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" Prevalence of Red Cell Alloantibodies among Multi Transfused Egyptian Patients "

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Abstract:

Introduction

Blood Transfusion-dependency doesn't come without its serious hazards. RBCs Alloimmunization in multi-transfused is a major challenge. Alloimmunization is a late complication that affects 8% to 12% of recipients. This percentage increases if patients on continuing transfusion regimens, such as those with sickle cell anemia, thalassemia and chronic renal failure. The immune-mediated damage of circulating (RBCs) can happen by two mechanisms: the first is intravascular destruction by complement lysis of the IgM class. The second one is extravascular destruction by immune cells which recognize IgG and complement bound to RBC. Frequency, risk factors, and specificities of alloantibodies were our goals in this study, which was conducted on patients in Alexandria governorate, Egypt, with the goal of reducing the transfusion-related risks.

Materials and methods: A total of 400 patients with thalassemia, sickle cell and chronic renal failure attending Alexandria university hospital were evaluated. Alloantibody screening and documentation was accomplished by the DiaMed-ID micro-typing system. Results: prevalence of alloimmunization was 3.8% (15 of 400). The most shared alloantibody was RhD-related; anti-E was the recurrent alloantibody seen in five of the 15 patients (33.3%), followed by anti-D in four patients (26.7%), anti-kell in two (13.3%), anti-c, anti-C, Anti-Fya and Anti-Jka each in one patient (6.7%).

Conclusion: High alloimmunization rates were found in male patients, in patients with chronic renal failure (13.6%) followed by sickle cell anemia (13.3%) then thalassemia (2.8%). A statistically significant relation between splenectomy, age at starting blood transfusion and use of leukoreduced blood was detected ($P < 0.05$).

Keywords: Alloimmunization, thalassemia, transfusion, hemolysis

Introduction

Blood transfusion has become a communal practice in current medicine. Not only a supportive measure in surgical and critical care settings, but also a mainstay therapy for congenital or acquired hematological conditions which cause ineffective blood cell production or bone marrow failure. Although life-saving, transfusion therapy comes with its own list of hazards and complications which can be branded as acute or delayed, which is further classified as: non-infectious (as: Acute or delayed hemolytic reaction, anaphylactic or allergic reaction, coagulopathy in massive transfusion, febrile reactions, transfusion-related lung injury,

circulatory overload, iron overload) and infectious as hepatitis viruses and immune-deficiency virus[1] .Beta-thalassemia are hereditary anemia characterized by absent or decreased β globin chain. The relative extra α globin chains precipitates and lead to premature death. Different phenotypes are ranging from the mild form to the major thalassemia type. Patients with major subtype have severe microcytic anemia, and usually detected within the first two years of life. Management is with iron chelation therapy and regular transfusions [1].

Alloimmunization is a late complication that affects 8% to 12% of recipients. This percentage increases if patients on continuing transfusion regimens, such as those with sickle cell anemia, thalassemia and chronic renal failure [2] .It may also contribute to severe fatal hemolysis [3], RBC alloantibodies in pregnancy may possibly cause hemolytic disease of the newborn [4] although RBC autoantibodies is less frequent they can lead clinical hemolysis [5]. Allo -and auto-antibodies can affect pre-transfusion compatibility testing. RBC antigenic differences, genetic predisposition, the recipient's clinical state and quantity of transfused units affect ialloimmunization risk [6] .Blood products leukoreduction and phenotyping of most immunogenic blood groups as Rh and Kell, followed by cross matching should be the standard of care for these patients [7] .The International Society of Blood Transfusion (ISBT) identifys 347 red cell surface antigens. ABO, Rh, Kell, Duffy, Kidd, Lewis and MNS systems are the most clinically important systems [8].The immune-mediated damage of circulating (RBCs) can happen by two mechanisms: the first is intravascular destruction by complement lysis of the IgM class.The second one is extravascular destruction by immune cells which recognize IgG and complement bound to RBC **[**9**].**

Materials and Methods:

The study involved 400 Egyptian patients who had received multiple transfusions. Informed consent was acquired from parents or patients. All patients' clinical, laboratory, and transfusion regimens were reviewed, with special attention paid to the following factors: patient age and gender, transfusion interval from start of blood transfusion, age at starting transfusion, transfusion frequency and number of transfused units per year, spleen presence or removal, and history suggestive of transfusion reactions.4 ml of peripheral venous blood was drawn; under aseptic precautions for (ABO, D) phenotyping and antibody detection; Red cell alloantibodies Screening using indirect anti-globulin (IAT) test by column agglutination technique and panels of 11 red cell specimens selected for antibody identification [10].Direct antihuman globulin (DAT) test was done for exclusion of patients with autoantibodies. All serological tests were done using DiaMed-ID micro-typing

system.Antibody identification: Identifying antibodies formed against RBCs in patients' plasma based on an anti-globulin test by using 11 cell panels. Alloantibodies to red cell antigens may be initially identified in any test that uses a serum ((including ABO testing, the antibody detection test; (the antibody screen), or the cross match)). Ordinarily, when detecting an antibody, its clinical significance and specificity should be judged. Procedure:

- 14 columns were prepared & marked.
- (1-11) for panel cells
- (12) for auto control
- (13, 14) for +ve &-ve controls.
- 5% cell suspensions of the identification panel cells were ready to use.
- The wells were opened.
- One drop of cell suspensions of the identification panel cells was added to each well.
- Two drops of patient plasma were added to each well.
- Incubation for 10 min at 37º C was performed to allow reaction between Ag, and Ab.
- Centrifugation for 9 min was performed.
- Results were read and interpreted (taking into consideration the results of the patient's antibody screen test).

Statistical analysis: Data was fed and evaluated using IBM SPSS software version 20.0. (Armonk, NY: IBM Corp). Qualitative records was designated by numbers and percent. Significant results were mediated at the 5% level. Chi-square test and Fisher's Exact or Monte Carlo correction are tests used in analysis.

Results

Our study was conducted on 400 chronically transfused patients. Among them, 365 were diagnosed as β thalassemia major, 15 as sickle cell disease and 20 as chronic renal failure patients. The studied cases included 189 males (47.3%) and 211 females (52.8%). and a statistically significant difference was detected regarding sex and antibody screening results (P= 0.039). **Table** 1.

Table (1): Relation between antibody screening results and sex

	Antibody screening results				χ^2	**P**
	Negative (n = 385)		**Positive (n = 15)**			
	No.	**%**	**No.**	**%**		
Sex						
Male	178	46.2	11	73.3	4.254*	0.039*
Female	207	53.8	4	26.7		

Age of our patients ranged from 10-65 years with a mean of 37.59 with a significant difference between the age of the cases and the studied disease categories (P= < 0.001). With a P value of 0.001, **Table** 2

Table (2): Relation between disease categories and age

	Disease categories						χ^2	^{MC}p
	Thalassemia (n = 363)		Sickle cell disease (n = 15)		Chronic renal failure (n = 22)			
	No.	%	No.	%	No.	%		
Age (years)								
≥ 10 – 15	300	82.6	2	13.3	0	0.0		
> 15 – 35	63	17.4	13	86.7	2	9.1	175.476*	<0.001*
> 35 – 65	0	0.0	0	0.0	20	90.9		

A statistically significant relation between the ages of the cases and the results of the antibody screening was discovered. **Table** 3

Table (3): Relation between antibody screening results and age

	Antibody screening results				χ^2	^{MC}p
	Negative (n = 385)		Positive (n = 15)			
	No.	%	No.	%		
Age (years)						
≥ 10 – 15	297	77.1	5	33.3		
> 15 – 35	69	17.9	9	60.0	13.997*	0.001*
> 35 – 60	19	4.9	1	6.7		

In terms of antibody screening results, 385 patients (96.3%) had negative antibody screening while only 15 patients (3.8%) were positive. According to antibody screening results, the percentages of patients with positive antibody screening results in chronic renal failure patients (13.6%) were significantly higher than in sickle cell patients (13.3%).And a percentage of (2.8%) was detected in thalassemia patients. **Table** 4

Table (4): Relation between disease categories and antibody screening result

	Disease categories						χ^2	^{MC}p
	Thalassemia (n = 363)		SCD (n = 15)		CRF (n = 22)			
	No.	%	No.	%	No.	%		
Antibody screening results								
Negative	353	97.2	13	86.7	19	86.4	9.263*	0.010*
Positive	10	2.8	2	13.3	3	13.6		

.A statistically significant relation between disease categories and antibody screening results was detected (P= 0.010). **Figure** 1

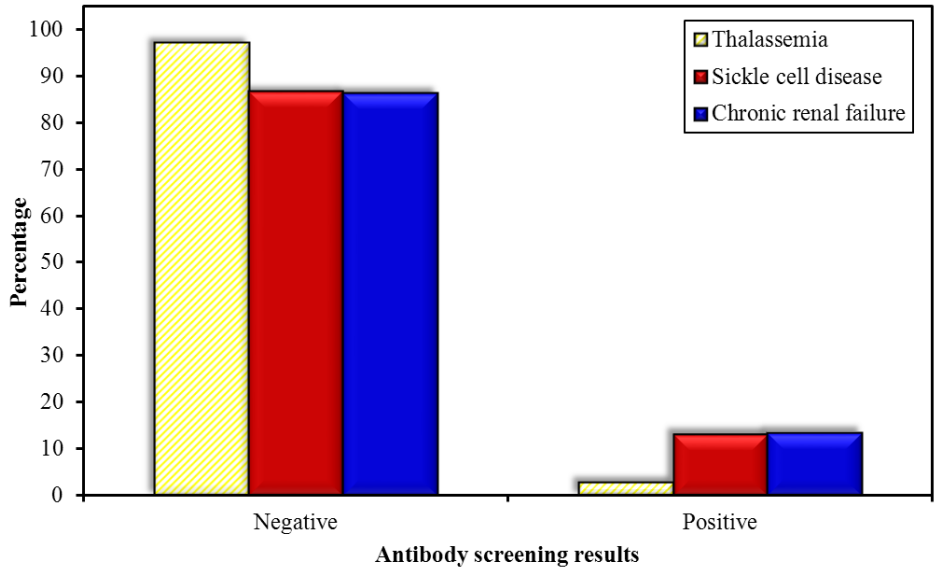

Figure (1): Relation between disease categories and antibody screening results

Regarding the age of starting transfusion, 121 cases (30.3%) received their first blood transfusion before the age of 1 year, while the remaining started later after one year of age. A significant statistical difference between antibody screening results and age of transfusion was detected (P=0.046). **Table** 5

Table (5): Relation between antibody screening results and age at start of transfusion

	Antibody screening results				χ^2	^{FE}p
	Negative (n = 385)		Positive (n = 15)			
	No.	%	No.	%		
Age at start of transfusion (years)						
< 1	120	31.2	1	6.7	4.108*	0.046*
≥ 1	265	68.8	14	93.3		

Regarding use of filtered blood, 150 patients (37.5%) received filtered blood units while 250 patients (62.5%) were not maintained on filtered blood. that came with a significant statistical relation between continued use of filtered blood or not and antibody screening results (P= 0.012). **Table** 6

Table (6): Relation between antibody screening results and transfusion of filtered blood

	Antibody screening results				χ^2	P
	Negative (n = 385)		Positive (n = 15)			
Transfusion of filtered blood	No.	%	No.	%		
Always filtered	149	38.7	1	6.7	6.322*	0.012*
Not always filtered	236	61.3	14	93.3		

Regarding spleen status, 209 of the patients (52.3%) had their spleen preserved while 191 patients (47.8%) were splenectomized. A significant relation between antibody screening results and spleen status was detected (P= 0.043). **Table** 7.

Table (7): Relation between antibody screening results and spleen status

	Antibody screening results				□□	p
	Negative (n = 385)		Positive (n = 15)			
	No.	%	No.	%		
Spleen status						
Preserved	205	53.2	4	26.7	4.088*	0.043*
Splenectomized	180	46.8	11	73.3		

χ^2: Chi square test for comparing between the two categories

Regarding the number of transfused units per year, we found that 119 patients (29.8%) received less than 24 units of packed red cells per year while 281 patients (70.3%) received more than 24 units per year. As regards relation to antibody screening results, a statistically significant relation with the number of transfused units per year, was detected (P= 0.047). **Table** 8

Table (8): Relation between antibody screening results and number of transfused blood units

Number of transfused blood units/ year	Antibody screening results				χ^2	^{FE}p
	Negative (n = 385)		Positive (n = 15)			
	No.	%	No.	%		
<24	118	30.6	1	6.7	3.973*	0.047*
≥ 24	267	69.4	14	93.3		

Regarding the frequency of transfusion reactions in patients with positive screening results, two didn't experience any. Every now and then, reactions were reported by 6 patients and time reactions were reported by 7 patients. Regarding antibody identification results of positively screened cases (n=15), the identified alloantibodies were: anti-E (33.3%), anti-D (26.7%), anti-kell (13.3%), anti-C (6.7%), anti-c (6.7%), anti-Fy^a (6.7%), anti-Jk^a (6.7%). **Table** 9

Table (9): Distribution of the studied cases according to antibody identification results (n=15)

Antibody identification results	No.	%
Anti-E	5	33.3
Anti-D	4	26.7
Anti-C	1	6.7
Anti-c	1	6.7
Anti-kell	2	13.3
Anti-Fy^a	1	6.7
Anti-Jk^a	1	6.7

Discussion

ß-thalassemia is common hemolytic anemia in Egypt as many other countries. [1, 10] Frequent (RBC) transfusions is the chief management for severe cases of thalassemia. However, this may lead to transfusion-related complications. [11]Alloimmunization is one of the most common and serious complications of repeated transfusions Moreover, (DHTR) is a life-threatening complication associated with alloimmunization. [12] It results in a severe decrease in recipient's hemoglobin levels, secondary to immune hemolysis. [13]

Results from previous studies have demonstrated alloantibodies frequencies in patients ranging from 5% to 30%. Also RBC autoantibodies can cause hemolysis and cross-matching difficulties. [14, 15]

Few Egyptian researches have studied the causes and frequency of alloimmunization. In our work, we observed the predominance and the shared alloantibodies in multitransfused persons. We also assessed the associated reasons that affect the occurrence of alloimmunization and established an incidence rate of 3.8%, with 7 alloantibodies identified in 15 patients.

The low frequency of alloimmunization in this study can be ascribed to the similar ethnic group. It may be also due to study size. [16] In Alexandria University, blood group serology by automated gel method was introduced 8 years ago. .

Similar Lower rates of alloimmunization of 4.9% to 10.1 % were described in nations with similar populations, in Pakistan, Greece, Iran, Italy, and India. [16] Meanwhile, Politis et al showed a rate of alloimmunization of 11.6%. [17] Chao et al [18] And Shenoy et al [19] stated that alloimmunization frequencies were 9.4 and 9.5%.

Meanwhile, Abdelrazik et al found alloimmunization prevalence was 7.98%. [20]. Another Egyptian study by Ahmed et al found 11.3% of patients established alloantibodies; 9.7% of these alloantibodies were clinically noteworthy. [21]. RBC alloantibodies were found in (10.5%) patients by El Sewefy and colleagues [22] The Egyptian studies with higher alloimmunization level were directed in the main capital that contain many different communities . For example, El Danasoury et al who found that (19.5%) of their patients were alloimmunized with a lower alloimmunization was detected in the LDEP (limited donor exposure program) group; 8.3% compared to 21.6% in the non-LDEP one. [23]

Hussein et al also showed a high rate of alloimmunization of 22.8% and stated that it was possibly affected by the antigenic difference between the recipient and donors' RBCs [24].The high rate described in the Singer et al study among the Asian population (22% and 20.8%) was accredited to RBC antigenic dissimilarity of white blood donors and Asians recipients [25]. The specificity of recorded alloantibodies in this work was against rh and kell systems (73.4%) which is similar to preceding reports of Zaman S (74.7%) [26], Bhatti F et al [27] and Karimi M (76.3%) [28]Another support comes from Iranian studies. [29] In Western countries alloantibodies are focused against C, E, and Kell antigens. [30]. Alloimmunization with Anti-D **was** also described.[11]

In Rh-negative patients, the high frequency of anti-D antibodies detected in our study is likely related to transfusions of weak D antigens units. Regarding the AABB Recommendations, weak-D testing is necessary in blood donors but is not mandatory when testing a patient or a pregnant woman [31].

Hussein and Teruya [32] established an anti-D antibody incidence of 63.5% in Rh-negative thalassemia children. Blood groups Genotyping has simplified the documentation of different D variants. Anti-D alloimmunization has been recognized for persons with partial D and weak D types 4.0, 4.2 (DAR), 11, and 15 [33, 34]. In blood constituents, leukocytes may cause a higher B-cell function that increase alloimmunization to RBCS antigens [35, 36]. 62.5 % of our patients were exposed to non-leukoreduced blood. Unfiltered blood transfusion had a higher alloimmunization rate (93.3%). The leukoreduction rate in Egypt is less than 5 * 10^6/μL . [37-39]. In our study, splenectomized persons had a higher proportion of alloimmunization. Splenectomy may boost the immune response to antigens [40]. Chou and colleagues [11] establish a significantly increased alloimmunization risk in splenectomized persons.

We also establish that the occurrence of alloimmunization was related to the starting age of transfusions. Singer et el informed that early transfusion at ages younger than 1 to 3 years may have some immune defense against alloimmunization [25]. Female gender has been recognized as a risk reason for alloimmunization [41]. But, our work did not determine that; in our study males had a higher alloimmunization rate (73.3%).In our work, a significant relationship between alloimmunization and number of transfused units ($P < 0.047$) was detected. Among our 400 patients, there were 22 chronic renal failure patients. Three of them (13.6%) have formed alloantibodies. Our results were supported by Babiker et al where they

establish the incidence of alloantibody in CRF patients was 13.1% [42]. This finding is higher than the frequency of other studies [43, 44].

Conclusions

From this study, we concluded that the frequency of red cell allo-antibodies was low (3.8%) but had a significant relation with clinical diagnosis of the patient, male sex, older age at start of transfusion, transfusion of unfiltered blood, number of transfused PRBCs units and patients being splenectomized. The majority of those antibodies were related to Rh and Kell systems. And most of them were clinically significant.Our study shows the requisite for a cost-effective transfusion program in developing nations. Production of antigen-negative or antigen-matched blood can be prepared to multiply transfused persons. Early transfusion is of help for reducing alloimmunization.

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