMMUCOR, INC. Norcross). If the presence of an unexpected antibody was proven, 11-panel cells (IMMUCOR INC, Norcross. USA) was utilized for antibody identification.

2.3. DNA extraction

Genomic DNA was extracted from patients' whole blood-separated buffy coats using a DNA extraction kit (FAVORGEN kit (Biotech Corp, Cat. No.: FABGK 001, Taiwan). DNA extracted quantity and quality were analyzed using the NanoDrop (Thermo Scientific, USA). Sample concentration within the range of 100–200 ng/μL and the DNA purity ratio of 260/280 nm in 1.8–2.0 was accepted.

2.4. Molecular genotyping and primers design

Polymerase chain reaction-Sequence Specific Primers (PCR-SSP) were used for the amplification of genomic DNA. The specific primers for studied alleles including the HLA-DRB1*07:01, HLA-DRB1*10, *12, *13:01 was designed according to the Olerup protocol (13). Primer specificity and reliability were investigated by BLAST NCBI and also by positive samples obtained from the Iranian blood transfusion organization. Moreover, to approve the reliability of the PCR reaction, a primer pair as an internal control was designed and added to each reaction tube.

2.5. Multiplex PCR

The PCR reaction was conducted using Peqstar Thermocycler. The total reaction in each microtube was 25 μL, which consisted of 12.5 μL of master mix 2X (Amplicon, Denmark), 1 μL DNA template (100 ng), 0.8 μL forward primer, 0.8 μL reverse primer, 0.4 μL forward internal control primer, 0.4 μL reverse internal control primer, and 9.1 μL H2O. The amplification of DNA products at PCR reaction was carried out at 95°C for 5 min (initial denaturation), followed by 30 cycles of 95°C for 60 s, 60°C for 40 s, 72°C for 35 s and 72°C for 1 min for the final extension. All the assays were performed in duplicate with positive and negative controls. Finally, to determine the PCR-SSP amplicons Gel electrophoresis was carried out using 1.5 % agarose gel in 0.5X tris-borate-EDTA (TBE) buffer in 90 voltage for 40 min.

2.6. Sequencing

The PCR-SSP products must be confirmed. So that, the Sanger sequencing method was carried out. For this purpose, all positive samples for each allele were amplified in 50 μL volume and considered for sequencing by ABI-3130 XL (USA). The sequencing results were assessed by the Blast NCBI tool and Chromas software.

2.7. Statistical analysis

Statistical analysis was performed by SPSS software (V25.0,) and GraphPad Prism 6.07 software. The normal distribution of data was examined by the Shapiro-Wilk and Kolmogorov-Smirnov test. The *t*-test or Mann-Whitney samples from parametric and nonparametric tests were used for data analysis. Assessment of the relationship between qualitative variables and case /control groups was determined by chi-square and Fisher exact tests. In addition, the odds ratio (OR) with a 95 % confidence interval (95 %CI) was obtained. $p < 0.05$ was considered as the significant level.

3. Results

3.1. Profile of patient sample specifications and alloantibody characteristics

The present study was conducted on 120 major thalassemic TDT patients who were referred to the Zafar Adult Thalassemia Clinic for repeated transfusion. Patients were included 60 cases (responder patients, 50 %) and 60 controls (non-responders, 50 %) and consist of 54.2 % female and 45.8 % male. There were no significant differences in age and sex of subjects between two groups in this study and all were patients from Iranian population. Moreover, the responder group was divided into two groups including 16 mono-responders (produced one alloantibody) (26.7 %) and 44 hyper-responders (produced more than one alloantibody) (73.3 %) respectively. The results of antibody screening showed that the most frequent alloantibody was Anti-K (46.7 %), and followed by Anti-E (32.5 %), Anti-C (15.8 %), Anti-D (13.3 %), Anti-e (8%), and Anti-c (5.8 %), respectively. In addition, in hyper-responder patients, the most frequently detected combined alloantibodies were K + E alloantibodies (29.2 %).

3.2. HLA-DRB1 genotyping

3.2.1. PCR-SSP of HLA-DRB1*07:01

HLA-DRB1*07:01 was detected in 18.3 % of responder subjects and 28.3 % of non-responder patients. *HLA-DRB1*07:01* allele was more detected in hyper-responders than mono-responders (63.6 % vs 36.4 %) but a significant statistical relationship was not found (p = 0·195, OR = 0.568, 95 %CI) (Table 1). There was not also an association between antibody specificities and DRB1*07:01.

3.2.2. PCR-SSP of HLA-DRB1*10

HLA-DRB1*10 allele was only detected in 5 patients (6·7 %). The frequency of DRB1*10 was not highly different between the responder and non-responder groups (5 % vs 3.3 %), and a significant difference was not observed (p = 0·648, OR = 1.526, CI: 0.246−9.478) (Table 1).

3.2.3. PCR-SSP of HLA-DRB1*12

HLA-DRB1*12 allele GSA was only detected in four patients (all were belonging to hyper responder groups) (6·7 %). Further analysis revealed there is an association between HLA-DRB1*12 and alloimmunization (p = 0·05, OR = 2.071, CI: 1.716–2.501). All 4 positive patients for the DRB1*12 allele belonged to patients who had anti-K and anti-E simultaneously. It was significantly different between these patients and non-alloimmunized ones (for anti-K: p = 0.02. fisher exact test = 0.03, OR = 0.925. CI: 0.856−0.998) (for anti-E: p = 0.003. fisher exact test = 0.01, OR = 0.897. CI: 0.807−0.998).

3.2.4. PCR-SSP of HLA-DRB1*13:01

HLA-DRB1*13:01 allele was detected in 6 patients including 2 (3.3 %) of responders and 4 (6.7 %) of non-responder subjects. There was not any significant correlation between HLA-DRB1*13:01 allele and alloimmunization (p = 0.402, OR = 3.290; 95 %CI) (Table 2).

All bands in PCR amplification were specific without any non-specific bands or contamination (Fig. 1).

4. Discussion

Alloimmunization in TDT patients, as well as other blood recipients, is one of the major complications that against other transfusion reactions has not yet dissolved [17–19]. The rate of alloimmunization is different based on population ethnicity (5–30 %) [20] and according to the darvishi et al. publication, the rate of alloimmunization among Iranian TDT patients is approximately 10 % [4]. To tackle this problem a large number of studies were performed and found that it is attributed to several factors that are divided into two general groups including donor-related and recipient-related risk factors [6,21,22]. The first category consists of the time between blood collection and blood processing, the efficiency of leukocyte reduction methods, the amount of platelet residue in RBC products, and so on [6,21]. Genetic characteristics, recipient health condition, and previous antigen exposure are receptor-related factors. One important risk factor is receptor genetic traits such as HLA alleles that have a clear effect on receptor immunity. HLA system is characterized by its high degree of polymorphism due to differences in an amino acid setting that finally results in the expression status of major histocompatibility complex (MHC) peptides [6,22]. The difference in the expression of MHC peptides in patients has been investigated, and in recent years HLA restriction has been introduced for RBC antigens such as Rh and Kell. Furthermore, studies showed that some types of HLA-DRB1 alleles are also more likely to be associated with a higher chance of response rate [10,11,23]. So, we surveyed the present study and genotyped the TDT patients for the HLA-DRB1 *07:01, *10, *12, *13:01 to evaluate the probable association between these two variables, alloimmunization, and HLADRB1 alleles. The results revealed that HLA-DRB1*12 positive patients are more susceptible to alloantibody development, whereas HLA-DRB1*07:01, *10, *13:01 have not a significant relationship with alloantibody production.

Table 1
Comparing frequency of alleles between Hyper-responder and mono-responder patients.

	Hyper responder	Mono responder	Total	p-value (Fisher exact test)
HLADRB1*07:01	7(63.6 %)	4(36.4 %)	28(23.3 %)	0.421(0.324)
HLADRB1*10	3(100 %)	0	5(4.2 %)	0.284(0.387)
HLADRB1*12	4(100 %)	0	4(3.3 %)	0.02 (0.03)
HLADRB1*13:01	2(100 %)	0	6(5 %)	0.386(0.534)

Antibody screening results showed that the most frequent alloantibodies were Kell, E, C, and D, respectively. This is in agreement with the systematic review publication of alloimmunization in the Iranian population which revealed that Kell (37 %) is the most prevalent alloantibody, and the second and third alloantibody in the mentioned meta-analysis were anti-D and anti-E, respectively [4]. Ali Ghasemi et al. also presented that the most prevalent alloantibodies in the Iranian population were Rh and Kell alloantibodies [24]. Although there was no significant relationship between DRB1*07:01 and alloimmunization in this study, it seems to be a protective allele in alloantibody production. Furthermore, the non-significant result may be due to the low sample size; so, HLA genotyping with a larger number of patients is supposed to get a significant correlation. On the other hand, in the responder group HLA-DRB1*07:01 allele was more observed in hyper-responders than mono-responders (63.6 % vs 36.4 %). It can be concluded that if a TDT patient expresses the HLA-DRB1*07:01 allele, he/she would be more susceptible to produce more than one alloantibody.

Our finding in this regard was inconsistent with the study of Henk Schonewille et al., which showed a high frequency of HLA-DRB1*07 in responder patients. Moreover, they revealed a significantly increased frequency of DRB1*07 in anti-c + E cases, whereas in our findings significant association was not found with antibody specification [13].

HLA-DRB1*10, *12, *13;01 alleles were only detected in fewer than 10 % of patients and it is postulated that they are low-frequency alleles in the Iranian population. In addition, the frequency of HLA-DRB1*10, *13;01 was not significantly different between case and control groups. This finding is in contrast with the study of Henk Schonewille et al., which demonstrated a significant relationship between HLA-DRB1*10, *13 and alloimmunization against kell antigen [13]. Nevertheless, it was in accordance with the report by Ebrahimi et al. that demonstrated a non-significant relationship between HLA-DRB1*13 with alloimmunization [11]. But Jacques Chiaroni presented a high frequency and significant relationship between HLA-DRB1*13 and Kell alloantibody production [25]. This reveals that against other nationality populations HLA-DRB1*13 allele has not an important role in predisposing to alloantibody production in TDT Iranian patients.

In contrast, all positive patients for HLA-DRB1*12 (6·7 %) belong to the responder group including hyper-responders, and there was a significant association between HLA-DRB1*12 and alloimmunization. It is worth noting that all of these patients had Kell + E alloantibodies simultaneously. In this regard Henk Schonewille et al., study is in line with our findings and demonstrated that cases with multiple antibodies had higher frequencies of HLA-DRB1*12 compared to the control group [13]. These findings could help health managers where personalized medicine is debated. Personalized medicine which described the implementing therapeutic strategy based on the characteristics of individual patients is also valuable for thalassemia patients as well. It can be applied by molecular investigations such as the discussed HLA studies before the starting transfusion protocol, so prevents alloantibody production, and subsequently reduces complications, costs and increases patient's quality of life.

5. Conclusion

The findings of this study confirmed that there is a significant

Table 2
The frequency of HLA-DRB1 alleles and their relationship with alloimmunization.

	Responder	Non-responder	Total	p-value	OR	CI 95 %	Fisher exact test
HLADRB1*07:01	11(18.3 %)	17(28.3 %)	28(23.3 %)	0.195	0.568	0.240–1.344	
HLADRB1*10	3(5 %)	2(3.3 %)	5(4.2 %)	0.648	1.526	0.246–9.478	0.5
HLADRB1*12	4(6.7)	0	4(3.3 %)	0.05	2.071	1.76–2.501	0.05
HLADRB1*13:01	2(3.3 %)	4(6.7 %)	6(5 %)	0.402	0.483	0.085–2.741	0.34
Male	29(48.3 %)	26(43.3 %)		0.58	0.81	0.39–1.67	
Female	31(51.7 %)	34(56.7 %)					

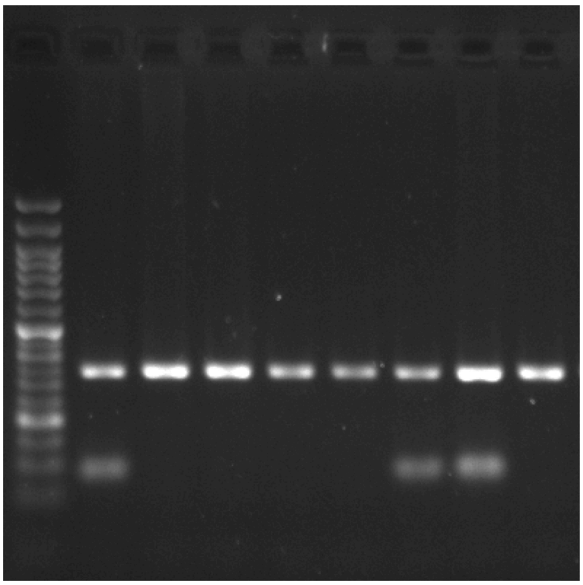

Fig. 1. Agarose gel electrophoresis results of multiplex PCR-SSP. The internal control band (328 bp) was positive in all samples. HLADRB1*07:01 allele (85 bp) was positive in wells 1, 6 and 7. Agarose gel electrophoresis for other studied alleles was as well as this picture containing positive and internal control.

relationship between HLADRB1*12 and alloimmunization, especially with Kell and E specification. Since Iran is categorized among countries with a high frequency of thalassemia patients, HLA genotyping at the beginning of repeated and lifelong transfusion can help patient management. This study suggests more HLA base researches on TDT patients with large sample volumes and more HLADRB1 alleles genotyping for the Iranian population.

Funding

This study was funded by the Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran.

Authors' contributions

Fateme Mezginejad and Azita Azarkeivan designed the study; Fateme Mezginejad and Gholamreza Anani Sarab and Kamran Atarodi and Arezoo Oodi contributed to experimental procedure; Gholamreza Anani Sarab and Kamran Atarodi and Arezoo Oodi contributed to biostatistics' analysis; Fateme Mezginejad contributed to manuscript writing; and Azita Azarkeivan contributed to final revision.

Ethics approval

This study was approved by the Medical Ethics Committee of Transfusion Medicine Ethics Committee (IR.TMI.REC.REC.1399.024) and written informed consent was obtained from all volunteered participants.

CRediT authorship contribution statement

Fatemeh Mezginejad: Conceptualization, Methodology, Investigation, Writing - original draft. **Gholam Reza Anani Sarab:** Investigation, Visualization, Validation, Formal analysis. **Kamran Atarodi:** Methodology, Visualization, Validation, Writing - review & editing. **Arezoo Oodi:** Investigation, Validation, Formal analysis, Writing - original draft. **Azita Azarkeivan:** Conceptualization, Supervision, Data curation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Acknowledgments

The ethics committee approval was obtained from High Institute for Research and Education in Transfusion Medicine Ethics Committee (IR.TMI.REC.REC.1399.024). We are thankful from Miss Mina Ebrahimi from Ahvaz University of Medical Sciences for help us in primer design and some troubleshooting's in laboratory procedures. We acknowledge the Iranian Blood Transfusion Organization; and the staff of Zafar Adult Thalassemia Clinic, Tehran, Iran, for their kind help. Written informed consent was obtained from all volunteered participants.

References

[1] Vaziri M, JavadzadehShahshahani H, Moghaddam M, Taghvaee N. Prevalence and specificities of red cell alloantibodies in transfusion-dependent beta thalassemia patients in Yazd. Iran J Pediatr Hematol Oncol 2015;5:93–9.
[2] Compernolle V, Chou ST, Tanael S, Savage W, Howard J, Josephson CD, et al. Red blood cell specifications for patients with hemoglobinopathies: a systematic review and guideline. Transfusion 2018;58:1555–66. https://doi.org/10.1111/trf.14611.
[3] Lal A, Wong TE, Andrews J, Balasa VV, Chung JH, Forester CM, et al. Transfusion practices and complications in thalassemia. Transfusion 2018;58:2826–35. https://doi.org/10.1111/trf.14875.
[4] Darvishi P, Azami M, Sayehmiri K, Sayehmiri F, Goodarzi A, Azarkeivan A, et al. Red blood cell alloimmunization in Iranian beta-thalassemia patients: a systematic review and meta-analysis. ISBT Sci Ser 2016;11:163–73. https://doi.org/10.1111/voxs.12299.
[5] Franchini M, Forni GL, Marano G, Cruciani M, Mengoli C, Pinto V, et al. Red blood cell alloimmunisation in transfusion-dependent thalassaemia: a systematic review. Blood Transfus 2019;17:4–15. https://doi.org/10.2450/2019.0229-18.
[6] Ryder AB, Zimring JC, Hendrickson JE. Factors influencing RBC alloimmunization: lessons learned from murine models. Transfus Med Hemother 2014;41:406–19. https://doi.org/10.1159/000368995.
[7] 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. https://doi.org/10.5858/arpa.2013-0016-OA.
[8] Kulkarni S, Choudhary B, Gogri H, Patil S, Manglani M, Sharma R, et al. Molecular genotyping of clinically important blood group antigens in patients with thalassaemia. Indian J Med Res 2018;148:713–20. https://doi.org/10.4103/ijmr.IJMR_455_17.
[9] Baleotti W, Ruiz MO, Fabron A, Castilho L, Giuliatti S, Donadi EA. HLA-DRB1*07:01 allele is primarily associated with the Diego a alloimmunization in a Brazilian population. Transfusion 2014;54:2468–76. https://doi.org/10.1111/trf.12652.
[10] Darvishi P, Sharifi Z, Azarkeivan A, Akbari A, Pourfathollah AA. HLA-DRB1*15:03 and HLA-DRB1*11: useful predictive alleles for alloantibody production in

thalassemia patients. Transfus Med 2019;29:179–84. https://doi.org/10.1111/tme.12531.

[11] Ebrahimi M, Maleknia M, Parav N, Mohammadi MB, Mortazavi Y, Saki N, et al. The HLA-DRB1*11 group-specific allele is a predictor for alloantibody production in the transfusion-dependent thalassemia patients. Transfus Apher Sci 2020;59:102729. https://doi.org/10.1016/j.transci.2020.102729.

[12] Raos M, Zunec R, Mocibob M, Gojceta K, Lukic M, Golubic Cepulic B. Susceptible and protective HLA-DR and HLA-DQ alleles for Fy a alloimmunization in the Croatian population. Transfusion 2019;59:1118–24. https://doi.org/10.1111/trf.15087.

[13] Schonewille H, Doxiadis IIN, Levering WHBM, Roelen DL, Claas FHJ, Brand A. HLA-DRB1 associations in individuals with single and multiple clinically relevant red blood cell antibodies. Transfusion 2014;54:1971–80. https://doi.org/10.1111/trf.12624.

[14] Tatari-Calderone Z, Gordish-Dressman H, Fasano R, Riggs M, Fortier C, Campbell AD, et al. Protective effect of HLA-DQB1 alleles against alloimmunization in patients with sickle cell disease. Hum Immunol 2016;77:35–40. https://doi.org/10.1016/j.humimm.2015.10.010.

[15] Qidwai A. Single center retrospective study in Karachi, 1; 2018. p. 2017–9.

[16] Ebrahimi M, Dayer D, Jalalifar MA, Keikhaei B, Tahan Nejad Asadi Z. Association between HLA-DRB1*01 and HLA-DRB1*15 with alloimmunisation in transfusion-dependent patients with thalassaemia. Transfus Med 2020;30:275–80. https://doi.org/10.1111/tme.12677.

[17] Davari K, Soltanpour MS. Study of alloimmunization and autoimmunization in Iranian β-thalassemia major patients. Asian J Transfus Sci 2016;10:88–92. https://doi.org/10.4103/0973-6247.172179.

[18] Davoudi-Kiakalayeh A, Mohammadi R, Pourfathollah AA, Siery Z, Davoudi-Kiakalayeh S. Alloimmunization in thalassemia patients: new insight for healthcare. Int J Prev Med 2017;8:101. https://doi.org/10.4103/ijpvm.IJPVM_246_16.

[19] Dhawan HK, Kumawat V, Marwaha N, Sharma RR, Sachdev S, Bansal D, et al. Alloimmunization and autoimmunization in transfusion dependent thalassemia major patients: study on 319 patients. Asian J Transfus Sci 2014;8:84–8. https://doi.org/10.4103/0973-6247.137438.

[20] Zalpuri S, Zwaginga JJ, van der Bom JG. Risk Factors for Alloimmunisation after red blood Cell Transfusions (R-FACT): a case cohort study. BMJ Open 2012;2:e001150. https://doi.org/10.1136/bmjopen-2012-001150.

[21] Romphruk AV, Simtong P, Butryojantho C, Pimphumee R, Junta N, Srichai S, et al. The prevalence, alloimmunization risk factors, antigenic exposure, and evaluation of antigen-matched red blood cells for thalassemia transfusions: a 10-year experience at a tertiary care hospital. Transfusion 2019;59:177–84. https://doi.org/10.1111/trf.15002.

[22] Gerritsma J, Oomen I, Meinderts S, van der Schoot CE, Biemond BJ, van der Bom JG, et al. Genetic association studies of red blood cell transfusion related alloimmunization: a systematic review and meta-analysis. Blood 2019;134:2459. https://doi.org/10.1182/blood-2019-128199.

[23] Tian L, Hou L, Wang L, Xu H, Xiao J, Ying B. HLA-DRB1*09:01 allele is associated with anti-E immunization in a Chinese population. Transfusion 2018;58:1536–9. https://doi.org/10.1111/trf.14568.

[24] Ghasemi A, Abbasian S, Ghaffari K, Salmanpour Z. Prevalence of alloantibodies and autoantibodies in transfusion dependent thalassemia patients. Iran J Blood Cancer 2016;8:80–5.

[25] Chiaroni J, Dettori I, Ferrera V, Legrand D, Touinssi M, Mercier P, et al. HLA-DRB1 polymorphism is associated with Kell immunisation. Br J Haematol 2006;132:374–8. https://doi.org/10.1111/j.1365-2141.2005.05868.x.