Red cell alloimmunization and autoantibodies in Egyptian transfusion-dependent thalassaemia patients

Azza Mohamed Ahmed[1], Nehal Salah Hasan[2], Shadia Hassan Ragab[2], Sonia Adolf Habib[1], Nahed Abdelmonem Emara[2], Azza Ahmed Aly[2]

[1]Department of Paediatrics, National Research Centre, Cairo, Egypt
[2]Department of Clinical and Chemical Pathology, National Research Centre, Cairo, Egypt

Corresponding author:
Nehal Salah Hasan
Department of Clinical
and Chemical Pathology
National Research Centre (NRC)
Cairo, 12622 Egypt
Phone: +202 336 07 15
Fax: +202 337 09 31
E-mail: nemyb@hotmail.com

Abstract

Introduction: The objective of this study was to explore the frequency of red cell alloantibodies and autoantibodies among β-thalassaemia patients who received regular transfusions.

Material and methods: This study included 501 patients with β-thalassaemia. This work planned to study the presence of alloantibodies and autoantibodies to different red cell antigens in multitransfused thalassaemia patients using the ID. Card micro typing system.

Results: Of a total of 501 β-thalassaemia patients included in the study, 11.3% of patients developed alloantibodies; 9.7% of these alloantibodies were clinically significant. The most common alloantibodies were anti-K, anti-E and anti-C. The rate of incidence of these alloantibodies was 3.9%, 3.3% and 1.7% respectively. Autoantibodies occurred in 28.8% of the patients and 22.1% of these antibodies were typed IgG. There was a significant association between splenectomy with alloimmunization and autoantibody formation (p = 0.03, p = 0.001 respectively). There was no significant association between alloantibody, autoantibody formation and number of transfused packed red cells.

Conclusions: Alloimmunization to minor erythrocyte antigens and erythrocyte autoantibodies of variable clinical significance are frequent findings in transfused β-thalassaemia patients. There is an association between absence of the spleen and the presence of alloimmunization and autoantibody formation.

Key words: β-thalassaemia, alloimmunization, autoantibodies.

Introduction

Thalassaemia is among the most common genetic disorders worldwide, particularly in the Mediterranean region, Africa, the Middle East and Southeast Asia [1]. Thalassaemia is a hereditary anaemia resulting from defects in haemoglobin production [2]. β-Thalassaemia, which is caused by a decrease in the production of β globin chains, affects multiple organs and is associated with considerable morbidity and mortality [3]. Accordingly, lifelong care is required and financial expenditures for proper treatment are substantial [4]. The recommended treatment for thalassaemia major involves regular blood transfusions, usually administered every 2 to 5 weeks to maintain a pre-transfusion haemoglobin level above 9-10.5 g/dl. One of the complications of blood transfusion is the formation of alloantibodies and autoantibodies against red blood cell (RBC) antigens [5].

Results from a number of studies have demonstrated various frequencies and percentages of alloantibody and autoantibody formation in multi-transfused patients [6].

Some alloantibodies may cause haemolytic transfusion reactions, which limits the possibility of safe transfusion, while others are clinically insignificant. Red cell autoantibodies appear less frequently but can result in haemolysis and difficulty in blood cross-matching [7].

Antibodies must be identified in the recipient's serum before each transfusion so that compatible blood can be provided. The causes of alloimmunization in thalassaemia patients are not fully understood. However, data suggest that the recipient's immune status, absence of spleen and difference in the red cell phenotype between donors and recipients are likely to contribute further to the phenomenon [8].

The aim of this study was to explore the frequency of alloimmunization and erythrocyte autoantibodies among β-thalassaemia patients receiving regular transfusions and the factors that might contribute to their development, and to investigate the RBC phenotype of Egyptians.

Material and methods

This study was conducted over a 2-year period from January 2006 to December 2007 at the Paediatric Haematology Unit of the National Research Centre (NRC). The study was approved by the Ethics Committee of the NRC. Written consent was provided for each patient.

A total of 501 β-thalassaemia patients who received regular blood transfusions were included in the study. They were 389 patients with β-thalassaemia major (228 males and 161 females) aged 2-24 years, 59 patients with β-thalassaemia intermedia (24 males and 35 females) aged 4-26 years and 53 patients with sickle cell-β-thalassaemia syndrome (27 males and 26 females) aged 4-19 years.

The diagnosis of thalassaemia was confirmed by standard haemoglobin electrophoresis and measurements of Hb A, A_2 and F.

Clinical transfusion records of 501 β-thalassaemia patients who fulfilled the criteria were analysed for ethnic background; age at start of transfusion and number of transfused packed red cells (the majority of our patients had been exposed to non-leukodepleted blood for a long time before introduction of the universal leukodepletion protocol for thalassaemia patients). All patients in our unit received fully blood matched for only ABO and Rh (D) antigens. Presence of the spleen was also recorded. There were no available data about the time from start of transfusion to antibody formation. Patients received supportive treatment in the form of folic acid (5 mg/day, oral), calcium (40-80 mg/day, oral) and vitamin D (400 IU/day, oral).

Patients with connective tissue disease or autoimmune disease, or receiving immunosuppressive or immunomodulatory drugs were excluded from the study.

Laboratory investigation

The practical part of this work aimed to study the presence of alloantibodies and autoantibodies to different red cell antigens in multitransfused thalassaemia patients using the ID – Card micro typing system (Gel tear, Switzerland). Dia Cell I, II and III were used to screen for the presence of red cell alloantibodies and the Dia Panel to identify these alloantibodies.

All pre-transfused patients were routinely tested for ABO and Rh D antigen.

Screening of the sera for erythrocyte antibodies and identification tests: the idea of this test was to detect alloimmune antibodies formed against any RBC antigen in the tested sera; this was done with two different tests: screening and identification tests.

The antibody screening test was done with a combination of 3 sets of commercial group O red blood cells which have been typed for clinically significant antigens as well as rare antigens. These 3 sets are known as Dia – cell I, II, III.

Dia cell I contains the following antigens: D, C, e, C^ω, k, kp^b, Js^b, Fy^a, JK^b, Fy^b, Le^b, P, N, S, Lu^b and xg^a.

Dia cell II contains the following antigens: D, E, c, k, Kp^b, Js^b, Fy^a, JK^a, Le^b, M, N, S, Lu^b and xg^a.

Dia cell III contains the following antigens: C, E, k, kp^b, Js^a, Fy^b, JK^b, Le^a, P, N, S, Lu^b and xg^a.

Positive sera (with Dia cell I or II or III or all) were examined by an identification test using the Dia panel, which consists of 11 different group O red cells: k, C, E, JK^b, Le^a, kp^a, M, I, Fy^b, N, S.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) program version 9 was used for analysis of data. Data were summarized as mean ± SD and percentage.

Comparison of multiple subgroups was done using one-way ANOVA.

Fisher's Exact Test Calculator for a 2 × 2 contingency table was used.

Analysis of two independent variables was done by using a non-parametric test (Mann-Whitney U test).

In all tests, the value of p was considered significant if > 0.05.

Results

Characteristics of β-thalassaemia patients are summarized in Table I. There was a highly signi-

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ficant difference between and within the 3 groups regarding age, start of transfusion and rate of transfusion ($p = 0.001$). Also, Table I shows the percentage of our patients that had splenectomy.

Table II demonstrates the frequency of red blood cell alloantibody formation in β-thalassaemia patients. Of the total 501 multi-transfused β-thalassaemia patients 57 patients (11.3%) developed alloantibodies. Forty-nine (9.7%) of these alloantibodies were clinically significant (i.e. capable of causing haemolytic transfusion reaction or haemolytic disease of newborn).

The most common alloantibodies were anti-K (Kell system), anti-E and anti-C (Rhesus system). The incidence of these alloantibodies was 20 (3.9%), 17 (3.3%) and 9 (1.7%) respectively of the total 501 patients with β-thalassaemia.

Furthermore, comparison between groups of β-thalassaemia patients revealed a highly significant association between β-thalassaemia major compared to sickle cell-β-thalassaemia syndrome, and β-thalassaemia intermedia compared to sickle cell-β-thalassaemia ($p = 0.001$) regarding both the total of clinically significant patients and total alloantibodies.

Table III demonstrates the frequency of red blood cell autoantibodies in β-thalassaemia patients. This study revealed that 145 (28.8%) of the total 501 patients with β-thalassaemia had autoantibodies, and 111 (22.1%) of them were typed immunoglobulin G (IgG). Immunoglobulin G was detected in 99 (25.4%) of 389 patients with β-thalassaemia major, 4 (6.7%) of 59 patients with β-thalassaemia intermedia and 8 (15%) of 53 patients with sickle cell-β-thalassaemia syndrome.

Also, regarding total autoantibodies Table III shows a highly significant association between groups of β-thalassaemia major compared to β-thalassaemia intermedia, and β-thalassaemia intermedia compared to sickle cell-β-thalassaemia syndrome ($p = 0.001$, $p = 0.04$ respectively).

Table IV represents the association of splenectomy with alloantibodies and autoantibodies in β-thalassaemia patients. Among the splenectomized group, 30 patients with β-thalassaemia major had alloantibodies and 123 patients with β-thalassaemia major had autoantibodies, while in the non-splenectomized group none of them had alloantibodies or autoantibodies ($p = 0.001$).

Overall, of the 233 β-thalassaemia patients with splenectomy, 35 (15%) patients became alloimmunized and of the 269 patients without splenectomy, 22 patients (8.1%) became alloimmunized ($p = 0.03$). Also, of the 233 patients with splenectomy, 140 patients (60%) developed autoantibodies and of 269 patients without splenectomy, 5 patients (1.8%) developed autoantibodies ($p = 0.001$).

Analysis using the Mann-Whitney test shows that there was no significant association between alloantibody or autoantibody formation and the number of transfused packed red cells ($p = 0.5$, $p = 0.9$ respectively). Also, there was no significant association between alloantibody or autoantibody formation and age at start of transfusion ($p = 0.3$, $p = 0.5$ respectively) (Table V).

Discussion

Only a few studies in the world have investigated the frequency and causes of alloimmunization and autoimmunization [9]. In the present study we examined these elements and defined the common RBC phenotypes among Egyptians which have not been previously described.

In this study the frequency of alloimmunization was 11.3% in transfusion-dependent thalassaemia patients. Previous data on presumed homogeneous populations in Italy showed an overall low rate (10%) of alloimmunization [10]. This is consistent

Table I. Characteristics of β-thalassaemia patients

Items	β-Thalassaemia major (n = 389)	β-Thalassaemia intermedia (n = 59)	Sickle cell-β thalassaemia (n = 53)	Value of p
Age [years] Mean ± SD	(2–24)* 9.29 ±3.98	(4–26)* 12.73 ±4.58	(4–19)* 10.49 ±2.97	0.001
Start of transfusion [months] Mean ± SD	(6–30)* 13.37 ±4.21	(24–72)* 43.02 ±7.73	(10–48)* 29.26 ±12.31	0.001
Rate of transfusion [units/year] Mean ± SD	(4–12)* 9.43 ±2.41	(3–12)* 5.05 ±1.58	(2–12)* 6.46 ±2.19	0.001
Splenectomy: • Yes • No	214 (55%) 175 (45%)	11 (18.6%) 48 (81.4%)	10 (18.9%) 43 (81.1%)	

*minimum to maximum, SD – standard deviation, p value < 0.05 is significant, p value < 0.01 is highly significant

Table II. Frequency of red blood cell alloantibody formation in β-thalassaemia patients

Alloantibody	β-Thalassaemia major (n = 389)	β-Thalassaemia intermedia (n = 59)	Sickle cell-β thalassaemia (n = 53)	Total (n = 501)	Value of p
Anti-K*	14 (3.5%)	2 (3.3%)	4 (7.5%)	20 (3.9%)	$p_1 = 1$ $p_2 = 0.2$ $p_3 = 0.4$
Anti-C*	6 (1.5%)	–	3 (5.6%)	9 (1.7%)	$p_1 = 0.5$ $p_2 = 0.08$ $p_3 = 0.1$
Anti-E*	8 (2%)	2 (3.3%)	7 (13.2%)	17 (3.3%)	$p_1 = 0.6$ $p_2 = 0.01$ $p_3 = 0.09$
Anti-Fya	2 (0.5%)	–	4 (7.5%)	6 (1.2%)	$p_1 = 1$ $p_2 = 0.002$ $p_3 = 0.05$
Anti-M	1 (0.2%)	–	1 (1.8%)	2 (0.4%)	$p_1 = 1$ $p_2 = 0.2$ $p_3 = 0.2$
Anti-Kpa*	2 (0.5%)	–	1 (1.8%)	3 (0.6%)	$p_1 = 1$ $p_2 = 0.01$ $p_3 = 0.1$
Total	33 (8.4%)	4 (6.7%)	20 (37.7%)	57 (11.3%)	$p_1 = 0.8$ $p_2 = 0.001$ $p_3 = 0.001$
Total of clinically significant patients	30 (7.7%)	4 (6.7%)	15 (28.3%)	49 (9.7%)	$p_1 = 1$ $p_2 = 0.001$ $p_3 = 0.01$

*Indicates clinically significant, p_1 – comparison of β-thalassaemia major vs. β-thalassaemia intermedia, p_2 – comparison of β-thalassaemia major vs. sickle cell-β-thalassaemia, p_3 – comparison of β-thalassaemia intermedia vs. sickle cell-β-thalassaemia, $p < 0.05$ is significant, $p < 0.01$ is highly significant

Table III. Frequency of red blood cell autoantibodies in β-thalassaemia patients

Alloantibody	β-Thalassaemia major (n = 389)	β-Thalassaemia intermedia (n = 59)	Sickle cell-β thalassaemia (n = 53)	Total (n = 501)	Value of p
IgG	99 (25.4%)	4 (6.7%)	8 (15%)	111 (22.1%)	$p_1 = 0.005$ $p_2 = 0.23$ $p_3 = 0.23$
IgM	27 (6.9%)	1 (1.6%)	6 (11.3%)	34 (6.8%)	$p_1 = 0.23$ $p_2 = 0.16$ $p_3 = 0.06$
Total	126 (32.3%)	5 (8.4%)	14 (26.4%)	145 (28.9%)	$p_1 = 0.001$ $p_2 = 0.6$ $p_3 = 0.04$

Ig – immunoglobulin, p_1 – comparison of β-thalassaemia major vs. β-thalassaemia intermedia, p_2 – comparison of β-thalassaemia major vs. sickle cell-β-thalassaemia, p_3 – comparison of β-thalassaemia intermedia vs. sickle cell-β-thalassaemia, $p < 0.05$ is significant, $p < 0.01$ is highly significant

with our study. Similarly, Ho *et al.* [7] in Hong Kong found that the alloimmunization rate in thalassaemia patients was 7.4%. Other studies by Noor Haslina *et al.* [8] and Karimi *et al.* [9] reported that the incidence of alloimmunization in Malay and Shiraz, Southern Iran, was 8.6% and 5.3% respectively.

Our low alloimmunization rate in this study and other similar studies can probably be explained by the similarity in the ethnicity between patients and donors. All of our alloimmunized patients were Egyptian and most of our blood donors were also Egyptian. 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 [7].

On the other hand, the alloimmunization rate in two studies done in Greece [11] and Kuwait [6]

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Table IV. Relationship of splenectomy with alloantibodies and autoantibodies in β-thalassaemia patients

Items	With splenectomy ($n = 232$)	Without splenectomy ($n = 269$)	Value of p
Alloantibody			
β-Thalassaemia major	30 (13%)	–	0.001
β-Thalassaemia intermedia	2 (0.8%)	3 (1.1%)	1
Sickle cell-β-thalassaemia	3 (1.2%)	19 (7%)	0.003
Total	35 (15%)	22 (8.1%)	0.03
Autoantibody			
β-Thalassaemia major	123 (53%)	–	0.001
β-Thalassaemia intermedia	8 (3.4%)	1 (0.3%)	0.01
Sickle cell-β-thalassaemia	9 (3.9%)	4 (1.5%)	0.1
Total	140 (60.3%)	5 (1.8%)	0.001

p < 0.05 is significant, p < 0.01 is highly significant

Table V. Relationship of red cell alloantibodies and autoantibodies with number of transfused packed red cells and age at start of transfusion

Item	Alloantibodies (Value of p)	Autoantibodies (Value of p)
Number of transfused packed red cells [units/year]	0.5	0.9
Age at start of transfusion [year]	0.3	0.5

Value of p > 0.5 is not significant

was 22% and 30% respectively. 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.

Also, other studies by Sylvia *et al.* [12] and Gader *et al.* [13] found a high alloimmunization rate of 20.8% and 22.06% among Asians and in Saudi Arabia because the majority of patients received multi-ethnic blood.

In the current study anti-K (Kell system) was seen most frequently, followed by anti-E and anti-C (Rhesus system), which are all potentially haemolytic antibodies. All of our patients received compatible blood for ABO, Rh D.

In an Italian study [14] alloantibodies were confined to the common antigens of the Kell, Rhesus and Kidd systems. This is similar to our study. Also, in studies from Iran and Kuwait, Karimi *et al.* [9] and Ameen *et al.* [6] found that the most common alloantibodies were anti-Rh C, E and D. Meanwhile, several studies have shown that anti-E antibodies were the most prevalent alloantibodies among transfusion-dependent thalassaemia patients [7, 8, 12]. Spanos *et al.* [15] demonstrated that for prevention of alloimmunization, transfusion of blood matched for Rh and K antigens resulted in a significant difference in the alloimmunization rate.

The factors for alloimmunization are complex and involve at least 3 main contributing elements: the RBC antigenic difference between the blood donor and the recipient, the recipient's immune status, and the immunomodulatory effect of the allogenic blood transfusion on the recipient's immune system [12]. A low rate of alloimmunization may be expected when there is homogeneity of RBC antigens between the blood providers and recipients [16]. Other factors beyond the antigenic discrepancy, in particular the immune system, are undoubtedly involved. An overall normal $CD_4 : CD_8$ ratio was shown in thalassaemia patients transfused with post-storage leukocyte-depleted blood [17]. However, a marked absolute lymphocytosis mostly due to an increase in B lymphocytes predominantly in splenectomized patients was reported with the use of both non-leukoreduced and leukoreduced blood [18]. The lymphocytosis is accompanied by an increase in serum immunoglobulins, immune complexes, and cells bearing surface immunoglobulins which are the result of the immunomodulatory effect of blood elements, absence of spleen and recipient immune status [19, 20]. The majority of patients in the present study had long-term exposure to non-leukodepleted blood.

In this study the incidence of autoantibodies was 28.8%. Our data were similar to an Asian study where 25% of their thalassaemia patients developed autoantibodies [12]. Also a Kuwait study observed that 11% of their patients developed autoantibodies [6].

However, a study in Iran found that the rate of autoantibodies was 1.4% [9]. Other studies in Hong Kong [7] and Malaysia [8] reported that only one patient developed autoantibodies.

The pathogenesis of erythrocyte autoantibody formation following transfusion is not well understood. However, clinical evidence of auto-immune haemolytic anaemia has been seen with high amounts of RBC-associated IgG [21]. It was

also suggested that alloantibody binding to the RBCs could lead to conformational changes of the antigenic epitope that ultimately stimulates production of autoantibodies [22]. 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 [23].

In this study there was a significant association of splenectomy with alloimmunization and auto-antibodies (p = 0.03, 0.001 respectively). In agreement with our study, Singer *et al.* and Sylvia *et al.* observed that Asian patients with splenectomy had a higher rate of alloimmunization compared with patients who did not have splenectomy (p = 0.06).

Also our results were supported by Wiener *et al.*, [24] who revealed that significant elevations in RBC-bound IgG were seen in thalassaemia patients and it was more abundant in splenectomized than non-splenectomized subjects. They found that the absence of a spleen may further enhance the immune response to the infused foreign antigens which are not effectively filtered. In contrast, Noor Haslina *et al.* [8, 21] reported that in Malaysia despite a higher rate of patients with splenectomy none of them had alloantibodies and only one patient had autoantibodies.

In the present study there was no association between alloantibodies, autoantibodies and age at start of transfusion. Similarly, in Malaysian studies Noor Haslina [8, 21] found that there was no association between alloantibodies, autoantibodies and age at start of transfusion (p = 0.5, p = 0.8 respectively).

In our study there was a significant association between groups of β-thalassaemia patients (β-thalassaemia major vs. sickle cell-β-thalassaemia; β-thalassaemia intermedia vs. sickle cell-β-thalassaemia) regarding allo- antibodies and also (β-thalassaemia major vs. β-thalassaemia intermedia; β-thalassaemia intermedia vs. sickle cell-β-thalassaemia) regarding autoantibodies. A vulnerable immune status of the recipient may predispose to an altered or increased immune response to various antigens. This aspect of immune status has never been explored in our patients, and needs to be looked into. This agrees with Fluit *et al.* [25] who found that some antigen-negative patients may not produce antibodies at all or may form only one antibody despite exposure to antigen-positive cells. Other patients may have serological findings of a clinically significant alloantibody without evidence of a haemolytic transfusion reaction, and this agrees also with Sylvia *et al.* who reported that with the growing knowledge base of the immune effects of current blood transfusion and limited data on immune status of thalassaemia patients, a large study addressing the complex interaction of these factors is needed.

In conclusion, alloimmunization to minor erythro-cyte antigens and erythrocyte autoantibodies of variable clinical significance are frequent findings in transfusion-dependent β-thalassaemia patients. The overall frequency of alloantibody and auto-antibody formation in our patients is 11.3% and 28.8% respectively. The most common alloantibodies are anti-K (Kell system), anti-E and anti-C (Rhesus system). There is a significant association between absence of the spleen and the presence of alloim-munization and autoantibody formation. The difference in the RBC antigen profile between donors and recipients together with splenectomy in this study likely contributes to these phenomena.

We recommend obtaining an RBC antigen phenotype for all thalassaemia patients who are started on transfusions and providing universal leukodepleted blood matched for antigens of the ABO, Kell and Rh system. Recruitment of Egyptian blood bank donors can increase the availability of compatible blood for beta thalassaemia patients.

Acknowledgments

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