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-
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 37°C 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-
opposed only 1 antibody, which were anti-E and anti-K. One patient developed 2 antibodies, which were anti-E and anti-j ka, 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).

**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 1**

Demographic data of thalassemia patients who received regular blood transfusions.

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

**Table 2**

Data of patients with alloantibodies.

<table>
<thead>
<tr>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis</td>
<td>Hb E/β thal major</td>
<td>Hb E/β thal major</td>
<td>Hb E/β thal major</td>
<td>Hb E/β thal major</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>ABO Blood Group Systems</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhesus Genotype</td>
<td>R1R1</td>
<td>R1R1</td>
<td>R1R1</td>
<td>R1R1</td>
</tr>
<tr>
<td>Splenectomy</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Age at start of transfusion</td>
<td>18 months old</td>
<td>1 year old</td>
<td>5 years old</td>
<td>10 years old</td>
</tr>
<tr>
<td>Number of packed cell transfused</td>
<td>65 units</td>
<td>114 units</td>
<td>38 units</td>
<td>12 units</td>
</tr>
<tr>
<td>Type of antibody</td>
<td>Anti-E</td>
<td>Anti-E, -c, -S, -N</td>
<td>Anti-E</td>
<td>Anti-E, -j ka</td>
</tr>
</tbody>
</table>

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 thalassemia 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.

REFERENCES


Blumberg N, Gettings KF. WBC reduction of RBC transfusions is associated with decreased incidence of RBC alloimmunization. Transfusion 2003; 43: 945-52.

Blumberg N, Goeken N, Knox L. Should chronic transfusion be matched for antigens other than ABO and Rh (D)? Vox Sang 1984; 47: 205-8.


Floss AM, Strauss RG, Goeken, Knox L. Multiple transfusions fail to provoke antibodies against blood cell antigens in human infants. Transfusion 1986; 26: 419-22.


George E. Thalassaemia carrier diagnosis in Malaysia. Thalassaemia diagnosis services (ThalIDS), 1998.


Martelli AM, Tazzari L, Bortol R, et al. Nuclear matrix protein is released from apoptotic white cells during cold (1-6 degrees C) storage of concentrated red cell units and might induce antibody response in multiply transfused patients. Transfusion 2000; 40; 169-77.


Uhlmann EJ, Wallhermfetchel M, Goodnough LT. Prestorage universal WBC reduction of RBC units does not effect the incidence of transfusion reactions. Transfusion 2001; 41: 997-1000.