



ARAŞTIRMA/RESEARCH

Red blood cell alloimmunization in patients with sickle cell disease in Turkey: a single center retrospective cohort study

Türkiye’de orak hücre hastalığına sahip hastalarda eritrosit alloimmünizasyonu: tek merkez geriye dönük kohort çalışması

Soner Solmaz¹; Pelin Karacaoğlu²; Çiğdem Gereklioğlu³; Süheyl Asma³; Aslı Korur³; Nurhilal Büyükkurt¹; Mutlu Kasar¹; Mahmut Yeral¹; İlknur Kozanoğlu⁴; Can Boğa¹; Hakan Özdoğu¹

¹Adana Hospital of Başkent University, Department of Hematology, ²Department of Internal Medicine, ³Department of Family Medicine, ⁴Department of Hematology Research Laboratory, Adana, Turkey

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Abstract

Purpose: We aimed to investigate erythrocyte alloimmunization frequency and related factors in our region where SCD is common.

Material and Methods: This study was planned as a single center, cross-sectional and retrospective cohort study. A total of 216 patients who had been followed up due to SCD [Hemoglobin (Hb) SS, Hb S-β thalassemia, Hb S-α thalassemia] were included in this study. Patients were divided to two groups according to amount of transfusion. The patients who had received less than 6 transfusions per year and who did not have the history of erythrocytapheresis were allocated to Group 1, and the patients who had received 6 or more simple transfusion per year or who had undergone erythrocyte exchange were allocated to Group 2.

Results: Of 216 SCD patients included in the study. Alloimmunization was detected in 67 (31.0%) out of 216 patients who underwent transfusion, and in 17 (30.4%) out of 56 patients in Group 1 and in 50 (31.3%) out of 160 patients in Group 2. When the patients were analyzed according to alloimmunization development, our study revealed that neither SCD complications are a risk factor for alloimmunization nor alloimmunization increases mortality rates.

Conclusion: High alloimmunization frequency found in our study suggests the insufficient adherence of alloimmunization-prevention policies in RBC transfusions performed except experienced institutions. Therefore alloimmunization may be reduced or prevented through performing extended red cell typing among SCD patients.

Key words: Sickle cell disease, transfusion, alloimmunization

Öz

Amaç: Orak hücre hastalığının sık görüldüğü bölgemizde eritrosit alloimmünizasyon sıklığını ve ilişkili durumları araştırmayı amaçladık.

Gereç ve Yöntem: bu çalışma tek merkezli, zamansal kesitli ve geriye dönük kohort çalışması olarak planlanmıştır. Toplamda 216 orak hücre hastalığı [Hemoglobin (Hb) SS, Hb S-β talasemi, Hb S-α talasemi] tanısı olan hasta çalışmaya dahil edilmiştir. Hastalar transfüzyon miktarına göre iki gruba ayrılmıştır. Yılda 6’dan daha az transfüzyon alan ya da eritroferez öyküsü olmayan hastalar Grup 1’e, yılda 6 ve daha fazla basit transfüzyon alan ya da eritroferez işlemine alınan hastalar Grup 2’ye dahil edilmiştir.

Bulgular: Çalışmamıza 216 hasta dahil edilmiştir. Transfüzyon tedavisi alan toplam 216 hastanın 67 (%31.0)’sinde, Grup 1’deki 56 hastanın 17’sinde (%30.4), Grup 2’deki 160 hastanın 50’sinde (%31.3) tespit edilmiştir. Hastalar alloimmünizasyon gelişimi açısından analiz edildiğinde, çalışmamız ne orak hücre komplikasyonlarının alloimmünizasyon gelişimi için ne de alloimmünizasyonun ölüm için bir risk faktörü olmadığını göstermiştir.

Sonuç: Çalışmamızda bulunan yüksek alloimmünizasyon sıklığı, tecrübeli merkezler dışında yapılan transfüzyonlarda alloimmünizasyonu önleyici politikalara yeteri kadar uyulmadığı konusunda fikir vermektedir. Bu nedenle orak hücre anemili hastalarda alloimmünizasyon, ayrıntılı eritrosit antijen tanımlama işlemi yapılarak azaltılabilir veya önlenebilir.

Anahtar kelimeler: Orak hücre hastalığı, transfüzyon, alloimmünizasyon

Yazışma Adresi/Address for Correspondence: Dr. Soner Solmaz, Adana Hospital of Başkent University, Department of Hematology, Adana, Turkey. E-mail: drssolmaz@gmail.com
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INTRODUCTION

Red blood cell (RBC) transfusion therapy is an important management strategy in sickle cell disease (SCD) for several acute and chronic indications¹. Despite improved patient outcomes with hydroxyurea administration, indications for chronic transfusions have increased in the last 10 years and are associated with considerable reduction in morbidity and mortality². However, alloimmunization to RBC blood group antigens remains a major complication for patients with SCD and often presents significant challenges in their medical management³. Alloimmune reactions can be associated with significant morbidity in SCD, including delay in urgent treatment of stroke and acute chest syndrome due to difficulty to find compatible blood, delayed transfusion reactions, hyperhemolysis, and autoantibody formation⁴.

The incidence of alloimmunization in patients with SCD ranges from 7% to 47%, depending on age, RBC exposures, and extent of antigen matching for blood groups other than ABO and RhD3. Sixty six percent of the alloantibodies identified in most of the reports were those of the Rhesus (Rh) and Kell systems in SCD⁵. Some studies identified alloantibodies of the Kidd and Duffy systems in addition to the Rh and Kell systems⁵. Factors implicated in RBC alloantibody formation include recipient sex and age, history of pregnancy, number and timing of blood transfusions, recipient clinical diagnosis and treatment, genetic factors related to antigenic response, and racial differences between donors and recipients⁶.

Alloimmunization frequency varies among communities depending on various factors including ethnic, genetic differences and transfusion policies. To the best of our knowledge, sufficient data are not available in Turkey, therefore we aimed to investigate erythrocyte alloimmunization frequency and related factors in our region where SCD is common.

MATERIAL AND METHODS

Study Group and Data Collection

This study was planned as a single center, cross-sectional and retrospective cohort study. A total of 216 patients who had been followed up due to SCD

[Hemoglobin (Hb) SS, Hb S- β thalassemia, Hb S- α thalassemia] in our center between 01 October 2011 and 01 November 2015 were included in this study. A total of 160 patients whose detailed medical data could not be accessed and who did not have a definite diagnosis of SCD, who never underwent RBC transfusion, who underwent bone marrow transplantation and who died due to the other causes than SCD were excluded from the study.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1975 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by Başkent University Institutional Review Board and Ethics Committee (project no: KA15/329).

Definitions of Sickle Cell Disease Related Conditions

Hydroxyurea regular use of 15 mg/kg/daily hydroxyurea for at least one month was considered. The patients who did not require medication due to painful conditions for 4 weeks were considered to be in steady state. Painful crisis was defined as the patient's admission to hospital due to pain which was considered not to be related any other causes than SCD, intervention with parenteral NSAIDs, metamizol and narcotics^{1,2}.

Degree of crisis frequency was defined as rare if the patient experienced <3 painful crisis a year, frequent if the patient experienced ≥ 3 painful crisis a year. Nephropathy was defined as the presence of at least one of renal dysfunction as microalbuminuria and proteinuria, hypercchogenity and/or thinning in renal cortex on ultrasonography, low creatinine clearance⁴. Pulmonary hypertension was defined as >25 mmHg mean resting pulmonary artery pressure or >30 mmHg following exercise and <15 mmHg pulmonary capillary pressure⁷. Avascular necrosis was defined as the presence of radiologically evident typical bone lesions⁴.

Transfusion therapy was defined as Group 1 for the patients who had received less than 6 transfusions per year and who did not have the history of erythropheresis, Group 2 for the patients who had received 6 or more simple transfusion per year or who had undergone erythrocyte exchange.

Laboratory Studies

For indirect antiglobulin screening, the sera were used by gel technology using commercial panel of cells "ID-DiaCell" (DiaMed ID, Micro Typing System, DiaMed, Cressier sur Morat, Switzerland) with polyspecific anti-human globulin (rabbit anti-immunglobulin G and monoclonal anti-C3d). Patients were considered to be alloimmunized if antibodies to one or more erythrocyte antigens were identified.

Statistical Analysis

Patient characteristics were examined using descriptive statistics. Continuous variables were given as mean \pm standard deviation (SD), and categorical variables were defined as percentage. Chi-square test and t-test were used to compare proportions and means for categorical and continuous variables, respectively. For univariate analysis of possible associations between parameters, chi-square test or Fisher's exact test was used for discrete variables. The variables which have significant p values ($p < 0.05$) and marginal insignificant p values ($p < 0.1$) in univariate analysis were included in multivariate analysis. All test significances were two-tailed. SPSS statistical software (SPSS 17.0 for Windows, Inc., Chicago, IL, USA) was used for all statistical calculations.

RESULTS

Of 216 SCD patients included in the study, 112 (51.9%) were female and 104 (48.1%) were male with mean age of 33.9 ± 9.7 years (18-64) (32.5 ± 8.5 years for males, 35.1 ± 10.7 years for females). HbSS was detected in 158 (73.1%), HbS β + in 30 (13.9%) and HbS β 0 in 28 (13%) and 54.6% of the patients were detected to have been using hydroxyurea.

When 216 patients were divided to groups according to transfusion therapy, 56 (25.9%) were in Group 1, 160 (74.1%) were in group 2. Alloimmunization was detected in 67 (31.0%) out of 216 patients who underwent transfusion and autoantibody development was detected in 14 (20.9%) out of these 67 alloimmunized patients. Alloimmunization was detected in 17 (30.4%) out of 56 patients in Group 1 and in 50 (31.3%) out of 160 patients in Group 2. A statistically significant difference was not detected between groups with regard to alloimmunization ratios ($p > 0.05$).

Although Group 2 received more intense transfusion therapy, this was not found to lead to an additional risk for alloimmunization compared to Group 1 patients. Laboratory data of the patients are given in Table 1 and data of the patients about SCD complications are given in Table 2. A statistically significant difference was not detected between groups with regard to HbA, HbS, HbA2, Hb F, hemoglobin, hematocrit, white blood cell, platelet values, age, gender, hydroxyurea use, SCD complications ($p > 0.05$). Our study revealed that neither SCD complications are a risk factor for alloimmunization nor alloimmunization increases mortality rates (Table 3).

DISCUSSION

Sickle cell anemias are inherited disorders of hemoglobin synthesis that result from the substitution of glutamic acid at the sixth position of its β -globin chain by valine (HbS)⁷. The HbS gene is found in high frequency in many parts of Africa, India, the Middle East, the Mediterranean area, and parts of Turkey⁸. The primary manifestations of SCD are chronic hemolytic anemia and episodes of severe pain crises due to vasoocclusion⁹. Repeated vasoocclusive crisis can affect multiple organ systems, and individuals with SCD have increased risks of stroke, renal dysfunction, pulmonary hypertension, retinal disease, and avascular necrosis⁹. RBC transfusions remain a cornerstone treatment for acute and chronic complications in SCD¹⁰. Approximately 90% of adults with SCD will have received at least one RBC transfusion⁹. Transfusion therapy facilitates blood and tissue oxygenation and reduces the propensity for sickling by diluting the host cells and temporarily suppresses the production of HbS-containing red cells¹¹. Despite the beneficial effects of transfusion therapy in SCD, there are still adverse effects associated with transfusion that can lead to serious short and long-term complications including alloimmunization¹¹. Prevalence of alloimmunization varies between 7-47% in SCD patients in literature³.

We detected alloimmunization prevalence as 31% in our study which was conducted due to the absence of data about alloimmunization in SCD patients in our region where SCD is prevalent. Alloimmunization was not found to be related with the other SCD-related complications like acute chest syndrome or stroke, consistently with the study of Telenet al⁴.

Table 1. Laboratory data of the patients

	Total (N=216)	Non-immunized patients (N=149)	Alloimmunized patients (N=67)	P value
WBC (x10 ³ /mCL)	12.3±7.1	11.9±5.1	13.1±10.0	0.255
Hgb (gr/dL)	8.7±1.7	8.8±1.6	8.5±1.9	0.260
Hct (%)	25.6±5.4	25.8±5.4	25.0±5.6	0.284
Plt (x10 ³ /mCL)	426±223	440±223	395±223	0.172
Hb A (%)	8.2±13.4	9.4±14.9	5.6±8.8	0.078
Hb A ₂ (%)	3.5±2.4	77.8±15.4	80.9±13.2	0.456
Hb F (%)	8.8±10.1	8.6±10.2	9.3±10.0	0.669
Hb S (%)	78.8±14.8	77.8±15.4	80.9±13.2	0.201

WBC, white blood cell count; Hgb, hemoglobin; Hct, hematocrit; Plt, platelet

Table 2. Data of the patients about SCD complications

	Total (N=216)	Non-immunized patients (N=149)	Alloimmunized patients (N=67)
Frequent Painful Crisis	29.6%	30.2%	28.4%
History of Avascular Necrosis	5.6%	5.4%	6.0%
History of Acute Chest Syndrome	9.7%	10.1%	9.0%
History of Arterial Hypertension	8.4%	8.1%	9.0%
History of Heart Failure	4.6%	4.0%	6.1%
History of Nephropathy	31.0%	31.5%	29.9%
History of Pulmonary Hypertension	44.0%	46.3%	38.8%
History of Cerebrovascular Disease	6.9%	6.7%	7.5%
History of Pulmonary Thromboembolism	5.1%	4.7%	6.0%
History of Deep Venous Thromboembolism	3.7%	4.0%	3.0%
Death	7.4%	6.0%	10.4%

Table 3. Comparison of groups and relative risk in alloimmunized patients.

	Inter-group comparison	Alloimmunized patients (N=67)
	p	RR (95% CI)
Sex	0.901	0.971 (0.614-1.536)
Hydroxyurea use	0.287	1.158 (0.875-1.533)
Transfusion Requirement	0.765	0.926 (0.560-1.530)
History of Erythropherisis	0.610	1.065 (0.833-1.362)
Frequent Painful Crisis	0.784	1.065 (0.678-1.674)
History of Avascular Necrosis	0.515	1.368 (0.518-3.613)
History of Acute Chest Syndrome	0.799	1.124 (0.456-2.770)
History of Arterial Hypertension	0.835	0.905 (0.355-2.310)
History of Heart Failure	0.521	0.669 (0.195-2.292)
History of Nephropathy	0.780	1.064 (0.688-1.646)
History of Pulmonary Hypertension	0.388	1.157 (0.825-1.623)
History of Cerebrovascular Disease	0.851	0.905 (0.322-2.546)
History of Pulmonary Thromboembolism	0.702	0.792 (0.240-2.615)
History of Deep Venous Thromboembolism	0.701	1.358 (0.281-6.555)
Death	0.253	1.049 (0.958-1.150)

Interestingly, the authors detected a relationship between alloimmunization and short life span in the

same study, this possible association has not been previously studied⁴. We did not detect a relationship

between alloimmunization and mortality.

Ugwu et al.¹¹ reported that the prevalence of red cell alloantibody among multi-transfused patients with SCD was found to be 9.3% in Nigeria, and this finding further reaffirms that blood transfusion is associated with the development of alloantibodies¹¹. Miller et al.¹² reported alloimmunization prevalence as 14.4%. However mean age was lower in both studies (26 and 19.3 years, respectively) compared to our study (33.9 years)

Elega and Niel¹³ reported high ratio (16%) of alloimmunization development in French Guiana. The authors explained that since blood cannot be collected locally, use of blood mostly from French Caucasian blood donors is causing alloimmunization in the Guianese population due to ethnic/genetic differences¹³. Natukunda et al.⁶ reported that the rate of RBC alloimmunization in Ugandan SCD patients was 6.1%. The homogeneity between donors and SCD patients plus the low transfusion load may explain this low immunization frequency⁶. Chou et al.³ found alloimmunization prevalence as 58% among the patients who undergo chronic transfusion, 15% among the patients who undergo episodic transfusion. The authors suggest that altered Rh alleles in both patients and donors contributed to Rh alloimmunization³. Aly et al.¹³ reported alloimmunization prevalence as 21.4% in 42 Egyptian SCD patients. They concluded that low alloimmunization could probably be explained with the similarity in the ethnicity between patients and donors¹⁴. These studies emphasize the importance of ethnic/genetic differences between donors and recipients¹⁴. Therefore we also consider that this ethnic heterogeneity may be one of the factors responsible for high alloimmunization prevalence in our study population.

Ameen et al.¹⁵ used two approaches in their study conducted with Kuwaiti Arab SCD patients. In the first approach (Group 1), the donor-recipient matching was random and limited to the conventional procedure for ensuring ABO and D antigen compatibility with nonleukoreduced blood until the patients develop RBC alloantibodies. For the second approach (Group 2), donor-recipient matching using poststorage-leukoreduced blood was limited to phenotype-matched blood for Rh (D, C, E, c, e) and K1 (partial phenotype matching)¹⁵. They found alloimmunization prevalence as 65.5% in Group 1, 23.6% in Group 2¹⁵. They concluded that sex and blood transfusion protocol had significant

effects on the rate of alloimmunization, consistently with the other studies and this study confirms the importance of selecting partial phenotype blood to prevent formation of antibodies related to the Rh systems¹⁵. We suggest that similar ratios of alloimmunization Group 1 (30.4%) and Group 2 (31.3%) in our study is associated with our standard transfusion policy (leuko-depleted blood matched for antigens of the ABO, Rh and Kell systems and routine antibody screening for alloimmunized patients) on the contrary to the expectations that alloimmunization should be more frequent among the patients who received more transfusions.

As discussed above, alloimmunization prevalence varies between populations in SCD patients. Many factors should be considered to minimize alloimmunization prevalence in this group of patients who need more frequent transfusions. The main factors which increase alloimmunization risk include increased patient age^{3,11}, female gender^{2,14}, history of pregnancy^{2,6}, not applying leukoreduction¹⁵, use of long term stored blood products^{14,15}, the number of transfused RBC units^{2,14}, transfusion for acute vasoocclusive indications and elevated inflammatory status of the patients², ethnic/genetic differences^{3,6,13}.

Our study has some limitations despite it yields significant data for our country. We could not sufficiently evaluate RBC transfusions that were performed out of our institution, a detailed stratification of the amount of transfusions could not be done and thereby the influence of this factor on alloimmunization could not be exactly evaluated and finally we could not provide alloantibody pattern as we could not obtain alloantibody screening data of all alloimmunized patients as this routine procedure was introduced in only recent years.

In conclusion, high alloimmunization frequency found in our study suggests the insufficient adherence of alloimmunization-prevention policies in RBC transfusions performed except experienced institutions. Additionally, it is thought that the antigenic differences between recipients and donors could be effective depending on ethnic/genetic factors resulting from the heterogeneity of our population. Therefore, alloimmunization may be reduced or prevented through performing extended red cell typing among SCD patients. So we suggest that the policies of Blood Transfusion Centers should be meticulously followed in order to prevent

red cell alloimmunization. Further studies are required to reveal the influence of ethnic and genetic factors on alloimmunization, to evaluate alloantibody pattern in our country.

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