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Red Blood Cells Alloimmunization in Transfusion-dependent B-thalassemic Children at Zagazig University Hospital

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ABSTRACT

Background: The thalassemias are hereditary hemolytic anemias characterized by reduced or absent synthesis of one or more of globin chains of hemoglobin leading to globin chain imbalance. The most important forms of thalassemia result from autosomal mutant genes that reduce the rate of synthesis of α and β chains of hemoglobin (Hb) A, leading to α and β thalassemias respectively. In Egypt, β-thalassemia is the commonest form of chronic hemolytic anemia among Egyptian children. Formation of alloantibodies resulting in clinical hemolysis, and difficulty in cross-matching blood, and shortening of the duration of RBC's survival. **Objective:** To detect the presence of alloantibodies in regularly transfused beta-thalassemic patients and to identify type of these antibodies. **Patients and Methods:** This is a cross sectional study on one hundred transfusion dependent B thalassemic patients for presence of alloantibodies. The study included children with thalassemia who attended to outpatient clinic at Zagazig University Hospital aged from 1 to 16 from June 2019 to June 2020. **Results:** Alloantibody was negative in 82% of cases, and positive in 18%. Of positive cases 61.1% had anti E, 16.7% non-specific, 11.1% anti E and anti-JKB and 11.1% anti E and anti C. There was no statistically significant difference between patients with negative and positive alloantibodies as regard sex, age or consanguinity, **Conclusion:** Alloimmunization to red blood cell antigens are frequent finding and quite relevant among Egyptian transfusion-dependent thalassemic patients. The most frequent antibodies detected were anti-E. The majority of alloantibodies detected in the current study were clinically significant.

Keywords: Children, Red Blood Cells Alloimmunization, Transfusion-Dependent B-thalassemic.

INTRODUCTION

Thalassemia is a congenital hemolytic anemia, caused by a partial or complete defect in alpha or beta globin chain synthesis. It results from autosomal mutant genes that reduce the rate of synthesis of α and β chains of hemoglobin A in α and β thalassemia, respectively [(1)]. The globin chains that are produced in relative excess can damage the RBCs [(2)].

Thalassemia is considered the most common genetic disorder worldwide with high frequency in a broad belt, extending from the Mediterranean basin through the Middle East, India, and Southeast Asia. In absence of stem cell transplantation, it is treated by life-long RBC transfusion to keep the hemoglobin level 9 to 11.5 g/dL [(1)]. Alloimmunization is a complication among transfusion-dependent patients, which causes hemolytic transfusion reactions with serious morbidity with difficulty in identifying compatible blood. Some antibodies are clinically significant such as anti-A, anti-B, anti-D, and anti-Kell [(3)].

The identification of alloantibodies in recipient's serum makes transfusion safer. Blood compatibility for RBC antigens must include ABO, Rh, and minor antigens. Lack of phenotypic compatibility between donor and recipient blood may result in potential life-threatening complications [(4)].

Factors for alloimmunization are mainly: the RBC's antigenic difference between donor and recipient, the recipient's immune status, and the immunomodulatory effect of the allogenic blood transfusions on the recipient's immune system [(5)].

Formation of alloantibodies resulting in clinical hemolysis, and difficulty in cross-matching blood, and shortening of the duration of RBC's survival [(6)].

Transfusion of phenotypically matched blood for Rhesus and Kell systems (i.e., matched for D, C, E, c, e, K, FYa, FYb, JKa, and JKb antigens) compared with blood phenotypically to the standard ABO-D system proved to be effective in preventing alloimmunization [(7)].

The aim of this study was to detect the presence of alloantibodies in regularly transfused beta-thalassemic patients and to identify type of these antibodies.

PATIENTS AND METHODS

This is a cross sectional study on one hundred transfusion dependent B thalassemic patients for presence of alloantibodies. The study included children with thalassemia who attended to outpatient clinic at Zagazig University Hospital aged from 1 to 16 from June 2019 to June 2020.

Ethical consent:

An approval of the study was obtained from Zagazig University Academic and Ethical Committee. Every caregiver of a patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Inclusion criteria: Transfusion dependent thalassemic children aged 1-16 years, and both sexes were included. **Exclusion criteria:** Children less than 1 year or older than 16 years, known thalassemic patients with autoimmune disease, and other chronic hemolytic anemia.

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All patients were evaluated for:

- Age at first transfusion.
- Time interval from the start of blood transfusion therapy.
- Frequency and amount of transfusion therapy.
- Exposure to non leukodepleted blood.
- History of splenectomy.
- The presence of alloimmunization and antibody identification.
- History suggests reaction to blood products.
- Laboratory investigations including: ABO and D blood grouping, RBC antigen phenotyping, antibody screening, identification, using column agglutination technology by microtyping system reagent.

Antibody identification was done for patients with positive screening test. Commercial RBC panel composed of 11 vials containing papainized human RBCs of group (O) blood group in low ionic strength saline, was used to cover Rh, Kell, duffy, Kidd, MNS and Lewis systems.

The principle of the test is based on the gel technique described by **Lapierre *et al.*** [8]**.** Direct agglutination test was performed using a polyspecific antihuman globulin.

Immunohematological Tests:

Antibody identification was carried out on samples that tested positive in antibody screening procedure. For the identification of antibodies, panel cells of antibody identification (DiaMed-Dia Panel) and DiaMed Gel cards were used [8]**.**

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0**. (**Armonk, NY: IBM Corp**).** Qualitative data were represented as frequencies and relative percentages and were compared by chi square test (χ^2). The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation, median, and interquartile range (IQR) and were compared by Mann-Whitney test. Significance of the obtained results was judged at the 5% level.

RESULTS

Demographic data of the studied group are shown in **Table 1**.

Table (1): Demographic data of the studied group (n=100)

	No.	%
Sex		
Female	48	48.0
Male	52	52.0
Age (years)		
Min. – Max.	1.50 – 16.0	
Mean ± SD.	7.90 ± 3.78	
Median (IQR)	7.0 (5.0 – 10.0)	
Consanguinity		
Negative	46	46.0
Positive	54	54.0

This table shows no significant difference between patients with positive and negative alloantibodies as regards sex, age and consanguinity **(Table 2)**.

Table (2): Comparison between patients with negative and positive alloantibodies according to demographic data

	Total sample (n = 100)		Alloantibody				Test of Sig.	P
			Negative (n = 82)		Positive (n = 18)			
	No.	%	No.	%	No.	%		
Sex								
Female	48	48.0	41	50.0	7	38.9	χ^2= 0.730	0.393
Male	52	52.0	41	50.0	11	61.1		
Age (year)								
Min. – Max.	1.50 – 16.0		1.50 – 16.0		5.0 – 13.0		U= 709.50	0.797
Mean ± SD.	7.90 ± 3.78		7.95 ± 4.04		7.67 ± 2.25			
Median (IQR)	7.0 (5.0 – 10.0)		7.0 (5.0 – 11.0)		7.0 (6.0 – 10.0)			
Consanguinity								
Negative	46	46.0	35	42.7	11	61.1	χ^2= 2.018	0.155
Positive	54	54.0	47	57.3	7	38.9		

χ^2: **Chi square test** **U: Mann Whitney test.** P: p value for comparing between **negative** and **positive** alloantibodies

This table shows that there was 18 patients positive alloantibody in which 11 patients with anti E (61.1%) **(Table 3).**

Table (3): Frequency of patients developing alloantibodies (n = 100)

Presence of alloantibodies	No.	%
Negative	**82**	**82.0%**
Positive	**18**	**18.0%**
Total	**100**	**100%**
Types of alloantibodies	**No.**	**%**
Anti E	11	61.1
Anti E and Anti JKB	2	11.1
Anti E and Anti C	2	11.1
Non specific	3	16.7
Total	18	100%

This table shows the frequency of alloantibodies in the studied patients, the most frequent antibodies was anti-E (68.1%) **(Table 4)**.

Table (4): Frequency of alloantibodies in the studied patients

Alloantibodies	Frequency	%
Anti E	**15**	**68.2%**
Anti C	**2**	**9.1%**
Anti JKB	**2**	**9.1%**
Non specific	**3**	**13.6%**
Total	**22**	**100%**

DISCUSSION

Eighteen patients (18.0%) of the total number of patients (100) were found to have alloantibodies to red blood cell, of these patients 11 patients with anti E (61.1%), 3 patients with non-specific antibodies (16.7%), 2 patients with anti E and anti JKB (11.1%) and 2 patients with anti E and anti C (11.1%).

These data are in accordance with **El-Masry *et al.*** [9] who found a rate of alloantibody development is 18.0% in multi-transfused Egyptian thalassemic patients and **Saied *et al.*** [10] reported 28.4% alloantibody. **Jansuwan *et al.*** [11] found the rate of alloantibody development is 17.5% in multi-transfused thalassemic patients. **Gader *et al.*** [12] found the rate of alloantibody development is 22.06% in multitransfused patients in Saudi Arabia. **Singer *et al.*** [5] found a rate of 20.8% among Asian patients.

A low rate of alloimmunization may be expected when there is homogeneity of RBC antigens between donor and recipient as showed by data in Greece and Italy (5% to 10%) alloimmunization rate [13].

The high rate in our study may be because the majority of patients were transfused with blood matched for ABO and D antigens only, and the majority of red blood cell alloantibody formation was against Rh and KIDD blood group system. Previous reports have shown that reduction of red blood cell alloimmunization can be achieved by matching the blood for Rh and Kell antigens in transfusion dependent thalassemia patients [14]. Also the majority of patients had long term exposure to non leucodepleted or post storage leucodepleted blood. The presence of residual donor white blood cells could have a potential influence on the rate of alloimmunization [7].

When we study the specificity of alloantibodies Rh related alloantibodies was the most frequent alloantibody. The most frequent antibodies were anti-E (68.1%), anti C (9.09%), anti JKB (9.09%) and non-specific antibodies (13.6%). Similar to our study in other Egyptian study **El-Masry *et al.*** [9] found that anti Kell and Rh related alloantibodies was the most common. Similar to our study, in an Italian study alloantibody were confirmed to the common antigens of the Kell, Rhesus and Kidd systems [15]. In most Western countries, the most common alloantibodies in thalassemia patients are directed against C, E, and Kell antigens [14]. Similarly, **Hussein *et al.*** [16] showed rates of alloimmunization incidence were 22.8% and the most common alloantibody was Rh-related and anti Kell. In an Indian study, the most frequent alloantibody was anti-E [11].

In our study gender was not a significant factor in the development of alloimmunization (among alloantibody positive patients 61.1% were male and 38.9% were female). This is similar to the result of **Ameen *et al.*** [7] **and El-Masry *et al.*** [9] who reported that there was no statistically significant differences between male and female regarding the immunization rate.

In the present study there was no statistically significant difference between patients according to difference in age (alloimmunized patients aged from 5-13 y with mean ± SD 7.67 ± 2.25, non alloimmunized patients aged from 1.5-16 y with mean ± SD 7.95 ± 4.04). This is in accordance with the result of **Saied *et al.*** who found no differences according to age regarding the immunization rate [10].

Patient age at the initiation of transfusion may also affect the immune response. Transfusion at early age (less than 1-3 years old) may offer some immune tolerance and protection against alloimmunization in thalassemia patients [17]. We found that the incidence of alloimmunization was not influenced by the age at which transfusion was started. Similar results were noted in a study by **El-Masry *et al.*** [9] **Karimi and Ghavanini** [18].

CONCLUSION

According to the result that have been extracted from the current study, it was concluded that alloimmunization to red blood cell antigens are frequent finding and quite relevant among Egyptian transfusion-dependent thalassemic patients. The most frequent antibodies detected were anti-E. The majority

of alloantibodies detected in the current study were clinically significant.

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REFERENCES

1. **Wang L, Liang D, Liu H *et al.* (2006):** Alloimmunization among patients with transfusion-dependent thalassemia in Taiwan. Transfusion Medicine, 16(3): 200-203.
2. **Sadeghian M, Keramati M, Badiei Z *et al.* (2009):** Alloimmunization among transfusion-dependent thalassemia patients. Asian Journal of Transfusion Science, 3(2): 95-99.
3. **Meny G (2004):** Transfusing incompatible RBCs-clinical aspects. Immunohematology, 20: 161-166.
4. **Santos F, Magalhães S, Mota R *et al.* (2007):** Post-transfusion red cell alloimmunisation in patients with acute disorders and medical emergencies. Revista Brasileira de Hematologia e Hemoterapia, 29(4): 369-372.
5. **Singer S, Wu V, Mignacca R *et al.* (2000):** Alloimmunization and erythrocyte autoimmunization in transfusion-dependent thalassemia patients of predominantly Asian descent. Blood. The Journal of the American Society of Hematology, 96(10): 3369-3373.
6. **Young P, Uzieblo A, Trulock E *et al.* (2004):** Autoantibody formation after alloimmunization: are blood tranfusions a risk factor for autoimmune hemolytic anemia? Transfusion, 44(1): 67-72.
7. **Ameen R, Al-Shemmari S, Al-Humood S *et al.* (2003):** RBC alloimmunization and autoimmunization among transfusion-dependent Arab thalassemia patients. Transfusion, 43(11): 1604-1610.
8. **Lapierre Y, Rigal D, Adam J *et al.* (1990):** The gel test: A new way to detect red cells antigen - antibody reactions. Transfusion, 30:109–113.
9. **El-Masry H, Sadek A, Hassan M *et al.* (2018):** Metabolic profile of oxidative stress and trace elements in febrile seizures among children. Metab Brain Dis., 33(5):1509-1515.
10. **Saied D, Kaddah A, Badr Eldin R *et al.* (2011):** Alloimmunization and Erythrocyte Autoimmunization in Transfusion-dependent Egyptian Thalassemic Patients. Journal of Pediatric Hematology/Oncology, 33(6): 409-414.
11. **Jansuwan S, Tangvarasittichai O, Tangvarasittichai S (2015):** Alloimmunization to red cells and the association of alloantibodies formation with splenectomy among transfusion-dependent β-thalassemia major/HbE patients. Indian Journal of Clinical Biochemistry, 30(2): 198-203.
12. **Gader A, Al Ghumlas A, Al-Momen A *et al.* (2008):** Transfusion medicine in a developing country alloantibodies to red blood cells in multi-transfused patients in Saudi Arabia. Transfus Apher Sci., 39:199–204.
13. **Gupta R, Singh D, Singh B *et al.* (2011):** Alloimmunization to red cells in thalassemics: Emerging problem and future strategies. Transfus Apher Sci., 45: 167–170.
14. **Thompson A, Cunningham M, Singer S N *et al.* (2011):** Red cell alloimmunization in a diverse population of transfused patients with thalassaemia. Br. J. Haematol., 153:121–128.
15. **Sirchia G, Zanella A, Parravicini A *et al.* (1985):** Red cell al-loantibodies in thalassemia major. Results of an Italian cooperative study. Transfusion, 25: 110–2.
16. **Hussein E, Desooky N, Rihan A *et al.* (2014):** Predictors of red cell alloimmunization in multitransfused Egyptian patients with β-thalassemia. Archives of Pathology and Laboratory Medicine, 138(5): 684-688.
17. **Spanos T, Karageorga M, Ladis V *et al.* (1990):** Red cell alloantibodies in patients with thalassemia. Vox Sanguinis, 58(1): 50-55.
18. **Karimi M, Ghavanini A (2001):** Seroprevalence of hepatitis B, hepatitis C and human immunodeficiency virus antibodies among multitransfused thalassaemic children in Shiraz, Iran. J Paediatr Child Health, 37(6):564-66.