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Evaluation of Red Cell Alloimmunization in Thalassemic Patients with Repeated Blood Transfusion

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Abstract

Background: Thalassemia is a group of inherited disorders that arise as a result of certain mutations in hemoglobin (Hb) genes, affecting the synthesis of globin chain (α, β), which leads to certain pathophysiological disorders as chronic hemolytic anemia and ineffective erythropoiesis. Patients who suffered from thalassemia receive blood transfusion regularly every month or even less than month according to the severity of hemolysis. Repeated blood transfusion results in many complications including formation of alloantibodies or autoantibodies against transfused RBCs.

Aim of the Study: To evaluate alloantibodies and autoantibodies formation in thalassemic patients with repeated blood transfusion and try to avoid hemolytic transfusion reaction which may occur during future blood transfusion.

Patients and Methods: The present study was carried on a hundred (100) pediatric thalassemic patients. All included subjects were submitted to full history taking, clinical examination and laboratory investigations. Screening gel tests for allo and autoantibodies.

Results: We found that the frequency of development of red cell alloimmunization in thalassemic patients receiving multiple blood transfusions is 26% and autoantibodies 0%. The most frequently occurring alloantibodies was anti k (13%), anti Jsb (13%), anti Lub (13%), anti D (5%), anti Jka (5%), anti M (5%), anti S (5%), anti Kpa (4%), anti N (4%), anti C (3%), anti Lea (3%), anti c (1%), anti Fyb (1%), anti Lea (1%) and anti p1 (1%).

Conclusion: Thalassemic patients with repeated blood transfusion develop a significant rate of alloantibodies. Some of these antibodies are clinically significant and may affect the safety of further blood transfusion and cause hemolytic transfusion reaction. So antibody screening test should be performed to patients with chronic transfusion to avoid the hazardous of future transfusion.

Key Words: *Alloantibodies – Autoantibodies – Hemolytic transfusion reaction.*

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Introduction

THALASSAEMIA is among the most common genetic disorders worldwide, particularly in the Mediterranean region, Africa, the Middle East and Southeast Asia. Thalassaemia is a hereditary anemia resulting from defects in haemoglobin polypeptide chain production [1,2].

The lack of polypeptide chain results in interference in erythoroid maturation, function and ineffective erythropiosis. Thalassaemia major is characterized by major or total suppression of (Beta) chain synthesis in the homozygous form of the disease. Lifelong Red Blood Cell (RBC) transfusion is the treatment of thalassaemia major patients which alleviates the anemia and suppress the compensatory mechanism responsible for clinical disease including deaths in these patients [3].

Regular blood transfusions, usually administered every 2 to 5 weeks to maintain a pre-transfusion haemoglobin level above 9-10.5g/dl. One of the complications of blood transfusion is the formation of alloantibodies and autoantibodies against Red Blood Cell (RBC) antigens [4].

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 hemolysis and difficulty in blood cross-matching [5].

Patients with autoantibodies may have a higher transfusion rate and often require immunosuppressive drugs, a splenectomy, or alternative treatments [6].

The causes of alloimmunization in thalassaemic 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 [7].

Aim: It was to evaluate alloantibodies and autoantibodies formation in thalassemic patients with repeated blood transfusion and try to avoid hemolytic transfusion reaction which may occur during future blood transfusion.

Patients and Methods

The present study was carried at Clinical Pathology Department in Tanta University on a hundred β-thalassemic children who presented to Pediatric Hematological Unit of Tanta University Hospitals from May 2016 – May 2017. They were 56 males and 44 females, their ages ranged from 8 months to 15 years. All patients were subjected to the following: A- Detailed Clinical evaluation including history taking and clinical examination: Focusing on number of blood transfusion per year and splenomectomy. B- Laboratory investigations: Complete blood count, Blood group (ABO and Rh), Alloantibody screening anti D/-C/-c/-E/-e/-K/-k/-Fya/-Fyb/-Jka/-Jkb/-Lea/-Leb/-M/-N/-S/-s/-P and Direct Coombs test.

Methods:

Antibody screening test: The principle of the test is based on the gel technique described by Y.Lapierre for detecting red blood cell agglutination reactions. The agglutination occurs when the red blood cell antigens contact the corresponding antibodies, present in the reagent or in the plasma sample. The DG gel card consisting of 8 microtubes. Each microtube contain dextran mixed with anti-human globulin. The microtubes that contains anti-human globulin act by agglutinating the red blood cells sensitized by IgG antibodies or complement fractions. During centrifugation and depending on their size, the red blood cell agglutinations are trapped in the surface or along the gel column. The non-agglutinated red blood cells descend to the bottom of the microtube. 50 µl of reagent red blood cell (I, II, III) were carefully dispensed into the corresponding microtubes. 25 µl of the plasma of each patient were added to the three microtubes containing the red blood cell reagent (I, II, III). The DG gel card was incubated for 15 minutes at 37ºc and then the card was centrifuged in centrifuge for DG gel cards. The results have been read as shown in Fig. (1) and (Table 1) as negative or positive with different grades. According to which red blood cell reagent either I, II or III give positive or negative result, the antibody which cause the agglutination was detected.

Direct coombs test: Wash the red blood cell of the patient three times with saline. Prepare a 1% red blood cell suspension in DG gel solution. Centrifuge in centrifuge for DG gel cards. Detect positive or negative results as in Fig. (1) and Table (1).

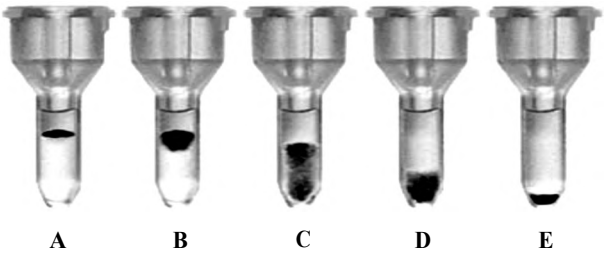

Fig. (1): Result interpretation of gel cards.

Table (1): Result interpretation of gel cards.

A	Positive	+ 4	• Band of agglutinated red blood cells in the upper part of the column.
B	Positive	+ 3	• Upper band of medium size agglutinations in the upper half of the column.
C	Positive	+ 2	• Small or medium size agglutinations throughout the column.
D	Positive	+1	• Some small agglutinations in the column.
E	Negative		• Band of red blood cells at the bottom of the column and no visible agglutinations in the rest if the column.

Statistical analysis: Data analysis were performed using SPSS 10.0 (Statistical package for science and society). Student's *t*-test was used for comparison between groups regarding quantitative data and Chi-square test (χ^2) for comparison between two groups regarding qualitative data. A two-tailed *p*-value less than 0.05 was considered statistically significant and highly significant results at *p*-value less than (0.01).

Results

This study was conducted on a hundred β-thalassemic children who presented to Pediatric Hematological Unit of Tanta University Hospitals. All patients were diagnosed as β thalassemia, 72 patients were diagnosed as thalassemia major and 28 patients were diagnosed as thalassemia intermedia. All patients in this study were conducted to autoantibody screening by coombs test and the result was 0% but alloantibody screening show 26% positive.There was 22 out of 72 patients were

positive for alloantibodies in thalassemia major (30.6%) and 4 out of total 28 patients were positive for alloantibodies in thalassemia intermedia (14.3%) but the negative alloantibody screening represent 50 out of 72 patients in thalassemia major (69.4%) and 24 out of 28 patients (85.7%) in thalassemia intermedia as shown in Fig. (2).

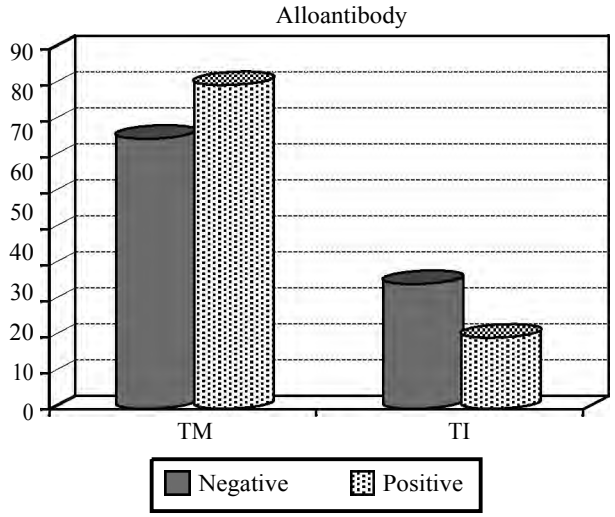

Fig. (2): Alloantibody formation in both thalassemic groups.

This study revealed that the most frequently detected alloantibodies was anti k (13%), anti Jsb (13%), anti Lub (13%), anti D (5%), anti Jka (5%), anti M (5%), anti S (5%), anti Kpa (4%), anti N (4%), anti C (3%), anti Lea (3%), anti c (1%), anti Fyb (1%), anti Leb (1%) and anti $_{p1}$ (1%).

In this study we try to find the factors which affecting alloantibody formation:

Sex: There is no significant association between sex of patient regarding alloantibody formation (p=0.840) as shown in Table (2).

Table (2): Relation between sex of patient and alloantibody formation.

Sex	Negative Alloantibody	Positive Alloantibody	Total
Male: N (%)	41 (55.4%)	15 (57.7%)	56 (56%)
Female: N (%)	33 (44.6%)	11 (42.3%)	44 (44%)
Chi-square: χ^2	0.041		
p-value	0.840		

Age: There was a significant association between age of patient and alloantibody formation (p=0.002) as shown in Table (3).

Table (3): Comparison between age of thalassemic patients/year and alloantibody frequency.

Age	Negative Alloantibody		Positive Alloantibody
Range	0.83-16		0.67-16
Mean ± SD	6.61±3.7		9.60±4.9
t-test		10.148	
p-value		0.002*	

Age at first blood transfusion: This results revealed that there is significant relation between age at first transfusion and alloantibody formation (p=0.024) as shown in Table (4).

Table (4): Comparison between age at the first transfusion and alloantibody formation.

Age at the first transfusion	Negative Alloantibody		Positive Alloantibody
Range	4-24		6-24
Mean ± SD	8.35±3.47		6.69±2.02
t-test		5.283	
p-value		0.024*	

Number of blood transfusion/year: There is no relation between number of transfusion/year and alloantibody formation (p=0.101) as shown in Table (5).

Table (5): Comparison between number of transfusion and alloantibody formation.

Number of transfusion/year	Negative Alloantibody		Positive Alloantibody
Range	2-36		2-28
Mean ± SD	12.70±7.79		15.65±7.88
t-test		2.745	
p-value		0.101	

Splenectomy: There is significant difference between positive alloantibody and negative alloantibody groups regarding splenectomy (p=0.001) as shown in Table (6).

Table (6): Comparison between number of transfusion and alloantibody formation.

Splenectomy	Negative Alloantibody	Positive Alloantibody	Total
Yes: N	18	16	34
No: N	56	10	66
Chi-square: χ^2	11.874		
p-value	0.001*		

Discussion

This study was conducted to find out the frequency of red cell alloantibodies and autoantibodies in thalassemic children in Tanta university hospital so that the need for pre-transfusion screening in these patients can be evaluated. According to this study, the frequency of development of red cell alloimmunization in thalassemic patients receiving multiple blood transfusions is 26% and autoantibodies 0%.In this study, the most frequently occurring alloantibodies was anti k (13%), anti Jsb (13%), anti Lub (13%), anti D (5%), anti Jka (5%), anti M (5%), anti S (5%), anti Kpa (4%), anti N (4%), anti C (3%), anti Lea (3%), anti c (1%), anti Fyb (1%), anti Lea (1%) and anti $_{p1}$ (1%).

A different rate was reported in a study by Cheng and his collaegues in China where a total of 382 patients were studied. Eighty-eight patients (23.0%) were reported to have RBC antibodies [8].

A high rate of autoantibody was detected by Dhawan and his colleges in India who performed a study on 319 patients. 5.64% developed alloantibodies and 28.2% developed autoantibodies. Age at first transfusion was significantly higher in alloimmunized than non-immunized patients [9].

Different studies were conducted across the world and reported different frequency of alloantibody and autoantibody formation in thalassemic patients who received repeated blood transfusion. High alloimmunization in thalassemia patient was reported from Taiwan (37%) [10], Arab (30%) [11], and Pakistan (9.2%) [12].

In this study we tried to find out the factors which affect the formation of alloantibodies including age, sex, ethinicity, age at first transfusion, number of blood transfusion and splenectomy. The results revealed that there was a significant association between alloantibody formation and age, age of patient at the first blood transfusion and splenectomy. Previous studies have shown that transfusion at an early age (<1-year-old) may lead to development of immune tolerance in recipients, and this phenomenon provides protection from alloimmunization. Bhatti agreed with Zaidi that the red cell alloantibody formation was not influenced by age at first transfusion, number of blood transfusions and ethnicity [13].

But Karimi et al., agreed that there was a significant association between age and alloantibody formation as they reported that all the patients in their study who had developed alloantibody were in the age group of 6 years or more [14].

And our results also revealed that there was no significant association between alloantibody formation and number of blood transfusion and this was in agreement with Azza et al., [15]. Who reported that there was no significant association between alloantibody, autoantibody formation and number of blood transfusion. Female gender has been known as a risk factor for alloimmunization [16].

However, our results did not demonstrate higher immunization rates in females. On the other hand, males had a higher alloimmunization rate in a study performed by Hussein et al., [17].

The number of units transfused is an important predictor of alloimmunization in patients who receive long term blood transfusion [18]. But in this study there is no significant relation between number of transfusion and alloantibody formation.

Splenectomy leads to decreased removal of damaged red cells, which may result in exposure of new antigens and risk of alloimmunization [19].

In this study, the results revealed that there was a significant association between splenectomy and alloimmunization. Sadeghian et al., (2009) reported that splenectomy preceded the development of antibodies in most cases [20].

Azza et al., reported that the low immunization rate in their study could be explained by the similarity in ethnicity between patients and donors as all of them were Egyptian. On the other hand, the alloimmunization rate was high in Greece. The higher rate of alloimmunization was explained by the heterogeneity of the population living in Greece [21,22]. In this study all patients and donors were Egyptian and there was a high rate of alloimmunization (26%).

Conclusion:

Thalassemic patients with repeated blood transfusion develop a significant rate of alloantibodies. Some of these antibodies are clinically significant and may affect the safety of further blood transfusion and cause hemolytic transfusion reaction. So antibody screening test should be performed to patients with chronic transfusion to avoid the hazardous of future transfusion.

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Conflicts of interest:

No conflicts of interest declared.

Authors' contributions:

All authors had equal role in design, work, statistical analysis and manuscript writing. All authors have approved the final article work.

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تقييم الأجسام المضادة فى مرضى أنيميا البحر المتوسط المعالجين بنقل الدم المتكرر

أنيميا البحر المتوسط مرض وراثى ينتج من نقص فى تصنيع الهيموجلوبين مما يؤدى إلى تكسير لكرات الدم الحمراء مما يجعل المريض فى إحتياج دائم لنقل الدم المتكرر ونقل الدم المتكرر يؤدى إلى كثير من المضاعفات التى تضر المريض. ومنها تكون أجسام مضادة ضد خلايا دم المريض أو خلايا دم المتبرع.

الهدف من الدراسة: هو تقييم تكون هذه الأجسام المضادة فى المرضى الذين يتلقون نقل الدم باستمرار لمحاولة تجنب المضاعفات التى تنتج عن تكون هذه الأجسام المضادة.

طرق البحث: أجريت الدراسة على مائة طفل مريض يعانون من الثلاسيميا ويعالجون بنقل الدم المتكرر وبعد إستبعاد المرضى المصابين بأمراض المناعة أو يتلقون أدوية تؤثر على الجهاز المناعى تم أخذ تاريخ مفصل عن المرض وبالتحديد عدد مرات نقل الدم وعملية إزالة الطحال وتم عمل فحص شامل للمرضى وقد خضعوا للفحوصات الآتية: صورة دم كاملة، فصيلة الدم والأجسام المضادة.

النتائج: أظهرت النتائج وجود أجسام مضادة فى ٢٦٪ من إجمالى مائة طفل يعانون الثلاسيميا وكان بيانها كالتالى: أنتى كى (١٣٪) أنتى جى إس بى (١٣٪) أنتى إل يو بى (١٣٪) أنتى دى (٥٪) أنتى إم (٥٪) أنتى إس (٥٪) أنتى كى بى إى (٤٪) أنتى سى (٣٪) أنتى إل إى ايه (٣٪) إنتى سى (١٪) أنتى اف واى بى (١٪) أنتى إل إى بى (١٪) أنتى بى (١٪). وبمقاونة النتائج وجدنا أن سواء عمر المريض أو عمره عند أول مرة نتلقى فيها نقل دم أو إزالة الطحال قد تكون من العومل التى تؤثر على تكون هذه الأجسام المضادة فى حين أنه لا توجد علاقة بين عدد مرات نقل الدم أو جنس المريض وتكون هذه الأجسام المضادة.

الاستنتاج: نقل الدم المتكرر يؤدى إلى تكون أجسام مضادة قد تؤدى إلى مضاعفات قد تؤدى بحياة المريض عند نقل الدم مرة أخرى وبالتالى فإن تحديد وجود الأجسام المضادة فى دم المريض من علمه قد يمكننا من تجنب هذه المضاعفات عند نقل الدم مرة أخرى.