RED CELL IMMUNIZATION IN MULTIPLY TRANSFUSED MALAY THALASSEMIC PATIENTS

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Abstract. The development of red blood cell (RBC) isoimmunization with alloantibodies and autoantibodies complicate transfusion therapy in multiply transfused thalassemia patients. Thus, the frequency, causes and prevention of these phenomena were studied among these patients. Clinical and serological data from 58 Malay multiply transfused thalassemic patients who sought treatment at Hospital University Sains Malaysia were collected and analyzed prospectively. Blood samples were subjected to standard blood bank procedures to screen for antibody and subsequent antibodies identification. All patients in our hospital received blood matched for only ABO and Rh (D) antigens. There were 46 (79.3%) patients with Hb E/β thalassemia, 8 (13.8%) with β thalassemia major, 3 (5.2%) with Hb H Constant Spring and 1 (1.7%) with Hb H disease. Overall, 8.6% of the patients had alloantibodies and 1.7% had autoantibodies. The alloantibodies identified were anti-E, anti-c, anti-K, anti-Jka, anti-N and anti-S. In conclusion, the transfusion of matched blood is essential for chronically multiply transfused patients in order to avoid alloimmunization. Considering the high frequency of anti E at our hospital, it is advisable to genotype patients and match the red cells for E antigens in multiply transfused thalassemia patients.

INTRODUCTION

Thalassemia, a major public health problem in Malaysia, is a heterogeneous group of inherited autosomal recessive disorders of hemoglobin synthesis, which is characterized by the absence or reduced output of one or more globin chains of hemoglobin (George, 1998).

The recommended treatment for thalassemia major involves regular blood transfusions, usually administered every 2 to 5 weeks, to maintain a pretransfusion hemoglobin level above 9-10.5 g/dl. One of the complications of blood transfusion is the formation of alloantibodies and autoantibodies against RBC antigens. Results from a number of studies have demonstrated various frequencies and percentages of alloantibodies and autoantibody formation in multi-transfused patients (Spanos *et al,* 1990; Singer *et al,* 2000; Ameen *et al,* 2003). Some alloantibodies may cause hemolytic transfusion reactions and limits the possibility of safe transfusion, while others are clinically insignificant. Red cell autoantibodies appear less frequently, but can result in hemolysis and difficulty in blood cross-matching (Ho *et al,* 2001).

Antibodies must be identified in the recipient's serum before each transfusion so that compatible blood can be provided. The causes of alloimmunization in thalassemia patients are not fully understood, however data suggests that the recipient's immune sta-

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tus, absence of spleen and difference in the red cell phenotype between donor and recipients are likely to contribute further to the phenomena (Sirchia *et al,* 1985). This paper reports the results of a study carried out in this center to determine the prevalence of RBC alloantibodies and autoantibodies and the factors that might contribute to their development.

MATERIALS AND METHODS

This prospective study was conducted over a 1-year period from January 2004 to December 2004 at Hospital University Sains Malaysia. The study was approved by the hospital ethics committee. Written consent was provided for each patient.

Patients

A total of 58 thalassemia patients receiving multiple blood transfusions at intervals of 2 to 4 weeks, or whom had received at least 10 transfusions, were included in this study. The diagnosis of thalassemia was confirmed by standard hemoglobin electrophoresis and measurement of Hb A, A2 and F.

Clinical transfusion records of 58 thalassemia patients who fulfilled the criteria were analyzed for the presence of allo- and autoimmunization, their antibody specificity and the time interval of RBC immunization from start of transfusion. Ethnic background, status of splenectomy, age at start of transfusion and the number of blood units received were also recorded.

Laboratory investigations

Using standard blood bank methods, serum was analyzed prior to each transfusion to detect new antibodies to RBC antigens. All pretransfusion sera were also tested to determine their phenotype for the following blood group systems: ABO; Rhesus (D, C, E, c, and e); Kell (K, k), Kidd (Kpa, Kpb) and Duffy (Fya, Fyb).

An antigen panel was used for the antibody screening procedure, where the serum was mixed with saline suspended red cells in LISS Coombs gel card incubated at 37ºC for 15 minutes. The antibody identification test was performed by a commercial RBC panel when the antibody-screening test was positive.

A polyspecific direct antiglobulin test was performed using a 0.8% cell suspension of the patient's RBC with anti-human globulin. Elution and absorption methods were employed in patients with suspected autoantibodies. commercial RBC panel was used for the eluates and adsorbed sera to detect any specificity of the autoantibodies and alloantibodies, respectively. The tests were done using the gel card method by Diamed ID (Switzerland).

Statistical analysis

Descriptive statistics and Fischer exact statistical test was performed and a p-value of less than 0.05 was considered significant. The results were analyzed using SPSS statistical software version 11.0.

RESULTS

A total of 58 multiply transfused thalassemia patients were included in this study. Demographic data are shown in Table 1. Twenty-two patients (37.9%) were blood group B, 16 (27.6%) were blood group O, 12 (20.7%) were blood group A, and 8 (13.8%) were blood group AB. All the patients were rhesus positive. Twenty-six patients (44.8%) were genotyped as R1R1, 25 (43.1%) were R1R2, 6 (10.4%) were R1r and one (1.7%) was R2r. Red cell alloantibodies were found in 5 of 58 patients (8.6%) and only one patient (1.7%) developed autoantibodies.

Alloimmunized patients

Details of the patients with alloantibodies are shown in Table 2. Three patients devel-

oped only 1 antibody, which were anti-E and anti-K. One patient developed 2 antibodies, which were anti-E and anti-Jka, and 1 patient developed 4 antibodies, namely anti-E, -c, -S and -N. The time to development of antibodies ranged between after 8 to 100 units of packed red cells transfused.

There was no significant association between alloantibody formation and gender (p=0.16), age at start of transfusion (p=0.58), number of packed red cells transfused (p=1.00) and splenectomy (p=0.31).

Table 1
Demographic data of thalassemia patients who received regular blood transfusions.

Demographic data	Number of patients	%
Total patients	58	
Diagnosis		
β thalassemia major	8	13.8
HbE/β thalassemia	46	79.3
Hb H Constant Spring	3	5.2
Hb H disease	1	1.7
Gender		
Male	34	58
Female	24	42
Splenectomy		
Yes	15	25.8
No	43	74.2

DISCUSSION

To the best of our knowledge this is the first report on the incidence of RBC immunization among multiply transfused thalassemic patients in the Malay population. The frequency of alloimmunization ranged from 5% to 30% in transfusion dependent thalassemia patients (Sirchia *et al,* 1985; Michail-Merianou *et al,* 1987; Spanos *et al,* 1990). However, the incidence of RBC alloimmunization and autoimmunization was low in our study, 8.6% and 1.7%, respectively. This study was consistent with a study by Ho *et al* (2001) in Hong Kong. Alloimmunization rates in two studies done in Greece and Kuwait were 22% and 30%, respectively (Singer *et al,* 2000; Ameen *et al,* 2003). The higher alloimmunization rate in these two studies was probably due to the heterogeneity of the populations living in Greece and Kuwait and mismatched RBC phenotypes between donors and recipients compared to our study population which were more homogenous.

In the present study, anti-E was seen most frequently followed by anti-c (Rhesus system), anti-S, anti-N (MNSs system), anti-

Table 2
Data of patients with alloantibodies.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Diagnosis	Hb E/β thal	β thal major	Hb E/β thal	Hb E/β thal	Hb E/β thal
Sex	Male	Female	Female	Female	Female
ABO Blood Group Systems	A	B	A	O	A
Rhesus Genotype	R1R1	R1R1	R1R1	R1R1	R2r
Splenectomy	No	No	No	No	No
Age at start of transfusion	18 months old	1 year old	5 years old	10 years old	20 years old
Number of packed cell transfused	65 units	114 units	38 units	12 units	30 units
Type of antibody	Anti-E	Anti-E, -c, -S, -N	Anti-E	Anti-E, -Jka	Anti-K

RBC = red blood cell; WBC = white blood cell; Ig = immunoglobulin; Hb = hemoglobin; Rh = rhesus; LISS = low ionic saline solution

Jka (Kidd system) and anti-K (Kell system). All of our patients received compatible blood for ABO and Rh D antigens. In an Italian study, the alloantibody was almost entirely confined to the common antigens of Rhesus, Kell, Kidd and Duffy systems (Sirchia *et al,* 1985). Several studies have shown that anti-E antibodies are the most prevalent alloantibodies among transfusion dependant thalassemia patients (Sirchia *et al,* 1985; Ho *et al,* 2001; Ameen *et al,* 2003).

Autoantibodies were found in an 11 year-old post-splenectomy Malay girl with HB E/β thalassemia. She developed autoantibodies without underlying alloantibodies, as determined by a persistent positive direct Coombs test with no specific pattern on the red cell elution test and panagglutination on the absorption test. The monospecific Direct Coombs test was positive for both IgG and C3d in this patient. No secondary causes were identified in this patient. A study in Kuwait observed that 11% of their patients developed autoantibodies positive for both IgG and C3d or IgG alone (Ameen *et al,* 2003). However, the majority of their RBC autoantibodies were associated with RBC alloantibodies. They reported that the presence of residual donor white blood cells (WBC) could have potentially influenced the rate of alloimmunization and autoimmunization seen among transfusion dependent thalassemic patients in Kuwait. RBC bound IgG was found more abundant in splenectomized than nonsplenectomized thalassemia patients (Chinprasertsuk *et al,* 1997). The antibodies were also found to have specificity for spectrin and band 3 proteins in thalassemia patients.

Our results show that there was no significant association between alloimmunization and gender, however, all the alloimmunized patients were females. Only 1 of our alloimmunized patients was an adult. The alloimmunization in her could have been due to a previous pregnancy or blood transfusion. Clinically significant alloantibodies have been reported to occur about twice as often in women compared to men (Walker *et al,* 1989). Of the 5-alloimmunized patients in our study, 3 were adults and 2 were children. There was no association between alloimmunization and age demonstrated in this study. A few studies have also reported no significant relationship between age and alloimmunization in transfusion dependent thalassemia patients (Fluit *et al,* 1990; Singer *et al,* 2000; Ho *et al,* 2001). Adult recipients, age 16 to 88 years, apparently do not lose their ability to respond to red cell alloantigens as they age (Walker *et al,* 1989).

Our low alloimmunization rate in this study probably can be explained by the similarity in the ethnicity between patients and donors. All of our alloimmunized patients were Malays and most of our blood donors were also Malays, which comprise about 83% of blood donors in our local population. In Hong Kong the majority of immunized patients were southern Chinese, and all the blood donors were predominantly of the same ethnic origin. A lower rate of alloimmunization in their study was explained by their access to phenotypically matched donors in Hong Kong (Ho *et al,* 2001).

In the present study, all 5 immunized patients were started on transfusions after the age of one year old. This finding is consistent with and supported by other studies. A study done in Kuwait found the majority of alloimmunized patients formed first alloantibodies between age 2 and 10 years (58%). They observed that most of the alloimmunized patients involved in their study developed alloantibodies at a younger age (Ameen *et al,* 2003). Few other studies reported a low frequency of alloimmunization found in patients with thalassemia major who started transfusion early. Those results also support the view that there is some form of immune tolerance induced by an immature immune response to repeated blood transfusions (Michail-Merianou

et al, 1987; Spanos *et al,* 1990; Singer *et al,* 2000; Ameen *et al,* 2003). Immune response may also be affected by the patient's age at the start of transfusion and the number of blood units a patient receives. One study observed, despite exposure to many RBC and WBC antigens, infants do not produce alloantibodies against blood cell antigens and immunologically mediated transfusion reactions are quite rare in young infants (Floss *et al,* 1986). However, our results showed there was no statistically significant association between alloimmunization rates and the age at start of transfusion. This was probably due to the small sample size.

In our study, we found the earliest development of antibodies was after 8 units of packed red cells transfused. However there was no significant relation between the number of packed red cells transfused and the alloimmunization rate ($p>0.05$). Spanos *et al* (1990) found the earliest sensitization appeared after 10 units transfused. Blumberg *et al* (1984) concluded that most blood group antibodies seen in multiply transfused patients were due to previous pregnancy and occurred during the initial first ten transfusions. However, there was increasing antibody formation with increasing numbers of transfusions.

In our study, despite a higher rate of patients with splenectomy, none of them had alloantibodies. This is in contrast to Singer *et al* (2000) who observed that patients who had a splenectomy had a higher alloimmunization rate. They found the absence of a spleen may further enhance the immune response to the infused foreign antigens, which are not affectively filtered.

In our study, the majority of patients received packed red cells age 2 to 7 days old, and all of them had long-term exposure to non-leukocyte depleted packed red cells. Frabetti *et al* (1998) noted apoptosis of WBC began to occur by 48 to 72 hours of storage. A study by Blumberg *et al* (2003) supported the hypothesis that WBC reduction may be associated with a reduced frequency of RBC alloimmunization. However, this result is in contrast with a study by Uhlmann *et al* (2001) who observed no significant difference in the transfusion reactions in patients receiving leukocyte depleted and non-leukocyte depleted RBC. However, another study observed that, nuclear matrix protein released from apoptotic white cells during the cold storage may induced an antibody response in multiply transfused patients (Martelli *et al,* 2000).

Our data show the rates of RBC immunization to red cell antigens are low in transfusion dependent Malay thalassemic patients, despite the use of non-leukocyte depleted blood. This probably can be explained by the ethnic homozygosity between the blood donors and thalassemia patients and the use of relatively fresh packed red cells. We found that age at the start of transfusion and splenectomy did not influence the formation of RBC antibodies.

In conclusion, due to a high incidence of anti-E in our study population, it is advisable to genotype patients and matched red cell units for E antigen in addition to ABO and D antigen. Antigen matched transfusions should effectively prevent alloimmunization for thalassemia patients who have a life long, transfusion dependent disease.

ACKNOWLEDGEMENTS

We thank the University Sains Malaysia for the short term grant (PPSP/304/6131313) provided for this project and to the staff of the Transfusion Medicine Unit, Hospital University Sains Malaysia for their support and help.

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