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Original Research Article

Frequency of autoantibody formation in multi-transfused thalassemia patients at a tertiary care hospital: a cross-sectional study

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ABSTRACT

Background: Autoantibodies are produced by the immune system against the body's own proteins, such as red blood cells, particularly after transfusions. This phenomenon, known as erythrocyte autoimmunization, has been a recognized complication of repeated transfusions. The purpose of this study was to evaluate the frequency of autoantibody formation in multi-transfused thalassemia patients at a tertiary care hospital. The aim of the study was to detect the frequency of autoantibody formation in multi-transfused thalassemia patients at a tertiary care hospital.
Methods: This cross-sectional study at the department of transfusion medicine, BSMMU, Dhaka, conducted from October 2017 to September 2018, included 384 thalassemia patients who received at least 10 units of red cell concentrate. Informed consent was obtained, and data collection involved patient interviews, clinical exams, and hematological tests. Statistical analysis using SPSS version 23.0 included Chi-square, Fisher's exact, and t-tests, with significance set at $p<0.05$.
Results: In the study of 384 patients, 43.5% were under 10 years old, with a majority of 52.9% male. Coombs' test showed 3.1% DCT positive, 7.5% ICT positive, and 4.2% positive for both. All 12 DCT-positive patients had a history of drug use, and 7.7% had undergone splenectomy.
Conclusions: In conclusion, regular blood transfusion in thalassemia patients is a key factor in autoimmunization, with lower antibody formation in females, younger patients, and those with blood group B, while Rh phenotype-matched blood and prestorage leukodepletion may help reduce autoantibody formation.

Keywords: Autoantibody formation, Blood transfusion, Immunization, Tertiary care, Thalassemia

INTRODUCTION

Autoantibodies are antibodies produced by the immune system that target the individual's own proteins (antigens). Erythrocyte autoimmunization, which occurs when autoantibodies develop against red blood cells following transfusion, was first documented over 50 years ago. Notably, a case described by Lalezari et al showed that erythrocyte autoantibodies, alongside an Rh alloantibody response, led to a severe hemolytic transfusion reaction.[1]

The term thalassemia originates from the Greek words thalassa, meaning "the sea", and emia, meaning "related to blood". It was first recognized in individuals of Mediterranean descent. This hereditary hemoglobinopathy is marked by reduced or absent synthesis of normal globin chains, as seen in thalassemia major, a transfusion-dependent condition, and thalassemia intermedia, which sometimes requires transfusions due to hemoglobin fluctuations.[2]

Lifelong red blood cell (RBC) transfusions remain essential for managing thalassemia major and symptomatic thalassemia intermedia. However, repeated transfusions can cause serious complications, including iron overload, transfusion-transmitted infections, and

autoimmunization.[3] Autoantibody formation, a significant issue in multi-transfused patients, requires screening for antibodies in recipients to avoid transfusion reactions.[4] Although the mechanisms of erythrocyte autoantibody formation are not fully understood, RBC-associated IgG has been implicated in autoimmune hemolytic anemia.[5] Some studies suggest that alloantibodies could alter RBC antigens, triggering autoantibody production.[6]

There is also evidence that genetic predisposition may make certain patients more susceptible to autoantibody formation, potentially due to immune system dysfunction.[7] Hyperhaemolysis, a severe complication associated with acquired RBC autoantibodies, warrants testing for underlying alloantibodies and potential immunosuppressive therapy.[8] In multi-transfused patients, donor white blood cells might further contribute to autoimmunization, as they can induce immune reactions by exposing new antigenic sites.[9]

The age at which transfusions start and the number of transfusions may influence autoantibody formation; early initiation of transfusions has been associated with a lower risk.[10] Yet, the correlation between the volume of blood transfused and antibody development remains unclear in thalassemia.[9] Thalassemia patients often show altered immune responses due to exposure to multiple antigens from transfusions, and those receiving frequent transfusions have shown different autoantibody profiles compared to less-transfused patients.[11,12] The direct antiglobulin test, commonly used to detect RBC coating by immunoglobulins or complement, helps identify autoantibodies that may develop from intrinsic red cell antigens or alloantibodies from mismatched transfusions in multi-transfused patients.[13] The purpose of this study was to evaluate the frequency of autoantibody formation in multi-transfused thalassemia patients at a tertiary care hospital.

Objectives

The aim of the study was to detect the frequency of autoantibody formation in multi-transfused thalassemia patients at a tertiary care hospital.

METHODS

This cross-sectional study was conducted at the department of transfusion medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, over a 12-month period from October 2017 to September 2018. The study population comprised 384 consecutive patients with thalassemia who had received at least 10 units of red cell concentrate.

Inclusion criteria

Age >1 year to <40 years. Both sexes. Diagnosed with thalassemia via Hb-electrophoresis. Received at least 10 units of blood transfusion. Able to provide written informed consent. Capable of understanding questions and communicating effectively.

Exclusion criteria

Age <1 year and >40 years. Presence of any autoimmune or connective tissue disease. Individuals refusing to participate or unable to comprehend the study for any reason.

Institutional approval for this study was obtained from the IRB of BSMMU, with ethical considerations addressed according to the Helsinki Declaration. Informed written consent was secured from all participants to ensure privacy and confidentiality. Each patient was initially interviewed, clinically examined, and underwent routine hematological tests, blood grouping, direct antiglobulin test (DAT), and indirect antiglobulin test (IAT) using a spin-tube technique. The study utilized research instruments including pre-tested questionnaires, specific reagents (anti-human globulin, anti-A, anti-B, anti-D), test tubes, glass slides, pipettes, normal saline, a centrifuge, and a microscope. Statistical analysis was conducted using SPSS version 23.0, with qualitative variables expressed as frequencies and percentages, and quantitative variables as mean±standard deviation. Associations between variables were analyzed using Chi-square, Fisher's exact, and unpaired t-tests, with odds ratios (OR) and 95% confidence intervals (CI) calculated, considering a p value <0.05 as statistically significant. Ethical clearance was obtained, and participants' rights and well-being were prioritized, with assurances of confidentiality and the freedom to withdraw at any time.

RESULTS

Figure 1 shows the age distribution of study donors. It was observed that 43.5% of the patients were under 10 years old, 33.9% were between 11 and 20 years, 11.7% were between 21 and 30 years, and 10.9% were over 30 years.

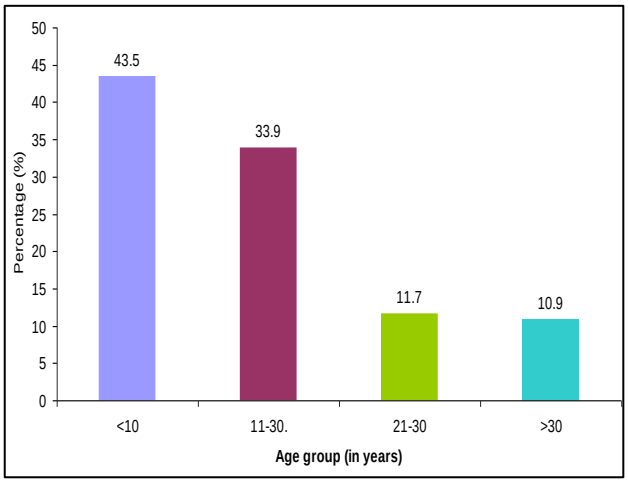

Figure 1: Age distribution of the study sample (n=384).

Figure 2 shows the sex distribution of the study population. It was observed that the majority, 203 (52.9%), were male, while 181 (47.1%) were female.

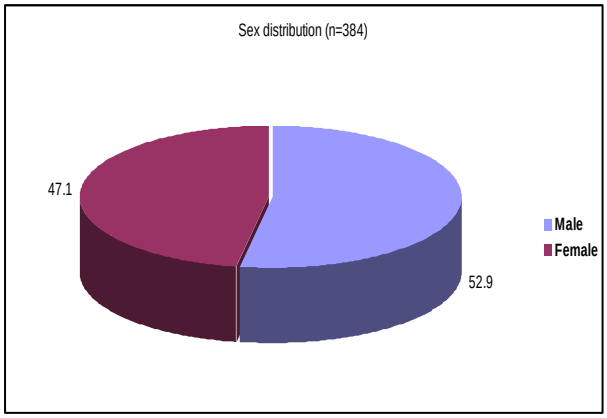

Figure 2: Sex distribution of the study sample (n=384).

Table 1 shows the distribution of the study population by Coombs' test results. It was observed that DCT was positive in 12 (3.1%) patients, ICT in 30 (7.5%) patients.

Table 2 shows the relationship between drug history and direct Coombs' test results. Among the study population, 12 (11.0%) patients with a history of drug use tested positive for the direct Coombs' test, compared to none of the patients without a drug history. A total of 3.4% of patients showed positive results, with a statistically significant association between drug use and test positivity ($p<0.001$).

Table 1: Distribution of the study respondents by coombs' test (n=384).

Test type	Number of patients	Percentage
Direct Coombs test (DCT) positive	12	3.1
Indirect Coombs test (ICT) positive	30	7.5

Table 3 shows the association between splenectomy status and direct Coombs' test results. Among the 7 patients who underwent splenectomy, 2 (28.6%) tested positive for the direct Coombs' test, compared to 10 (2.7%) among the 377 patients without splenectomy.

Overall, 12 (3.4%) of the study population had positive test results. A statistically significant association was observed, with patients who underwent splenectomy showing a higher rate of positivity ($p<0.001$).

Table 2: Distribution of the study patients by drug history (n=12).

Drug history	N	Direct Coombs' test		P value
		Positive	Negative	
		No. of patients (%)	No. of patients (%)	
Yes	109	12 (11.0)	97 (89.0)	<0.001[s]
No	275	0 (0.0)	275 (100.0)	
Total	384	12 (3.4)	372 (96.7)	

s= statistically significant ($p < 0.05$); P value calculated using the chi-square test.

Table 3: Distribution of the study patients by splenectomy status (n=12).

Splenectomy	N	Direct Coombs' test		P value
		Positive	Negative	
		No. of patients (%)	No. of patients (%)	
Yes	7	2 (28.6)	5 (71.4)	<0.001s
No	377	10 (2.7)	367 (97.3)	
Total	384	12 (3.4)	372 (96.7)	

s= statistically significant ($p < 0.05$); P value calculated using the chi-square test.

Table 4: Distribution of study patients by leukodepletion filter (n=12).

Leukodepletion filter	N	Direct Coombs' test		P value
		Positive	Negative	
		No. (%)	No. (%)	
Yes	6	2 (33.3)	4 (66.7)	<0.001s
No	378	10 (2.6)	367 (97.4)	
Total	384	12 (3.4)	372 (96.7)	

s= statistically significant ($p < 0.05$); P value calculated using the chi-square test.

Table 4 shows the relationship between the use of leukodepletion filters and direct Coombs' test results. Among the 6 patients who received leukodepletion filters, 2 (33.3%) tested positive for the direct Coombs' test, compared to 10 (2.6%) of the 378 patients who did not use leukodepletion filters. The overall positivity rate in the study population was 12 (3.4%). A statistically significant association was noted, with leukodepletion filter use linked to a higher rate of positive results ($p<0.001$).

DISCUSSION

The term thalassemia describes a disorder in globin chain synthesis, leading to ineffective erythropoiesis and a reduced lifespan of red blood cells. The current treatment involves regular monthly blood transfusions to maintain a hemoglobin level of 10-11 gm/dl. A complication of this treatment is the production of antibodies against the patient's own red blood cells, particularly in patients who have received multiple transfusions.

This study, conducted at Bangabandhu Sheikh Mujib Medical University from July 2017 to June 2018, included 384 thalassemia patients who received more than ten units of blood transfusion in the transfusion medicine department. The study aimed to determine the frequency of positive direct Coombs' tests among multi-transfused thalassemia patients.

Among the 384 patients, direct Coombs' test was positive in 12 patients (3.1%), indirect Coombs' test was positive in 30 patients (7.5%). The frequency of autoantibody formation varies by region: India at 0.47%, Pakistan at 1.87%, Malaysia at 1.7%, China at 4.7%, and Asian populations in the UK and US at 4.9%, with a significantly higher rate observed in this study population.

The study population included 203 males (52.9%) and 181 females (47.1%), with a male-to-female ratio of 1.1:1. Among the 12 patients with positive direct Coombs' tests, 9 (4.4%) were male, and 3 (1.7%) were female, indicating that thalassemia and autoantibody formation were more common in males. Other studies also highlight that male thalassemia patients are at a higher risk of developing alloantibodies and autoantibodies, potentially due to factors such as increased transfusion dependence or immune system responses that gender may influence the likelihood of developing transfusion-related autoimmunization in thalassemia patients.[14,15]

Of the 384 patients, most were younger, with 167 (43.5%) under 10 years and 130 (33.9%) between 11 and 20 years. The mean age of patients in the study was 15.62±12.19 years. Among the direct Coombs' positive patients, 3 (1.8%) were under 10 years, while 9 (6.9%) were between 11 and 20 years, indicating that autoantibody formation was more prevalent in children over 10 years. Other studies support this, showing a higher prevalence of autoantibody formation in children over 10. For example, Harmany et al found that thalassemia patients in the 10-20-year range had increased rates of autoimmunization, and Khan et al also observed a rise in autoantibody formation with age, particularly in children aged 10 and above.[16,17] These findings reinforce the trend that autoantibody formation becomes more common as children grow older.

Among the 12 patients in this study, only 1 had a history of splenectomy. The findings suggest that autoimmunization is more common in patients who have not undergone splenectomy. One function of the spleen is blood filtration, and previous studies have indicated that this process may play a role in autoimmunization. Another study by Dhawan et al also supports this, showing that patients who had not undergone splenectomy had a higher incidence of autoantibody formation, further suggesting the spleen's role in preventing autoimmunization.[18]

Among the 6 patients who received leukodepletion filters, 2 (33.3%) tested positive, while 10 (2.6%) of the 378 patients who did not receive the filter tested positive. The overall positivity rate in the study was 3.4%. A statistically significant association was found ($p<0.001$), suggesting that the use of leukodepletion filters is linked to an increased rate of positive direct Coombs' test results.

Our study shows that autoantibody formation is relatively common in multi-transfused thalassemia patients, particularly in males and those over 10 years old. The observed frequency of positive Coombs' tests in this population suggests regional factors may influence autoimmunization rates. Preventive measures, like Rh phenotype-matched blood and leukodepletion, could help reduce autoantibody risks in these patients, enhancing transfusion safety and effectiveness.

This study had several limitations. This study was limited to patient samples collected exclusively from BSMMU. Expanding the study to include a larger population could provide more representative data on the prevalence of autoimmunization in multi-transfused thalassemia patients. The study did not differentiate between IgG-mediated and complement-mediated autoantibodies. The specific antigens responsible for triggering these autoantibodies were not identified.

CONCLUSION

In conclusion, we found that regular blood transfusion is one of the causes of autoimmunization in thalassemia patients. The formation of antibodies is less common among females than males and is less frequent in children under ten years old. Autoantibody formation is more common among individuals with blood group B. The random use of red blood cells without knowing the Rh phenotype, as well as the cytokines released by white blood cells in stored blood, are common causes of autoantibody formation. Therefore, transfusion of Rh phenotype-matched blood and prestorage leukodepletion may help prevent autoantibody formation in multitransfused thalassemia patients.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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