Blood transfusion

Implementation of Red Cell Genotyping in Children with Beta Thalassemia in an Outpatient Setting in Turkey

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INTRODUCTION

Blood grouping serology may be inadequate for patients receiving chronic transfusion therapy.[1,2] The absence of β-chain synthesis results in short RBC lifespan in children with β-thalassemia major; these patients should receive regular transfusion therapy for regular growth and development. According to the International Thalassemia Federation (TIF) guideline and the Turkish national guideline, patients should have extended red cell antigen typing that includes at least ABO, C, c, D, E, e, and Kell before starting transfusion therapy.[3]

The prevalence of β-thalassemia carriers in Turkey ranges from 2.1% to 13.1%, with the highest prevalence in the Mediterranean and Aegean regions.[4] Since 2011, the Republic of Turkey has provided temporary protection status to individuals residing from Syria. Hemoglobin disorders are highly prevalent Syria, too.

Red cell genotyping has been served as a valuable adjunct to conventional serologic testing, providing accurate blood group determination in recently transfused patients or those with alloantibodies. In 2013, a red cell genotyping study in patients with thalassemia demonstrated that 51% of transfused patients had phenotype/genotype discrepancies in Turkey.[5] However, red cell genotyping has not become a routine diagnostic method in our country.

The primary aim of this study is to analyze the results of red cell genotyping in children and young adults with β-thalassemia major and to compare serologic results with genotyping results. The secondary aim is to compare patients with and without phenotype/genotype discrepancy in terms of transfusion frequency, iron accumulation, and organ damage.

METHODS

- All patients with β-thalassemia major followed at the main outpatient clinic in Ankara between January and September 2024 were studied.
- Red cell phenotyping (ABO, Kell and RhD,C,c,E,e) is performed by standard tube or gel matrix tests (Across, Dia Pro, Kocaeli, Turkey) prior to the first transfusion in all thalassemia patients.
- Transfusion data and EDTA-anticoagulated peripheral whole blood were obtained and shipped to the NIH.
- HEA BeadChip DNA array (Immucor, Warren, NJ) was used for red cell genotyping.
- Patients with and without phenotype/genotype discrepancy were compared in terms of iron accumulation and organ dysfunction by ferritin, liver functions, T2* and R2* magnetic resonance imaging (MRI), and echocardiography findings.

RESULTS and CONCLUSIONS

We enrolled 51 patients (M/F: 24/27) in the study, 25 were Turkish and 26 were Syrian (Table 1). There were no differences in age, weight, height, body mass index, percentiles, liver functions, ferritin, echocardiography or MRI findings between the two nationalities. Patient age was not associated with liver function tests, ferritin, echocardiography or MRI findings. The red cell genotyping results of the patients in consecutive order are presented in Figure 1.

We analyzed 6 antigens of the Rh and Kell systems and found 25 patients (49%) with at least 1 phenotype/genotype discrepancy (Table 2). Mixed-field reaction in serologic typing occurred in 3 Turkish and 3 Syrian patients; 3 other Turkish patients (5.8%) had alloantibodies; one had anti-E and anti-K, one had anti-E and one had anti-C. Transfusions, weight or height percentiles, ferritin, liver function tests, echocardiography and MRI findings did not differ in patients with or without phenotype/genotype discrepancy. DAT positivity was noticed in 21 patients (41.2%) at least once. Weight or height percentiles, ferritin levels, echocardiography and MRI findings did not differ between DAT positive and negative patients. However, DAT-positive patients were significantly older than DAT-negative patients (15.5±5.5 vs. 11.6±6.0 years, p=0.015).

Splenectomy was previously performed in 3 patients. Hepatomegaly or splenomegaly were not associated with phenotype/genotype discrepancy, liver function tests, echocardiography or MRI findings.

Cardiac iron overload, assessed by T2* MRI, occurred in 3 Turkish and 2 Syrian patients and was not associated with phenotype/genotype discrepancy (p=0.63). However, there was a trend toward increased hepatic iron overload in patients with phenotype/genotype discrepancies (84% vs. 58%, p = 0.071).

Our study provides a comprehensive analysis of immunohematological and clinical parameters in β-thalassemia patients, emphasizing the relevance of red cell genotyping.

Nearly half of the patients had a phenotype/genotype discrepancy and remained unchanged since a similar study in 2013. This result aligns with previous reports that conventional serology may fail to detect variant or partial antigens, especially in chronically transfused patients.[6,7] Because red cell genotyping has not been adopted, no improvement of care in this regard has occurred for thalassaemia patients in Turkey more than 10 years. Despite our extended antigen-matching policy, the alloimmunization rate was low.

Implementing red cell genotyping in routine transfusion practice could prevent alloimmunization. Establishing large-scale screening to identify antigen-negative donors could enhance transfusion safety and improve clinical outcomes for patients with β-thalassemia major in Turkey.

Table 1. Patient demographics

Parameter	Nationality§ Turkish	Syrian	p
Sex			
Male	11	13	
Female	14	13	0.66
Total	25	26	
Age (y)	14.9±6.6	11.6±5.1	0.055
Weight (SDS)†	-0.56±1.12	-0.72±1.36	0.43
Height (SDS)†	-1.18±1.00	-1.67±1.28	0.14
BMI (SDS)†	0.14±0.89	0.008±1.27	0.93
Hemoglobin (g/dL)	9.3±0.85	9.2±0.95	0.55
Transfusions (n)	73.0±34.4	63.8±24.7	0.56
AST (IU/L)	35.1±22.6	53.2±89.3	0.80
ALT (IU/L)	44.6±38.7	64.2±155.9	0.35
Total bilirubin (mg/dL)	2.53±1.52	1.92±0.59	0.15
Direct bilirubin (mg/dL)	0.70±0.27	0.59±0.16	0.26
Ferritin (mg/dL)	1585±1007	1755±1136	0.24
Liver iron (R2*, msec)	4.65±3.49	5.53±4.48	0.67
Hearth iron (T2*, msec)	20.2±4.9	21.9±11.9	0.18
Ejection fraction (%)	67.9%±4.8%	68.6%±3.2%	0.50

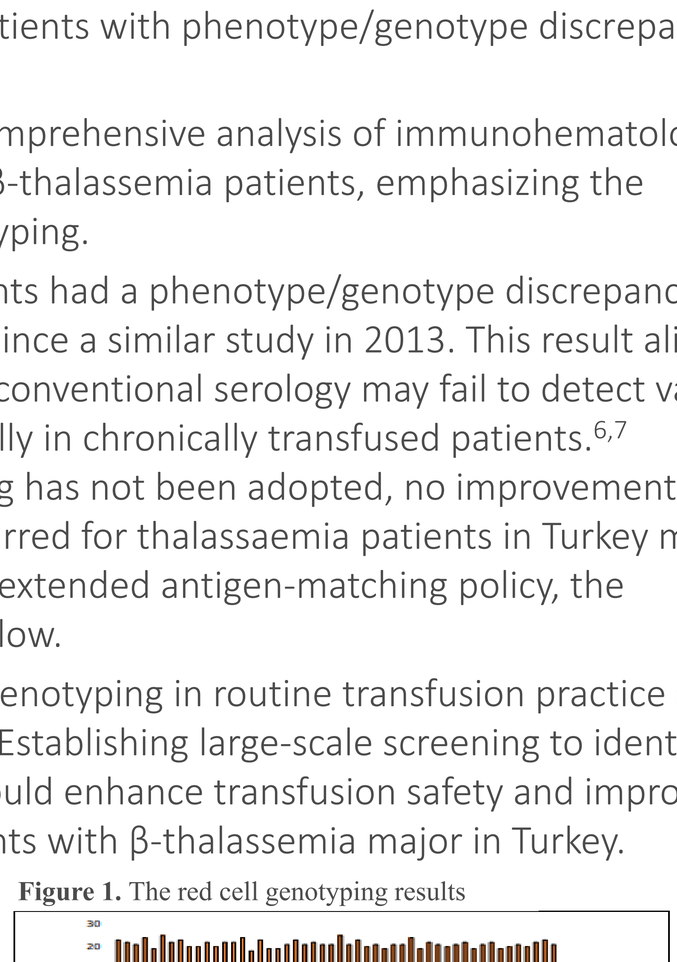
§ Mean ± standard deviation, except male/female
† Standard deviation score (SDS)

Table 2. Concordance between red cell genotyping and phenotype results in 51 patients with thalassemia

Genotyping	Phenotype† +/-	+/+	-/+
Rh system	C+c-	C+c+	C-c+
*RHCE*C/RHCE*C*	12	4	0
*RHCE*C/RHCE*c*	5	19	2
*RHCE*c/RHCE*c*	0	4	5
Rh system	E+e-	E+e+	E-e+
*RHCE*E/RHCE*E*	0	0	0
*RHCE*E/RHCE*e*	0	7	12
*RHCE*e/RHCE*e*	0	2	30
Kell system	K+k-	K+k+	K-k+
*KEL*01/KEL*01*	0	0	0
*KEL*01/KEL*02*	0	0	2
*KEL*02/KEL*02*	0	0	49

†The yellow cells indicate concordances between serologic reactivity and genotyping results. All results outside of the yellow cells represent discrepancies

Figure 1. The red cell genotyping results



Red boxes represent antigen present and white boxes presents antigen absent. The yellow box represents GATA1 positivity. The total number of positive antigens per patient is shown at the top. The prevalence of antigens is shown in the blue columns.

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