Red blood cell alloimmunisation among Chinese patients with β-thalassaemia major in Taiwan

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Background. The development of red blood cell (RBC) antibodies can significantly complicate transfusion therapy in transfusion-dependent patients with thalassaemia. However, few data are available on the frequency of RBC alloimmunisation in the Chinese population with β-thalassaemia major.

Materials and methods. In this retrospective study, we investigated the development of RBC antibodies among Chinese patients with β-thalassaemia major who had received long-term transfusion therapy with leucodepleted blood in our hospital over a period of 20 years.

Results. Of the 64 patients studied, six (9.4%) developed RBC alloantibodies, including four anti-E, one anti-C and one anti-"Mi^a". All of the six alloimmunised patients had experienced previous transfusion reactions, while only 12 of the 58 non-immunised patients had had previous transfusion reactions (100% *vs* 15.5%; $p < 0.001$). After subsequent transfusions with RBC which were negative for the antigens for the corresponding alloantibodies, all the RBC alloantibodies became undetectable within 1 year without additional interventions to eliminate them.

Conclusions. RBC alloantibodies in Chinese patients with β-thalassaemia major in Taiwan were different from those in other populations. The development of RBC alloantibodies was associated with previous transfusion reactions. Additional treatment may not be necessary for patients with alloantibodies.

Keywords: alloimmunisation, Chinese, leucodepletion, thalassaemia, transfusion.

Introduction

Thalassaemia is the most common inherited haemoglobinopathy in the world. It is also prevalent among Chinese population, especially in Southeast Asia, the southern area of China and Taiwan. Given the risk of haematopoietic stem cell transplantation and the improvements in chelation therapy for preventing iron overload-related complications[1-5], the choice of treatment for patients with β-thalassaemia major is a regular programme of life-long transfusions to maintain growth and development and to improve quality of life.

Transfusion therapy in transfusion-dependent thalassemia patients can, however, be significantly complicated by the development of red blood cell (RBC) antibodies. These alloantibodies, usually against minor blood group systems, may lead to delayed haemolytic transfusion reactions[6]. Multiple alloimmunisation can make the identification of compatible RBC difficult, limiting the availability of further safe transfusions. The expression of blood group antigens is variable among different populations and consequently different RBC antibodies are identified in patients undergoing long-term transfusion therapy.

Limited data are available on the frequency of RBC alloimmunisation in long-term transfused Chinese patients. In this study, we retrospectively investigated the development of RBC antibodies in Chinese patients with transfusion-dependent β-thalassaemia major in Taiwan over a period of 20 years.

Materials and methods

We retrospectively reviewed the clinical and transfusion records of patients with β-thalassaemia major who received regular transfusion therapy in our hospital from 1990 to 2010. All patients were transfused regularly with ABO- and RhD-compatible leucodepleted blood to maintain a pre-transfusion haemoglobin level greater than 10 g/dL. In Taiwan, the expression of Kell group antigens is uniform: all subjects express k antigen and none express K antigen[7]. It is, therefore, reasonable to assume that all of our patients also received RBC phenotypically matched for the Kell group. Once a patient developed an identified RBC alloantibody, subsequent transfusion were performed with RBC lacking the antigen for the corresponding alloantibody. No additional treatment (such as immunosuppressants) was used to eliminate the antibodies in patients with RBC alloantibodies. Chelation therapy with desferrioxamine and/or deferiprone was used to treat iron overload-related complications[5,8].

In addition to ABO grouping and Rh (D) typing, regular pre-transfusion testing consisted of antibody screening and major cross-matching using the manual Polybrene method. Antibody screening cells were supplied by the Taiwan Blood Services Foundation. If the screen was positive, further investigations to identify the antibody were performed using commercial panels of RBC, tested against the patient's serum using the following procedure. One part of RBC, two parts of serum and two parts of low ionic-strength saline were incubated at 37 °C for 15 minutes. The cells were then washed in normal saline, and polyspecific antihuman globulin serum was added before centrifuging and final reading.

Data were analysed using the chi-square test and t-test with SPSS 14.0. P values less than 0.05 were considered statistically significant.

Results

Sixty-four Chinese patients (33 females and 31 males; age 19.2±6.7 years) with β-thalassaemia major were studied. All patients began transfusion therapy in their first year of life. Of the 64 patients, six (9.4%) developed RBC alloantibodies 5-28 years after the initiation of transfusion therapy, including four anti-E, one anti-C and one anti-"Mi^a". After subsequent transfusions with leucodepleted RBC which were antigen-negative for the corresponding alloantibody, the RBC alloantibodies became undetectable within 1 year (range, 1.1 to 10.3 months). No additional treatment to eliminate the antibodies was used. The characteristics of the six Chinese patients who developed RBC alloantibodies are summarised in Table I.

By analysing factors associated with the development of RBC alloantibodies, we found that there was a statistically significant difference between the prevalence of previous transfusion reactions in the alloimmunised and non-immunised patients (100% *vs* 15.5%, respectively; $p < 0.001$). At least once, all alloimmunised patients had experienced previous transfusion reactions, including fever, skin rash and haemolysis. Only 12 of the 58 non-immunised patients had had transfusion reactions. Splenectomy, age of starting blood transfusions and duration of transfusion therapy were not significantly correlated with the development of RBC alloantibodies (Table II).

Discussion

There are significant variations in the expression of blood group antigens among different populations and different RBC alloantibodies may, therefore, develop after allogeneic blood transfusion. Studies have demonstrated the most commonly encountered alloantibodies in the world are those directed against antigens in the Rh and Kell systems[6,9-13]. In the present study, we identified six RBC alloantibodies in six Chinese patients with β-thalassaemia major. Four patients had anti-E and one had anti-C. One patient developed anti-"Mi^a", which refers to antibodies reacting with antibody-screening cells of the MiIII phenotype in Taiwan[14]. The frequencies of E and C in the Taiwanese population are about 45% and 89%, respectively[7]. Our findings correlated well with previous reports that anti-"Mi^a" and anti-E are the most common alloantibodies in Taiwan[7,15-17]. In our study, no alloantibodies were identified against antigens in the Kell system, which are common in other populations. This can be explained by the

Table I - Characteristics of the six Chinese patients with β-thalassaemia major who developed RBC alloantibodies.

Patient N.	1	2	3	4	5	6
Gender	Male	Male	Female	Female	Male	Female
RBC alloantibody	Anti-E	Anti-C	Anti-E	Anti-E	Anti-E	Anti-"Mi^a"
Age at initiation of transfusion therapy (months)	7.3	3.5	7.2	3.6	4.1	8.5
Age at detection of RBC alloantibody (years)	5.1	11.3	17.1	18.7	20.1	28.9
Amount of blood received prior to detection of the RBC alloantibody (units)*	220	392	352	250	448	464
Duration of RBC alloantibody persistence (months)	1.1	1.5	10.3	3.1	4.7	3.5
Splenectomy	No	Yes	No	No	No	No
Number of transfusion reaction episodes prior to detection of the RBC alloantibody	1	1	1	2	1	1
Manifestation of transfusion reaction	Fever, skin rash	Skin rash	Fever, skin rash	Skin rash	Skin rash	Haemolysis
Management of transfusion reaction	Diphenhydramine and acetaminophen	Diphenhydramine	Diphenhydramine and acetaminophen	Diphenhydramine	Diphenhydramine	Supportive treatment
Time from 1st transfusion reaction episode to detection of antibody (months)	28	33	17	50	20	25

Legend
*One unit of packed RBC is derived from 250 mL of whole blood in Taiwan.

Table II - Comparison of alloimmunised and non-immunised Chinese patients with β-thalassaemia major.

	Alloimmunised patients (n=6)	Non-immunised patients (n=58)	p value
Males	3/6 (50.0 %)	28/58 (48.3 %)	0.936
Age (years)	18.2±5.9	20.2±7.1	0.290
Age at initiation of transfusion therapy (months)	5.7±2.2	6.9±4.1	0.796
Duration of transfusion therapy (years)	17.8±4.3	18.2±4.8	0.852
Previous transfusion reactions	6/6 (100%)	9/58 (15.5%)	<0.001
Splenectomy	1/6 (16.7%)	12/58 (20.7%)	0.816

Values are presented as mean ± SD.

uniform expression of K system antigens in the Taiwanese population[7].

Variable rates of RBC alloimmunisation in transfusion-dependent thalassemia patients have been reported, ranging from 2.87 to 37%[6,9,10,12,13,18]. Several factors can contribute to the occurrence of alloimmunisation: the recipient's immune status along with the well-known immunomodulatory effects of blood transfusion and, obviously, the differences in the distribution of RBC antigens between donor and recipient[10-12,19,20]. Wang *et al.*[18] reported a high incidence of alloimmunisatio of 37% among thalassaemia patients in Taiwan. The much lower rate of 9.4% in the present study may be attributed to the consistent use of leucodepleted blood. Removal of leucocytes can reduce the degree of recipient immune activation due to allogeneic transfusion; this probably occurs through removal of donor's antigen-presenting cells and consequent lack of recipient's T-cell activation[11].

Factors associated with the development of RBC alloantibodies in transfusion-dependent thalassaemia patients have not been completely understood. In the present study, we found a strong relation between alloantibody formation and previous transfusion reactions. All of the six alloimmunised patients had experienced previous transfusion reactions,

but only 12 of the 58 non-immunised patients had done so (100% *vs* 15.5%, respectively; p <0.001). We speculated that previous transfusion reactions might be an important factor in the stimulation of the development of RBC alloantibodies.

Once an alloantibody has been identified, patients should receive RBC that do not carry the antigen for the corresponding alloantibody in subsequent transfusions in order to avoid haemolysis. Our patients who developed alloantibodies were thereafter transfused with compatible RBC and had no increase in transfusion requirements. To our knowledge, it has not been reported for how long RBC alloantibodies persist. It also remains to be discussed whether it is necessary to eliminate the antibodies by using additional therapeutic modalities, such as immunosuppressants. In our study, no additional treatment was adopted for patients with RBC alloantibodies. All the alloantibodies became undetectable within one year, and no additional antibodies were identified. Additional interventions to eliminate antibodies may, therefore, be unnecessary.

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