

# Red Blood Cell (RBC) Phenotyping in Major Thalassemia Patients in Bushehr

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## Abstract

**Background:** RBC phenotyping is essential to confirm the identity of suspected alloantibodies and to facilitate the identification of antibodies that may be formed in the future. We tested the phenotype detection of A, B, C, c, D, E, e, Le<sup>a</sup>, Le<sup>b</sup>, K, Fy<sup>a</sup>, Fy<sup>b</sup>, Jk<sup>a</sup>, Jk<sup>b</sup>, M, N, S, s and P1 antigens in the peripheral blood samples

**Methods:** RBC phenotyping in 72 transfused thalassemia patients were evaluated in Fatemeh Zahra Hospital in Bushehr in a cross-sectional study. K2-EDTA-anticoagulated blood samples were obtained for RBC antigen detection. Red cell antigens were detected using standard blood bank methods (saline, albumin and coombs phase).

**Results:** In our study the mean age of were 18.21±7.46 years. There were 37 (51.40%) males and 35 (48.60%) females. We observed the presence of e, FYa, FYb and M antigens in all of patients. c, N, S, Lua and Leb antigens were found in most of patients. s antigen was not positive in any patient.

**Conclusion:** If phenotype matching of the nonalloimmunized patient is done and if donor RBCs are selected to match the phenotype of the patient, then alloimmunization do not developed.

**Keywords:** RBC phenotyping, major thalassemia, Fatemeh Zahra Hospital, Bushehr

## Introduction

The differences in human blood are due to the presence or absence of certain protein molecules called antigens and antibodies. The antigens are located on the surface of the red blood cells (RBC) and the antibodies are in the blood plasma. Individuals have different types and combinations of these molecules(1). The distribution of various blood groups antigens varies amongst individuals in any given population(2). Blood group antigens are polymorphisms of proteins and carbohydrates on the outside surface of the RBC(3). The International Society of Blood Transfusion (ISBT) recognizes over 300 (302) red cell surface antigenic determinants; most of these (about 285) belong to one of 29 blood group systems(4).

Current blood transfusion practice requires that only ABO/Rh-compatible RBC units are transfused to a patient. Other Rh system antigens, such as C, c, E, and e, as well as antigens in the Kell, Kidd, Duffy, and many other systems, are not matched unless the patient has developed respective alloantibodies due to previous transfusions or pregnancies. Because RBC antibodies can cause hemolysis of transfused RBCs, differentiation of original blood group antigen status is mandatory for confirmation of antibody identification and further transfusions of compatible

RBC units in such cases(5). Hemagglutination has been the principal analytical tool of immunohematology since the discovery of blood group antigens(6).

The major risks of transfusions are unexpected incompatibility reactions, the transmission of infectious agents, Iron overload and alloimmunization. Alloimmunization leads to an increased risk of transfusion reactions, reducing the available pool of compatible blood for transfusion in subsequent crises. Alloimmunization is the source of a variety of problems during long-term medical and transfusion management, with the main problem being the identification of appropriate antigen-negative RBCs for transfusion (3).

Programs to prevent alloimmunization to RBC antigens have been designed and implemented to provide antigen-matched RBC transfusions to patients with b-thalassemia, particularly those who are alloimmunized and/or in need of chronic transfusion support. RBC phenotyping is essential to confirm the identity of suspected alloantibodies and to facilitate the identification of antibodies that may be formed in the future. Accurate antigen typing of transfused patients is often a difficult task due to the presence of donor RBCs in the patient's circulation.



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Thus, in these patients phenotyping can be time-consuming and difficult to interpret. It is also difficult to type cells when a patient's RBCs test positive for direct antiglobulin and no direct agglutinating antibody is available. Identification of antibodies that may be formed in the future. Accurate antigen typing of transfused patients is often a difficult task due to the presence of donor RBCs in the patient's circulation. Thus, in these patients phenotyping can be time-consuming and difficult to interpret. It is also difficult to type cells when a patient's RBCs test positive for direct antiglobulin and no direct agglutinating antibody is available(7).

In this study, we therefore tested the ability of detection of A, B, C, c, D, E, e, Le<sup>a</sup>, Le<sup>b</sup>, K, Fy<sup>a</sup>, Fy<sup>b</sup>, Jk<sup>a</sup>, Jk<sup>b</sup>, M, N, S, s and P1 antigens in the peripheral blood samples who repeatedly received multiple transfusion of blood matched for only the ABO and D antigens.

### Material and Methods

#### Patients:

All patients with major thalassemia that requiring long-term transfusion were eligible for study (72 patients). All of these patients were registered at thalassemia management center of Fatemeh Zahra Hospital in Bushehr. The transfusion records of all patients were examined for age, sex, age of the first blood transfusion. The study was carried out from May to August 2010 in Bushehr Blood Transfusion Organization.

#### Samples:

Blood samples were obtained for RBC antigen detection. K2-EDTA (Dipotassium ethylenediamine tetra acetate)-anticoagulated blood specimens were selected. When each sample was given from patient, antigen detection was performed immediately.

#### Laboratory protocol:

Our protocol included of red cell phenotype for the following antigens: A, B, C, c, D, E, e, Le<sup>a</sup>, Le<sup>b</sup>, K, Fy<sup>a</sup>, Fy<sup>b</sup>, Jk<sup>a</sup>, Jk<sup>b</sup>, M, N, S, s and P1. For this aim we used specific antibodies for every antigen from Biotest (Germani). Red cell antigens were detected using standard blood bank methods (saline, albumin and coombs phase).

### Results

#### Patient characteristics

All of the patients in this study had thalassemia major (mean age, 18.21 ± 7.46 years; range, 5-40 years). There were 37 (51.40%) males and 35 (48.60%) females.

The patients were being managed by regular transfusion of packed cell/whole blood keeping in consideration only ABO and Rh 'D' blood groups. In

none of the patients phenotypes other than these blood groups were known. Age of the first blood transfusion ranged from 40 days to 12 years (2.14±2.40 years).

#### Red Cell Antigens

In a total of 72 cases studied, red cell alloantibodies were detected in 9 (10%)(8). Frequency of ABO antigens of major thalassemia patients were collected in table 1. The frequency of O antigen was the most amount and after that A and B antigen were detected. Frequency of RBC antigens exception of ABO system in major thalassemia patients have been shown in table 2.

#### Discussion

There are few published data to support the conclusion that patients are receiving transfusions. The development of red cell antibodies (allo- as well as auto-antibodies) occurs in a variable number of multiply transfused thalassemia major patients. In majority of them, these antibodies are non-hemolytic; however, in others, they may be hemolytic. In such circumstances, transfusion therapy may become significantly complicated, as the patient may develop transfusion reaction. A low rate of allo-immunization (5-10%) would be expected if there is less heterogeneity of red cell antigens between donors and recipients. Extensive blood group phenotyping of ABO, Rh, Kell, Kidd, Duffy, and other blood groups is often needed after patients have received multiple transfusions. Because autologous blood group typing is hindered by nonidentical markers that have been transfused. In our study, we attempted to see whether our uniform cell typing protocol for simultaneous genotyping of ABO, RHD, RHCE, KEL, JK, and FY alleles and another RBC group, which can be performed in a single procedure within 3 to 4 hours, was convenient for such determination. Among the most common risks of RBC transfusion is the development of RBC alloantibodies. The incidence of RBC alloimmunization is not insignificant, ranging from 4% to as high as 60% in some patient populations(9).

This study was conducted to demonstrate the frequency of red cell antigens in patients with thalassemia major. We observed the presence of e, FYa, FYb and M antigens in all of patients. c, N, S, Lua and Leb antigens were found in most of patients. s antigen was not positive in any patient. A study was done by Castro and et al evaluated several protocols to determine the most reasonable approach to prevent alloimmunization in sickle cell disease patients; they concluded that the most common antibodies formed by these patients were to the C, E, and K antigens. When using the protocol that matched donor RBCs according to the patient's phenotype for C, c, E, e, and K, 87.5% of sickle cell patients in the study

would not have developed alloantibodies, as compared with 70.9% under the protocol of matching for ABO and D alone(10). Another study, by Vichinsky and et al, showed that phenotype matching for C, E, and K resulted in a drastic decrease in delayed hemolytic transfusion reactions (which dropped 90%), whereas the alloimmunization rate dropped from 3% to 0.5%(11).

**Conclusion**

If phenotype matching of the nonalloimmunized patient is done and if donor RBCs are selected to match the phenotype of the patient, then alloimmunization do not developed. A reduction in alloimmunization risk can reduction morbidity and mortality of multiple transfusion patients. Because if alloantibodies developed in these patients, the cost to treat complications of alloimmunization, such as acute and delayed hemolytic transfusion reactions was added.

**Conflict of interests**

The authors declare that they have no competing interests.

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**Table 1.** Frequency of ABO antigens in major thalassemia patients in Bshehr, Iran.

Variables		Frequency (Percent)
ABO system	A	18 (25.0%)
	B	18 (25.0%)
	AB	2 (2.8%)
	O	34 (47.2%)

**Table 2.** Frequency of RBC antigens exception of ABO system in major thalassemia patients in Bshehr, Iran

Systems	Antigenes	Positive	Negative
Rh	Anti D	67 (93.1%)	5 (6.9%)
	Anti C	69 (95.9%)	3 (4.1%)
	Anti c	71 (98.6)	1 (1.4%)
	Anti E	55 (76.4%)	17 (23.6%)
	Anti e	72 (100.0%)	0 (0.0%)
Kell	Anti K	3 (4.2%)	69 (95.8%)
Kidd	Anti Jka	62 (86.1%)	10 (13.9%)
	Anti Jkb	59 (81.9%)	13 (18.1%)
Lewis	Anti Lea	54 (75.0%)	18 (25.0%)
	Anti Leb	66 (91.7%)	6 (8.3%)
Lutheran	Anti Lua	71 (98.6)	1 (1.4%)
	Anti Lub	24 (33.3)	48 (66.7%)
Duffy	Anti Fya	72 (100.0%)	0 (0.0%)
	Anti Fyb	72 (100.0%)	0 (0.0%)
MN	Anti M	72 (100.0%)	0 (0.0%)
	Anti N	70 (97.2%)	2 (2.8%)
Ss	Anti S	70 (97.2%)	2 (2.8%)
	Anti s	0 (0.0%)	100 (100.0%)
P	Anti P1	66 (91.7%)	6 (8.3%)