Original Article

Impact of antigenic exposures and role of molecular blood grouping in enhancing transfusion safety in chronically transfused thalassemics

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Abstract:

Background: Red cell alloimmunization is an acknowledged complication of blood transfusion. Current transfusion practices for thalassemia do not cater to this risk. Serological phenotyping is usually not reliable in these cases unless performed before the first transfusion. Under such circumstances, molecular blood grouping is an effective alternative. **Aim:** To perform molecular blood group genotyping in chronically transfused thalassemia patients and assess the risk of antigenic exposure and incidence of alloimmunization with current transfusion protocols. **Materials and Methods:** Molecular blood group genotyping was performed for 47 chronically transfused thalassemia patients. Their 1-year transfusion records were retrieved to assess the antigenic exposure and the frequency thereof. **Results:** Of 47 patients, 6 were already alloimmunized (3 with anti-E and 3 with anti-K) and were receiving the corresponding antigen negative units. We observed that random selection of ABO and Rh D matched units resulted in 57.7% ±8.26% chance of Rh and Kell phenotype matching also. Forty-four patients had received one or more antigenic exposures at least once. The 6 already alloimmunized patients were further exposed to antigens other than the ones they were immunized to. During the study period, only one patient developed an alloantibody, anti-E with exposure to antigens C (92%) and/or E (32%) at each transfusion. **Conclusion:** Several factors apart from mere antigen exposure may influence the development of alloimmunization as most of our patients received antigenic exposures but not alloimmunized. Our data provide an impetus for future large-scale studies to understand the development of alloimmunization in such patients.

Key words:

Alloimmunization, antigenic exposure, molecular blood grouping, thalassemia

Introduction

Thalassemia is considered as a common genetic disorder worldwide with a particularly high frequency in a broad belt, extending from the Mediterranean basin through the Middle East (Iran), India, and Southeast Asia.[1] Overall prevalence of β-thalassemia in India is 3–4% with an estimate of around 8000–10,000 new births with major disease each year.[2,3] Most of these children have a severe clinical presentation, who, in the absence of stem cell transplantation are treated by life-long red blood cell (RBC) transfusions administered every 2–5 weeks to maintain the hemoglobin level between 9 and 11.5 g/dl.[4]

Development of one or more red cell alloantibodies (alloimmunization) is a common complication of such chronic transfusion therapies. The development of alloantibodies against red cell antigens can result in hemolytic transfusion reactions in the absence of appropriate pretransfusion testing facility employing sensitive techniques.[5] Besides, it can lead to difficulty in finding appropriate antigen negative blood. This provides a clinical impetus to consider shifting compatibility testing to an approach that allows for more complete and accurate red cell antigen matching. However, the best approach for prevention of alloimmunization is under considerable debate ranging from the provision of RBCs matched for all the major antigens associated with clinically significant antibodies to blood matched only for antibodies that have already been made.

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Current transfusion practices for thalassemics deals with compatibility of ABO and Rh D group alone but do not cater to the risk of development of alloimmunization. Serological phenotyping in multiply transfused patients is usually not reliable due to the presence of multiple cell populations unless performed before the first transfusion. Under such circumstances, molecular blood group analysis offers an effective alternative for typing red cell antigens so as to provide better-matched blood.[5]

This study was performed to assess the risk of alloantigenic exposures and incidence of alloimmunization with current transfusion protocols for thalassemics.

Materials and Methods

Forty-seven thalassemic patients registered with us for regular transfusion underwent Molecular blood group genotyping using BioArray HEA Bead Chip Technology (Immucor Inc., USA) for Rh, Kell, Duffy, Kidd, MNS, Lutheran, Dombrock, and other antigen systems in January 2014 at Department of Transfusion Medicine and Immunohematology, Indraprastha Apollo Hospitals, New Delhi. An EDTA (Ethylene di-amino acetic acid) vial sample was taken for DNA extraction and molecular typing for red cell antigens was performed as per the manufacturer's instructions. Before 2014, transfusion practice for thalassemia patients at our center, involved pretransfusion antibody screen and crossmatching of ABO and Rh D matched, leukoreduced red cell units. Known alloimmunized patients were provided appropriate antigen negative units [Figure 1]. Provision of extended Rh and Kell matched blood was not a part of the routine transfusion practice due to ambiguity in extended phenotyping results in chronically transfused patients bearing multiple cell populations.

With their molecular Rh and Kell profiles available in January 2014, 1-year transfusion records of all these patients from January 1, 2013, to December 31, 2013, were retrieved and reviewed retrospectively. Patient demographics and status of splenectomy were recorded and transfusion details including the age of initiation

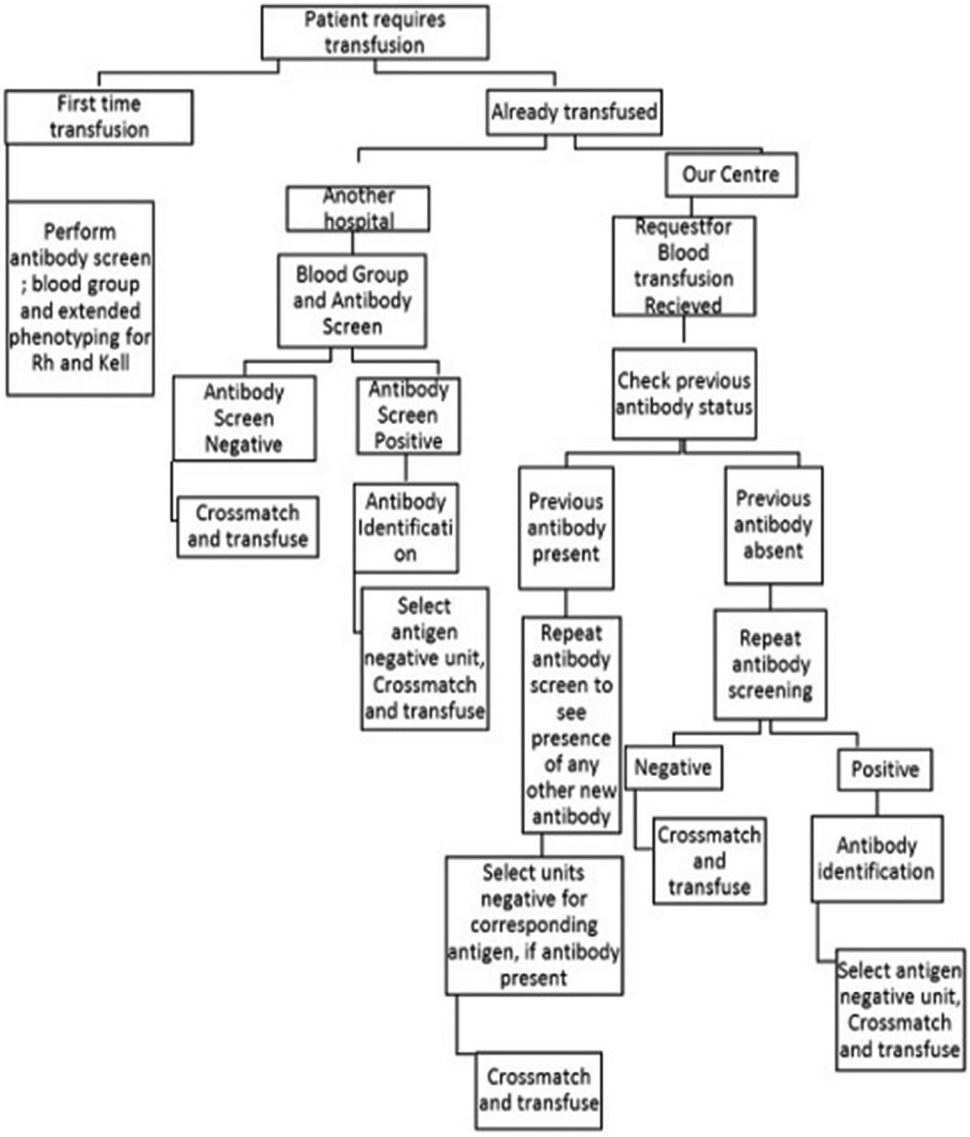

Figure 1: Transfusion policy for registered thalassemia patients at our center

of transfusions, units transfused between January to December 2013, pretransfusion antibody screening status before each transfusion episode and antibody identification results if any were assessed. As a departmental policy, all the donor units are typed for Rh (C, c, E, and e) and Kell (K) antigens which helped us in retrieving the Rh and Kell phenotypes of the units transfused and also compare the same with the molecular Rh and Kell profiles of the patients to assess the antigenic exposure, if any, received by each patient during the study period and the frequency thereof. The antibody screening and antibody identification were performed on Neo/Galileo (Immucor, Norcross, GA, USA) using the commercial panels (Immucor, Norcross, GA, USA).

Results

Forty-seven thalassemic patients were included in this study (11 females and 36 males). Age of initiation of transfusions of these patients is shown in Table 1. At the time of the study, six of these patients were already alloimmunized (3 had developed anti-E and 3 had anti-K) and were receiving the corresponding antigen negative units. In all, 1457 units of Packed Red Cells (PRC) (average of 31 units/patient, with a range of 9–65) were transfused during the 1-year analysis period. Following our transfusion protocol, 57.7% ±8.3% (n = 841) of PRC issued were Rh and Kell matched/compatible with the patients genotype and resulted in no antigenic exposures. Of all the patients, two were splenectomized and none of them developed any alloantibody.

Of the 47 patients, 44 received one or more alloantigenic exposures at least once during the study period [Table 2]. The 6 alloimmunized patients were also further exposed to antigens other than the ones they were already immunized to. During the study period, only one patient developed an alloantibody, anti-E. The patient was A Rh D positive with Rh and Kell genotype being ccee; K- and had received antigenic exposure for C (92%) and/or E (32%) during the study period. He was started on transfusions at the age of 2 years.

Discussion

Alloimmunization to red cell antigens is an immune response usually stimulated by the transfusion of blood components and is one of the complications of RBC transfusions. The factors for alloimmunization are complex and involve at least three main contributing elements: (1) The RBC antigenic difference between the blood donor and the recipient; (2) the recipient's genetic constitution and immune status; and (3) the immunomodulatory effect of the allogeneic blood transfusions on the recipient's immune system.[6]

Table 1: Age of initiation of transfusions

Age of initiation of transfusions (years)	Number of patients (%)
<1	35 (74.5)
1-3	10 (21.3)
>3	2 (4.3)

Various frequencies of alloimmunization (4–50%) have been reported worldwide in thalassemia patients depending on the homogeneity of the donor-recipient population, RBC phenotype matching policy, and age at initiation of transfusion.[7-9] The prevalence rates of alloimmunization in multi-transfused thalassemia patients in India were comparatively low, varying from approximately 3% to 10%.[9-16] This has been explained by the homogeneity of RBC antigens between the blood donors and recipients.[8] The most common antibodies are directed against Rh and Kell antigen systems.[5] In a previous study, by our center on thalassemia patients from New Delhi - NCR region, the most common antibody identified was also directed against Rh and Kell antigens.[11] Therefore, matching for Rh and Kell antigens goes a long way in preventing development of alloimmunization. However, in India, these patients are already started on transfusions even before they are referred to a center where a serological typing facility is available or in some instances are transfused as an emergency even before they are diagnosed as a case of thalassemia.

For a country like ours, molecular typing is not readily available and costs stand as the major limiting factor. We, at our center, performed molecular genotyping for thalassemics and observed that random selection of ABO and Rh D matched units resulted in only 57.7% ±8.3% of Rh and Kell phenotype matching. Hence, the lower rate of alloimmunization in our study population cannot be totally explained by similar antigenic profiles of patients and donors as there were large number of antigenic exposures.

The literature supports the concept that there are a subset of non-ABO alloantibody "responders," "non-responders," and "hyper-responders," i.e., transfused individuals who are capable of forming one or more antibodies after RBC exposure. Factors which may affect the "responder" status of an individual are manifold but not restricted to disease state, human leukocyte antigen (HLA) polymorphisms, underlying inflammation, and patient age at the initiation of transfusions.[17]

In multiply transfused patients, it has been shown that alloimmunization risk was significantly lower in patients who started transfusion therapy at a very young age (<3 years) compared with those who started later in life. An immature immune system and some form of acquired immune tolerance to allogenic RBC antigens are held responsible for the reduced alloimmunization risk.[17] In the present study, of 47 thalassemic patients, only 2 were of age >3 years when transfusion therapy was initiated and had received multiple antigenic stimulus for Rh and Kell antigens in all the transfusion episodes. Yet, none of them have developed any alloantibody. To the contrary, of the six patients

Table 2: Antigenic exposure to patients during one year study period (January-December 2013)

Antigenic stimulus	Antigen negative patients	Patients exposed (%)	Average frequency of exposure % (range)	Alloimmunization during study period
C	6	5 (83.3)	74.4 (14.7-94.7)	0
c	25	25 (100)	52.5 (35.5-96.7)	0
E	36	30 (83.3)	22 (7.9-44)	1
e	2	2 (100)	83.5 (73.2-93.8)	0
K	46	21 (45.6)	4 (1.9-8.3)	0

having pre-existing alloantibodies, 4 were started on transfusion therapies at age less than a year and 2 of them between 1 and 2 years of age. They subsequently received PRC units negative for the corresponding alloantibody but had been exposed to other antigenic stimuli. So far, no other alloantibodies have been detected. The patient who developed alloimmunization during the study interval was also started on transfusion therapy at the age of 2 years. Hence, considering mere age at which the transfusion was initiated might not affect the development of alloimmunization.

The relationship between the number of units transfused and alloimmunization is not clearly known in thalassemia. Some studies reported that alloimmunization is more likely in patients who receive more units of blood[9,18] while others found no significant association between alloantibodies formation and the number of transfused packed RBCs.[19] In our study, the association of development of alloimmunization and the number of units transfused were not statistically significant ($P = 0.117$).

Leukocytes present in the allogenic blood causes immunomodulatory effect in the recipient and increase the likelihood of alloimmunization against HLA antigens as well as those against red cell antigens. The soluble mediators released during storage from the leukocytes also contribute toward the immune modulatory effects. Prestorage leukodepletion has shown to downregulate the immune modulation system and has been repeatedly hypothesized to help in decreasing the alloimmunization rates. This hypothesis has shown discordant results in various studies so far.[20] Our center practices buffy coat method for component preparation and use of in line leukocyte depletion filters to achieve a 3–4 log leukoreduced PRC. This might be a reason which could be attributed to low frequencies of alloimmunization in spite of so many antigenic exposures.

In our study, 3 patients had preexisting anti-K formed. Of the remaining 44 patients, 43 were negative for "K" antigen and 18 of them were exposed to "K" stimulus once and 3 of them more than one time. Even though "K" antigen is the most immunogenic antigen second only to "D,"[21] none of the aforesaid patients developed any antibody against this highly immunogenic antigen. This is in accordance to Schonewille's[21] observation that for developing an antibody against "K" multiple transfusions are required. He also observed that the predominant antibody formed after 5 years of transfusion were anti-K and anti-Fy^a. Further follow-up of our patients exposed to Kell antigens could give a clear picture on this.

Antigen "c" is known about its antigenicity which follows "D" and "K" in the order.[5] Twenty-five of our patients lacked antigen "c" and all of them were exposed to the antigenic stimulus one or more number of times in multiple transfusion events. None of these patients developed any alloantibody against antigen "c" suggesting mere antigenicity cannot evoke an immune response, and other underlying recipient factors play a role. Similar is the case with antigens "e" and "C."

One patient who developed an alloantibody during the study period had anti-"E" specificity. He had received stimulus for "E" antigen in 32% of the transfusion episodes. Also, he had received stimulus for "C" antigen in 92% of the transfusion events but did not produce any anti-"C." This can be attributed to antigen "C" being one of the least immunogenic antigen among the Rh antigens.[5]

Conclusion

It was recognized that not all antigenic exposures result in antibody formations and several other factors also contribute to the development of alloimmunization. Molecular genotyping of the patients revealed the extent of homogeneity between the donor and patient population and the accurate frequency of antigenic exposures among chronically transfused patients. It will also go a long way in preventing any further development of alloimmunization in these patients by provision of antigen matched units at least for Rh and Kell antigens. Although the cost constraints for blood group genotyping is high, being one in a life time investigation and having incomparable benefits for patients on chronic transfusion support, it needs to be implemented at least in a few referral centers. Our data provide an impetus for future studies to understand the development of alloimmunization in such patients.

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Conflicts of interest

There are no conflicts of interest.

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