Red cell autoantibodies among thalassaemia patients in Hospital Universiti Sains Malaysia

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ABSTRACT

Introduction: Thalassaemia is one of the major public health problems in Malaysia. Regular monthly blood transfusion remains the main treatment for severe thalassaemia patients. One of the complications of blood transfusion is the formation by the recipients of alloantibodies and autoantibodies against red blood cell (RBC) antigen. The purpose of this study was to determine the prevalence of RBC autoantibodies among multiple-transfused thalassaemic patients in our institution and factors that contribute to its development.

Methods: A prospective study was conducted in Haematology Laboratory, Hospital Universiti Sains Malaysia between January 2004 and December 2004. A total of 63 thalassaemia patients, who received regular blood transfusion were included in this study. Clinical and serological data were collected and analysed prospectively. Blood samples were subjected to standard blood bank procedures for screening of antibodies and their subsequent identification using reagent of Diamed-ID Gel microtyping system.

Results: There were 49 (77.8 percent) patients with Hb E/beta-thalassaemia, ten (15.9 percent) beta-thalassaemia major, three (4.7 percent) Hb H Constant Spring and one (1.6 percent) Hb H disease. Only one (1.6 percent) patient had autoantibodies. There were no statistical associations found between the formation of autoantibodies with age at the start of transfusion, number of packed cell transfused and splenectomy.

Conclusion: Our data showed a low autoimmunisation rate in multiple-transfused thalassaemia patients in our hospital.

Keywords: autoimmunisation, autoantibody, blood transfusion complication, red cell autoantibody, thalassaemia

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INTRODUCTION

Thalassaemia is a common condition, particularly in the Mediterranean region and Southeast Asia.[1] The term 'thalassaemia' is used to describe a disorder with a significant decrease in the rate of synthesis of one or more globin chains.[2] The globin chain that is produced in excess is responsible for the ineffective erythropoiesis and shortened red blood cell (RBC) survival.[3] The present management consists of regular monthly blood transfusions to maintain the mean haemoglobin level of 10–11 g/dL, and this remains the main treatment for severe thalassaemia.[4] One of the complications of blood transfusion is the production of antibodies against RBCs, by the recipients. These risks apply mainly to those patients who have received multiple transfusions, including those suffering from thalassaemia. Such antibodies must be systematically identified in the recipient's serum before every transfusion, so that compatible blood can be provided. Otherwise, problems which sometimes could even threaten the patient's life, may occur.[1,5]

Formation of autoantibodies against RBCs has been documented in previous studies.[5-7] Autoantibodies are directed against the individual's own RBCs which can result in clinical haemolysis and difficulty in cross-matching blood. Most autoantibodies react with high incidence antigens; they agglutinate and sensitise the RBCs of random donors as well as those of antibody producers. This circulating humoral antibody may shorten the duration of RBC survival.[8] Patients with autoantibodies may have a higher transfusion rate and often require immunosuppressive drugs, splenectomy or alternative treatments. The present study was designed to explore the prevalence of autoantibodies in multiple-transfused thalassaemic patients in our institution and factors that contribute to its development. To the best of our

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knowledge, this is the first report on the prevalence of RBC autoantibodies among multiple-transfused thalassaemic patients in Malaysia, specifically in the Kelantan population.

METHODS

This prospective study was conducted over a one-year period from January 2004 to December 2004 at Hospital Universiti Sains Malaysia. The study had been approved by the hospital's ethical committee. Written consent from patients was also obtained. A total of 63 thalassaemia patients receiving multiple blood transfusions or had received at least ten transfusions were included in this study. Diagnosis of thalassaemia was confirmed by standard haemoglobin electrophoresis and measurement of Hb A, A2 and F. The clinical data was reviewed retrospectively for autoimmune screening (antinuclear antibody [ANA]/double-stranded deoxyribonucleic acid [dsDNA]). The exclusion criteria specified patients with connective tissue or autoimmune diseases. Clinical transfusion records of patients who fulfilled the criteria were analysed for the presence of autoantibodies, their antibody specificity and the time interval of RBC immunisation from start of transfusion. Ethnic background, status of splenectomy, age at start of transfusion, and the units of blood received were also recorded.

Using standard blood bank methods, serum was analysed prior to each transfusion for detection of new antibody to RBC antigen. All the pre-transfusion sera were also tested to determine their phenotype and genotype for ABO and rhesus (D, C, E, c, and e). An antigen panel was used for the antibody screening procedure where the serum was mixed with saline suspended RBCs in low ionic saline solution 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.

Polyspecific direct antiglobulin test was performed using 0.8% cell suspension of patient's RBC with anti-human globulin and it was done on all patients. The patient's own RBCs were included as a control. Elution and absorption methods were employed in patients with suspected autoantibody. A commercial RBC panel was used for the eluates and adsorbed sera to detect the specificity of the autoantibody. The tests were done by using reagent of Diamed-ID Gel microtyping system (Diamed AG, 1785 Cressier, S/Morat, Switzerland). Descriptive statistics, Mann-Whitney test and Fisher's exact test were performed and a p-value of less than 0.05 was considered significant. The results were analysed using the Statistical Package for Social Sciences version 11.5 (SPSS Inc, Chicago, IL, USA).

RESULTS

A total of 63 multiple-transfused thalassaemia patients were included in this study. Demographical data is shown in Table I. Majority of the patients (52.3%) in this study, whose ages ranged from three to 46 years, were more than 12 years of age. Most of them started their first transfusion at a paediatric age, which ranged from seven months to 20 years. The number of packed cells that had been transfused was between ten units to 116 units, with majority of patients receiving between 21 and 40 units of packed cells. The majority of patients were initially transfused every 3–4 months. Later, the frequency of transfusions increased to once every two months and finally monthly or once every three weeks. 33 of our patients were on three- to eight-weekly transfusions and the remaining were on 9–20 weekly transfusions.

Of 63 patients, six were positive for antibody screening. After completing the to antibody identification, only one patient had autoantibodies (Table II). She developed autoantibodies without underlying alloantibodies, as determined by persistent positive direct Coombs test. All the three screening cell panels and 11 cell panels for antibody identification showed 4+ reactions. There were no specific patterns in the RBC elution test and panagglutination in the absorption test. No secondary causes were identified in this patient. She has no history suggestive of any autoimmune disease and the results for ANA/dsDNA were negative. There was no significant statistical association between the formation of autoantibody with age at the start of transfusion ($p = 0.84$) and number of packed cells transfused ($p = 0.31$), when analysed using the Mann-Whitney test. Fisher's exact test also showed no significant statistical association between autoantibody formation and splenectomy ($p = 0.302$).

Table I. Demographical data of patients.

Demographical data	No. of patients (n = 63) (%)
Diagnosis	
ß thalassaemia major	10 (15.9)
HbE/ß thalassaemia	49 (77.8)
Hb H constant spring	3 (4.7)
Hb H disease	1 (1.6)
Ethnic group	
Malays	58 (92.1)
Chinese	5 (7.9)
Gender	
Male	34 (54)
Female	29 (46)
Splenectomy	
Yes	20 (31.7)
No	43 (68.3)

Table II. Data of one patient with autoantibodies.

Diagnosis	Hb E/β thalassaemia
Race	Malay
Gender	Female
Age (years)	11
ABO blood group system	AB positive
Rhesus genotype	R1R1
Splenectomy	Yes
Age at start of transfusion (years)	2
Number of packed cells transfused (units)	72
Frequency of packed cells transfused	Monthly
Rbc alloantibody	No
Type of antibody	No specificity
Time at development of antibody units	60
Direct Coombs test	
IgG	positive
C3d	positive

DISCUSSION

This prospective study of autoimmunisation to RBC antigens in multiple-transfused thalassaemia patients was the first carried out in the Kelantan population. In this study, we found that our data was similar with the study done in Hong Kong where only one patient with transfusion-dependent thalassaemia and who developed RBC autoantibodies, was found.[7] However, a study in Greece found that 16 of their transfusion-dependent thalassaemia patients developed autoantibodies which were associated with the presence of alloantibodies.[5] A study in Kuwait observed that 11% of their patients developed autoantibodies with underlying alloantibodies.[6] Our patient who had developed autoantibodies was diagnosed to have Hb E/β thalassaemia and had undergone splenectomy. She was positive for both immunoglobulin (Ig)G and C3d; however, no underlying alloantibody was detected. Our results were supported by Wiener et al, who revealed that significant elevations in RBC-bound IgG were seen in severe Hb E/β thalassaemia patients and it was more abundant in splenectomised than non-splenectomised subjects. This IgG showed specificity for spectrin in some β thalassaemia patients and for band 3 protein in several patients with α and β thalassaemia.[3] A study done in Thailand also found that Hb E/β thalassaemia patients had significant increases in RBC-bound IgG, and patients with Hb H disease showed lower proportions of IgG positive RBC. It was suggested that this was due to different membrane pathologies in α and β thalassaemia syndromes. In Hb H , IgG is likely to bind to band 3.[9]

The pathogenesis of erythrocyte autoantibody formation following transfusions is not well-understood. However, clinical evidence of autoimmune haemolytic anaemia has been seen with high amounts of RBC-associated IgG.[9] It was also suggested that alloantibodies binding to the RBCs could lead to conformational changes of the antigenic epitope that ultimately stimulates production of autoantibodies. There was no association between formation of autoantibody with age at the start of transfusion, number of packed cells transfused and splenectomy in our patient. It is possible that certain people are genetic "responders" who have an increased tendency to develop RBC autoantibodies and the tendency toward autoantibody formation could reflect an overall dysfunction of the immune system.[10] Hyperhaemolysis, due to acquired RBC autoantibodies was found to be an important complication. Patients who develop this complication should be tested for the presence of underlying alloantibodies and considered for immunosuppressive treatment.[11] Laboratory evidence of drug-induced lupus-like reaction was reported in patients who received the oral iron chelator, deferiprone (L1), where joint pain with ANA positivity was found in those patients.[12]

The role of leuco-depletion in preventing alloimmunisation and autoimmunisation was mentioned in a number of studies. It has been shown that storage of RBC at 1–6ºC would induce apoptosis in white blood cells (WBC).[13,14] The high percentage of apoptotic features of residual WBC and loss of viability were shown at day three after storage. This might lead to the release of potentially immunostimulatory antigens and soluble biological mediators from the dying cells, such as nuclear matrix protein and CTLA-4 epitopes. These released components would sensitise the immune system of the recipients and lead to the development of an autoimmune disease.[15] It has been reported that the presence of residual donor WBCs has potential influence on the rate of alloimmunisation and autoimmunisation seen among transfusion-dependent thalassaemic patients in Kuwait.[6]

All patients in this study had long-term exposure to nonleuco-depleted blood, and the majority of them received post-storage packed cells that were 2–7 days old. Therefore, the potential donor WBCs had influence on the rate of autoimmunisation in our multiple-transfused thalassaemia patients. Singer et al observed that senescent erythrocytes would have conformational changes (fragmentation, membrane deformation) and might expose new antigens and promote or enhance immune reaction. It was likely that the absence of the spleen, an efficient filtering system for the removal of damaged erythrocytes, enhanced the process of haemolysis.[5]

Age at the start of transfusion and number of packed cells transfused, play a role in the alloantibody and autoantibody formation. Our patients started transfusion at a mean age of two years and had received a mean of 72 units of packed cells. Previous studies revealed that a lower frequency of autoantibody formation was associated with the early onset of transfusion.[16,17] Singer et al observed that the immune response might be affected by the number of blood units received by patients. However, the relation between the number of blood units transfused and antibody formation is still unknown in thalassaemia.[5] Allogeneic blood transfusions have immunological effects on animal and human recipients. These include increased numbers of suppressor T cells, decreased natural killer-cell functions, decreased function of macrophages and monocytes, and induction of anti-idiotypic antibodies that suppress allogenic antigen recognition.[18] Thalassaemia patients exposed to multiple antigens through repeated blood transfusions showed lower potential autoantibodies expression than did those who had received fewer transfused units.[19]

In conclusion, we postulate that regular blood transfusion and underlying splenectomy were the possible causes of autoantibody formation in this patient. The prevalence of autoantibodies in multiple-transfused thalassaemic patients in our population is low, as compared to other developed countries. A limited number of multiple-transfused thalassaemic patients in the Kelantan population is one of the limitations in this study. In future studies, it is recommended that larger sample sizes should be obtained from other states in Malaysia to get better results, which could better represent thalassaemia patients in the Malaysian population.

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