Original Article

Red Cell Immunization in Multiply Transfused Thalassaemic Paediatric Patients- A Preliminary Study

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ABSTRACT

Background

Multiply transfused thalassaemia patients can develop red cell immunization that can potentially cause haemolytic transfusion reactions and pose problems with compatibility testing for future transfusions. Previous research on red cell immunization in transfusion-dependent thalassaemia patients has not focused on paediatric groups.

Objective

This study aims to evaluate the prevalence of red cell immunization in transfusion dependent paediatric thalassaemia patients and identify its potential risk factors.

Methods

Clinical and serological data of transfusion-dependent thalassaemia patients aged 18 years and below were collected retrospectively from the Hospital Laboratory Information System. Results of antibody identification of the alloantibody and/or autoantibody were collected and analysed.

Results

Of the 32 patients included in our study, 17(53.1%), (11)34.4%, (2)6.3%, (2)6.3%, patients had HbE/β thalassaemia, β-thalassaemia major, alpha thalassaemia, thalassaemia intermedia/homozygous delta beta thalassaemia respectively. Out of 32 patients, five have developed antibodies against RBC antigens, giving rise to the prevalence of red cell immunization at 15.6%, in which 12.5% were alloantibodies and 3.1% were autoantibodies. The alloantibodies detected were anti E, anti-e, anti-c and alloantibody against low-frequency antigen with an unknown specificity. The autoantibodies were nonspecific autoIgG. No significant association was observed between antibody formation and risk factors such as diagnosis, gender, blood group, age at first transfusion and number of packed cells transfused.

Conclusion

This study has successfully answered the prevalence of red cell immunization in multiply transfused paediatric thalassaemia. Our data showed prevalence of red cell immunization at 15.6%, in which 12.5% were alloantibodies and 3.1% were autoantibodies. We acknowledged that a bigger sample size with an extended study period is required.

Keywords

Red cell immunization; Alloantibodies; Autoantibodies; Thalassaemia; Multiple transfusions

INTRODUCTION

Thalassaemia is a congenital autosomal recessive disease with a significant incidence in the Mediterranean, Middle Eastern countries and Southeast Asia[1]. This disorder is characterized by a significant reduction or restricted synthesis of one or more globin chains of haemoglobin tetramers, which is responsible for ineffective erythropoiesis and shortened red blood cell (RBC) survival[2]. There are two main types of thalassaemia: alpha and beta, depending on the globin chain involved. In alpha thalassaemia, there is abnormal or absent production of alpha-globin chain that gives rise to four different types such as silent carrier with a single affected alpha-globin gene, alpha-thalassaemia trait with two affected alpha-globin genes, haemoglobin

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H (Hb H) disease involving three affected alpha-globin genes, and alpha-thalassaemia major or Hb Bart hydrops foetalis syndrome where typically there is deletion of all four alpha-globin genes[3]. In beta-thalassaemia, there is an absent or reduced production of the beta-globin chain that constitutes the adult haemoglobin. The consequence is the abnormal accumulation of alpha globin chain and defective erythropoiesis with red cell haemolysis[4]. Clinical manifestations of thalassaemia vary from asymptomatic carriers to severe anaemia requiring lifelong transfusion. Severely affected patients show extramedullary haematopoiesis with the manifestation of growth delay, skeletal deformity and complications such as heart failure, hepatosplenomegaly and iron overload[5].

World Health Organization (WHO) reported in 2008 that worldwide, around 40,000 children are born per year with β-thalassaemia, where around 25,500 are transfusion dependent[6,7]. The prevalence of thalassaemia in children is highest in East Asia, followed by Southeast Asia and South Asia[8]. In Malaysia, approximately 6.8% of people are thalassaemia carriers, resulting in varying degrees of anaemia[9]. The Malaysian Thalassaemia Registry from 2007 to 2018 showed that out of 7984 patients, the highest number of cases were reported in Sabah, accounting for 22.72%. It showed that the most affected groups are 5.0 to 24.9-year age group (64.45%), with Malay (63.95%) ethnicity and having a diagnosis of haemoglobin E (HbE)/β-thalassaemia (34.37%), followed by β-thalassaemia major (33.52%), haemoglobin H (HbH) disease (18.26%), β-thalassaemia intermedia (9.37%) and 'others' (4.48%) which includes other forms of HbH disease, Hb Lepore Hollandia, α-thalassaemia syndrome, δβ-thalassaemia and other thalassaemia disorders. The patients requiring regular blood transfusions were 56.73%, those receiving chelation therapy were 61.72%, and only 14.23% underwent splenectomy[9].

Current treatment consists of monthly blood transfusions to maintain mean haemoglobin levels of 10-11 g/dl, which remains the primary treatment for severe thalassaemia[10]. Regular red blood cell transfusions are essential for children, usually after one year of age, manifested with severe anaemia[11], when clinical complications arise from ineffective erythropoiesis, which can manifest as significant changes in facial bone structure including bossing of the skull, prominent malar eminence, stunted growth, hepatosplenomegaly, and symptomatic extramedullary haematopoiesis[12]. Transfusion therapy in thalassaemia alleviates anaemia symptoms and combats the underlying ineffective erythropoiesis, helping the child grow[12].

Although blood transfusion is the mainstay treatment for thalassaemia patients, some complications might arise from regular transfusion[13]. Iron overload resulting from lifelong blood transfusions in transfusion-dependent patients is prevented with iron chelation therapy[14]. The most common complication is alloimmunization, an immune response from exposure to foreign antigens. Red cell immunization is the development of red cell antibodies in an individual exposed to foreign blood cell antigens through transfusion or pregnancy[15]. Transfusion therapy, in general, can also lead to other complications of transfusion-transmitted infections, including hepatitis B, hepatitis C, and Human Immunodeficiency Virus (HIV) infection, as well as bacterial infections. Recipients are also at risk of adverse haemolytic reactions and non-haemolytic reactions of allergic or anaphylactic reactions or fever[16]. RBC alloimmunization in post-transfusion patients develops due to the disparity of antigens between donor and recipient, the recipient's immune status and immunomodulation following transfusions on the recipient's immune system[17]. The RBC alloimmunization can lead to clinical haemolytic reactions and cause difficulty in obtaining crossmatched blood[18].

While several studies have investigated alloimmunization among thalassaemia patients in Malaysia, none have explicitly focused on paediatric patients. Moreover, no study in our hospital has focused on paediatric groups of transfusion-dependent thalassaemia patients. Therefore, this study aims to determine the prevalence of red cell immunization in transfusion-dependent paediatric thalassaemia patients in our centre. We hope this study will highlight the types of alloantibodies and autoantibodies present and examine the association between potential risk factors, such as gender, diagnosis, blood group, age of onset of transfusion, number of transfusions, with the development of antibodies in these patients. This study can help identify proactive measures to prevent serious complications, such as haemolytic transfusion reactions.

MATERIALS AND METHODS

This retrospective cross-sectional study was conducted from October 2022 to September 2023. Approval for this study was obtained from the Research Ethics

Committee at Universiti Kebangsaan Malaysia (UKM) (Research number: FF-2023-209), ensuring that all research protocols met the highest ethical standards. The study population consisted of 32 paediatric thalassaemic patients who received treatment in Hospital Canselor Tuanku Muhriz (HCTM), Universiti Kebangsaan Malaysia and Hospital Pakar Kanak-Kanak (HPKK) during the study period. All the thalassaemic patients were aged 18 and below and were transfusion-dependent. Paediatric thalassaemic patients who were not transfusion-dependent were excluded from the study. Furthermore, patients with underlying conditions such as coagulopathy and autoimmune diseases and who were on immunomodulatory drugs were also excluded. Clinical and serological data of these patients were obtained from the hospitals' Thalassaemia registry and the Hospital Laboratory Information System (LIS).

Data was collected on the age of the patients, gender, ethnic background, diagnosis, age at the start of transfusion, the number of blood units received, blood group, and status of splenectomy. The antibody screening and antibody identification were performed during routine pretransfusion investigation for all patients together with the ABO and Rhesus blood grouping at the blood bank of the hospital and recorded in the hospital LIS. The clinical transfusion records of these patients were obtained together with the results of the pretransfusion investigation from the thalassaemia registry and the hospitals LIS. If any alloimmunization or autoimmunization was detected, the details of the alloantibody and autoantibody were also recorded.

The data was collected and analysed using the SPSS version 29. The descriptive results were expressed in numbers and percentages. The statistical analysis was performed to determine the association of risk factors (diagnosis, gender, blood group, age of first transfusion, number of packed cells transfused) with antibody formation using the Chi-square test. A p-value of less than 0.05 was considered to be statistically significant.

RESULTS

The data of 32 paediatric thalassaemia patients were analysed. Table 1 showed the demographical data where 19 (59.4%) participants were males, 13 (40.6%) were females, 24 (75%) were Malay, and 8 (25%) were Chinese, with no Indian participants. The age of the patients ranges from 2.5 months to 18 years. Of the 32 patients included in our study, 17(53.1%), (11)34.4%, (2)6.3%, (2)6.3%, patients had HbE/β thalassaemia, β-thalassaemia major, alpha thalassaemia, thalassaemia intermedia/homozygous delta beta thalassaemia respectively. All 32 patients had no history of splenectomy.

Table 1: Demographic data of transfusion dependent thalassaemic paediatric patients (n=32).

Demographic data		Number	Percent
Age range		2.5 months to 18 years	
Gender	Male	19	59.4
	Female	13	40.6
Ethnicity	Malay	24	75
	Chinese	8	25
Diagnosis	HbE/β thalassaemia β-thalassaemia major Thalassaemia intermedia /Delta beta thalassaemia	17 11 2	53.1 34.4 6.3
	Alpha thalassaemia	2	6.3
Splenectomy	Yes	0	0
	No	32	100

Table 2 showed the prevalence of red cell immunization Out of 32 patients, five have developed antibodies against RBC antigens, giving rise to the prevalence of red cell immunization at 15.6%, in which 12.5% were alloantibodies and 3.1% were autoantibodies.

Table 2: Prevalence of red cell immunization (n=32)

	Thalassaemic patients	Prevalence (%)
Total antibody positive	5	15.6
Alloantibody	4	12.5
Autoantibody	1	3.1

Table-3 showed patients with red cell antibodies. It showed all 5 (100%) patients who developed red cells antibody have beta thalassaemia. Here, 4 out of 5 (80%) were females, and only one (20%) was male. According to the blood group, out of 5 patients, 2 (40%) were of blood group O positive, 2 patients (40%) were blood group B positive and 1(20%) group A positive. The

age at first transfusion ranges from 2.5 months to 2 years, and the total packed red cell transfused ranges from 92 to 336 units. The association of factors such as diagnosis, gender, blood group, age of first transfusion, and number of packed red cells transfused with RBC alloimmunization were also investigated. However, there was no significant association detected between red cell immunization and diagnosis (p=1.00), gender (p=0.13), blood group (p=0.36), age of first transfusion (p=0.18), and number of packed red cell transfused (p=0.22).

The antibodies identified in these patients include both alloantibodies and autoantibodies. Overall, three patients (9.4%) developed alloantibody, one patient (3.1%) developed autoantibody and one patient (3.1%) developed both alloantibody and autoantibody. Four types of alloantibodies were detected: Anti-E, anti-e, anti-c and antibody against low-frequency antigen with unknown specificity, and the autoantibody detected was nonspecific auto-IgG antibody. Anti-E (2 of 5 patients, 40%) was the most common antibody detected (Figure1).

Table 3: Data of patients with red cell antibodies (Total five patients)

Variables	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Diagnosis	Beta thal major	Beta thal major	Beta thal major	Beta/Delta thal major	Thalassemia intermedia
Gender	Male	Female	Female	Female	Female
Blood group	O+ve	A+ve	B+ve	O+ve	B+ve
Splenectomy	No	No	No	No	No
Age at first transfusion	10 months	4 months	2.5 months	1 years old	2 years old
Number of packed cell transfused	199	304	133	336	92
Type of antibody	Allo and Autoantibody (anti-E, anti-c and non-specific auto-IgG)	Alloantibody (anti-E)	Alloantibody (anti-e)	Autoantibody (non-specific auto-IgG antibody)	Alloantibody (antibody against low frequency antigen with unknown specificity)

The p value of red cell immunization with risk factors: with diagnosis (p=1.00), gender (p=0.13), blood group (p=0.36), age of first transfusion (p=0.18), and number of packed red cell transfused (p=0.22).

DISCUSSION

This present study determines the prevalence of red cell immunization among paediatric patients and its association with risk factors of diagnosis, gender, blood group, age of onset of transfusion, and number of transfusions. Our study showed the prevalence rate as 15.6%, with 12.5% alloantibodies and 3.1% autoantibodies. There was no association with risk factors such as diagnosis, gender, blood group, age of onset of transfusion and number of transfusions.

The prevalence of red cell immunization in thalassaemia patients varies with the demography of the population. Few previous studies in Malaysia reported the prevalence rate as 36.4% in patients in Terengganu[19], 8.6% and 1.7% as alloantibody and autoantibody respectively in Malay patients in Kelantan[10] and 1.13% and 0.56% as alloantibody and autoantibody respectively among patients in Kelantan[20]. In Saudi Arabia, the prevalence rate of alloimmunization among thalassaemia patients was 11.97%[21]; in Iran,16.4%[22]; in Iraq, 5.8%[23]; in Pakistan, 22.7%[24] and in Egypt 22.8%[25]. Therefore, the

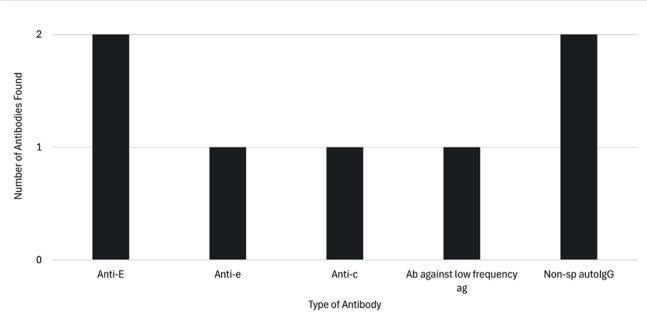

Figure 1: Number of different types of antibodies found among transfusion dependent paediatric thalassaemic patients in HCTM/HPKK. Here, number of anti-E detected was two, anti-e was one, anti-c one, antibody against low frequency antigen one and non-specific auto IgG one.

prevalence rate is variable in Malaysia and in many other countries. The heterogeneity among the different populations, study methods, and periods could explain the difference in RBC alloimmunization.

Our study showed that one patient had developed multiple alloantibodies of anti-E and anti-c along with an autoantibody. In contrast, other patients had developed a single antibody which are anti-E, anti-c, an antibody against a low-frequency antigen and a nonspecific autoantibody. Thus, most of these immunized patients have single rather than multiple antibodies against the red cells, of which anti-E is the most common (2/5 patients, 40%). Previous studies also showed a high frequency of anti-E alloantibody in thalassaemia patients [10, 25, 26, 27,28,29].

In our study, 4/5 patients were female who developed antibodies. Although there was no association of gender with antibody formation which is similar to the previous study[30]. Clinically significant alloantibodies have been reported to occur higher in females compared to males in Malaysia[10,20], in Punjab[31] and in Iran[32]. It is reported that the higher rate of alloimmunization in female patients could be attributed to pregnancy or following complications of pregnancy[17,20]. However, this reasoning cannot be applied to our paediatric population.

In our study, we did not find any association of alloimmunization with the age of onset of transfusion, which is similar to previous studies[25,33]. However, it is reported that transfusion at an early age (1-3 years old) may lead to developing immune tolerance in recipients and protection from alloimmunization[27,34]. It is well known that multi-transfused patients are at high risk of developing alloantibody[35]. This present study showed no association between number of transfusions and alloimmunization, which agrees with previous studies[36,37].

This study showed no significant differences between blood types and red cell immunization. In our study, there were two O-positive, two B-positive, and one A-positive patient. The relationship between antibody development and blood group is variable in different studies. A study in Pakistan showed that the highest frequency of antibody development is in blood group O[38]. The high frequency of alloimmunization in the O group could be related to highest frequency of group O in the Asian population[39]. However, Al-Joudi et al.[20] found significantly higher alloantibody development in A group people, although the reason for this association was unclear.

In our study participants, only two patients had alpha thalassaemia who had not developed any alloantibody. Among the remaining 30 patients with beta-thalassaemia, 5(16.7%) had developed antibodies. Our results of alloimmunization in alpha and beta thalassaemia patients are consistent with a study in Malaysia[10] where no alloimmunization was detected in alpha thalassaemia patients, while 9.3% of the beta thalassaemic patients has developed antibodies.

In this present study, there was no data on the history of splenectomy as none of our patients was splenectomised. The reason was that most of the paediatric patients were diagnosed at an earlier age of less than 5 years, on regular blood transfusion and splenectomy was not routinely done as seen in our patients.

We acknowledged that this study has some limitations. Due to limited study period and resources, only a limited number of transfusion-dependent thalassaemic patients, especially the paediatric population aged 18 and below, were included in this study. This study is limited to HCTM and HPKK only. Thus, in future studies, we recommend a bigger sample size with an extended study period, which can include a few other hospitals in the Klang Valley and, other hospitals from other states in Malaysia. This could better represent thalassaemia patients in our population.

CONCLUSION

In conclusion, the prevalence of red cell immunization was 15.6% in which 12.5% was alloantibody and 3.1%

was autoantibody. The type of antibody detected was anti-E, anti-e, anti-c, antibody against low frequency antigen with unknown specificity and a nonspecific auto-IgG. No significant association was observed between antibody formation and risk factors such as diagnosis, gender, blood group, age at transfusion and number of packed cells transfused. The development of red cell immunization is most likely due to the differences of antigens between donor and recipient, the immune status and the resulted immunomodulation that occurs in individuals after each transfusion. As red cell immunization can pose problems with compatibility testing for future transfusions, extended phenotyping of red blood cells is recommended to be done early for all transfusion-dependent thalassaemia patients and to transfuse phenotype-matched blood.

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Conflict of Interest: The authors declare no conflict of interest.

Authors' Contribution

All authors have participated equally in preparing this manuscript. All authors have approved the final version of the manuscript.

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