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Evaluation of Alloantibodies level in chronically transfused children with Thalassemia and Sickle cell anemia in Zagazig university hospitals.

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ABSTRACT

Background: alloimmunization can occur in sickle cell anemia (SCA) and thalassemic patients undergoing chronic blood transfusion may lead to developing of a delayed hemolytic transfusion reaction (DHTR), which can be life-threatening condition. Our study aimed to detect the frequency of alloantibodies in chronically transfused blood in children with thalassemia and sickle cell anemia presenting in Zagazig university hospitals**.**

Methods: our Comparative cross-sectional study included 190 of chronically transfused patients (thalassemia and SCA) in pediatric department, full clinical exam, lab investigations and Antibody screening and identification were carried for all participants.

Results: alloantibodies were presented by 16.3 % in thalassemic patients and by 14.7 % in SCA patients. there is statistically non-significant difference between presence of alloantibodies and gender, Rh typing, spleen status, blood groups and ABO blood groups.

Conclusion: Thalassemic and sickle cell anemic patients are at risk of alloantibodies formation and their complications

Keywords: Alloantibodies; Thalassemia; SCA; DHTR; RBCs; DHTR.

INTRODUCTION

Beta-thalassemia (β-thalassemia) results from deficiency of the hemoglobin subunit beta (hemoglobin beta chain) that results in microcytic hypochromic anemia, an abnormal peripheral blood smear with nucleated red blood cells, and reduced amounts of hemoglobin A (HbA). thalassemia major patients suffering from severe anemia and hepatosplenomegaly; it usually presents within the first two years of life. Without treatment, affected children have severe failure to thrive and shortened life expectancy. Treatment is achieved through a regular transfusion program and iron chelation therapy [1].

Sickle cell disease (SCD) is an inherited blood disorders. The most common type is known as sickle cell anemia (SCA). It results in an abnormal hemoglobin and a rigid, sickle-like shape RBCs under certain circumstances. Manifestations of sickle cell disease typically begin around 5 to 6 months of age such as attacks of pain ("sickle cell crisis"), anemia, swelling in the hands and feet, bacterial infections and stroke. Long-term pain may develop as people get older. The average life expectancy in the developed world is 40 to 60 years [2].

Repeated blood Transfusion can lead to erythrocyte alloimmunization with serious complications. The antibodies are often directed against antigens expressed on RBCs of white persons, which represent the majority of donors in Western countries. Finding compatible units lacking those antigens can sometimes be difficult and identifying and characterizing the antibodies can be time-consuming and laborious, causing transfusion delays. Genetic and acquired patient-related factors are likely to influence the process of alloimmunization [3].

Alloimmunization is the most serious adverse effect in thalassemia as it leads to the development of a delayed hemolytic transfusion reaction (DHTR), which can be life-threatening where the patient's hemoglobin level falls below the pre transfusion level, suggesting that, in addition to hemolysis of the transfused RBCs, the patient's own RBCs are lysed, a condition known as hyperhaemolysis. Hemolysis is worsened by additional transfusions and further worsen the degree of anemia., because alloimmunization is known to trigger autoantibody production.

However, DHTR/hyperhaemolysis cases have also been reported in the absence of detectable alloantibodies or autoantibodies [4].

Antibodies to minor RBC antigens have been reported in hematopoietic progenitor cells (HPC) transplantation at a rate of ~1% to 8.6%, with varying clinical effects. Various antibodies (anti-**Jk*b***, anti-M, anti-**Le*b***, anti-**Di*b***, anti-E, anti-**Jk*a***, and anti-K) have been identified, with a mean time of detection of approximately 1 month, although some may appear much later. This observation demonstrates that transplantation alloantibody formation is still possible in the peri transplant period, despite profound immunosuppression [5].

The clinical impact of non-ABO antibodies is generally insignificant; however, severe hemolysis from antibodies to the Kidd (Jk) blood group system have been identified. In these cases, previous sensitization of the donor to the Kidd antigen occurs but the pre-transplantation antibody screen did not detect the antibody. This phenomenon is common in patients with antibodies to the Kidd blood group system, and when re-exposed to the corresponding antigen, may result in an anamnestic response with concomitant hemolysis [6].

The aim of our study was to detect the prevalence of alloantibodies in chronically transfused blood in children with thalassemia and sickle cell anemia presenting in Zagazig university hospitals.

METHODS

Our Comparative cross-sectional study was designed to determine the prevalence of alloimmunization among chronically transfused children with β-thalassemia major and sickle cell anemia receiving regular blood transfusion at outpatient clinic of pediatric hematology unit at Zagazig University Hospitals during a period of 1 year from November 2017 to October 2018 and do alloantibodies screening for all patient then all patients with alloantibodies +ve by screening will subjected for antibody identification. 190 patients included in our study of both sexes. Age ranged from 1-18 years. Inclusion criteria include all patients diagnosed with Beta thalassemia and SCA and need repeated blood transfusion of both sexes between 1-18 years old. our exclusion criterion is the refusal of the Patient or his parents to participate in the study.

All cases enrolled in the study were subjected to Detailed medical history, clinical examination, and Laboratory investigations (CBC, LFTS, KFTS, Serum iron, Serum ferritin, Extended blood group phenotyping for children receiving blood.

Specific tests for Antibody screening and identification were carried out using column agglutination technology by micro-typing structure reagent (Identisera Diana P Grifols; Spain and Grifols DG gel card, Diana; Spain). Antibody identification was done for patients with positive screening test. Profitable RBC panel (Identisera Diana P Grifols, spain), 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. DAT was performed using a poly specific antihuman globulin (Grifols DG gel card, Diana; Spain).

Statistical analysis

Data analysis was performed using the software SPSS (statistical package for the social science) version 20. quantitative variables were described using their means and standard deviations. Categorical variations were described using their absolute frequencies and to compare the proportion of categorical data, chi square test and fisher exact test were used when appropriate. Data were collected and submitted to statistical analysis.

Kolmogrov-smirnov (distribution type) and Levene (homogeneity of variances) tests were used to verify assumptions for use in parametric test. To compare means of two groups, independent sample t test was used when appropriate nonparametric test (mann whitney) was used to compare means when data was not normally distributed and to compare medians in categorical data. To compare means of more than two groups, one way ANOVA was used for normally distributed data and kruskal wallis test was used for data which was not normally distributed. ROC curve analysis was used to assess the best cut off of studied parameters. the level statistical significance was set at 5% ($p<0.05$). highly significant difference was present if $p\leq0.001$.

Ethical declaration

Written informed consent was obtained from all participants" parents, the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University. The study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Approval was taken from the insititutional review board, faculty of medicine, Zagazig university

RESULTS

There is statistically significant difference between the studied groups regarding gender where the largest percentage of SCA patients were males. There is statistically non-significant difference between both groups regarding age (table. 1).

There is statistically non-significant difference between the studied groups regarding TLC, hematocrit, reticulocytic count and platelet count. There is statistically significant difference between them regarding MCH & Regarding hemoglobin level in each group, there is statistically significant increase before and after transfusions (table. 2).

There is statistically non-significant difference between the studied groups regarding TLC, hematocrit, reticulocytic count and platelet count. There is statistically significant difference between them regarding MCH & Regarding hemoglobin level in each group, there is statistically significant increase before and after transfusions (table 3).

Regarding serum alloantibodies level, there is statistically non-significant difference between the studied groups regarding serum alloantibodies level either by screening or identification (table. 3). The largest percentage of antibodies positive patients within each group was E Type Alloantibodies (80% in Thalassemia and 70% in SCA). In thalassemia patients, platelet count was higher in patients with positive alloantibodies. Also There is statistically non-significant difference between presence of alloantibodies in sickle cell anemia patients regarding all CBC findings (TLC, Platelet count, TLC, hematocrit, MCV, MCH and reticulocytic count. There is statistically non-significant difference between presence of alloantibodies in sickle cell anemia and thalassemia patients regarding serum iron and ferritin level.

Regarding qualitative data for alloimmunization in SCA and thalassemia patients, there is statistically non-significant difference between presence of alloantibodies and gender, Rh typing, spleen status, blood groups and ABO blood groups.

Regarding comparison between the frequency of transfusion in positive and negative alloimmunized thalassemic and SCA patients, there is statistically significant difference between presence of alloantibodies and frequency of blood transfusion in thalassemic patients (tables 4, 5).

When comparing between alloimmunization in SCA patients and age, hemoglobin level and transfusion index. In thalassemia patients, there is statistically significant difference between alloimmunization and age of first transfusion, transfusion index, hemoglobin before and after transfusion. But in SCA patients, there is statistically significant difference between alloimmunization and age of first transfusion but there is non-significant difference between it and age, hemoglobin before and after transfusion.

Finally, prevalence of alloimmunization by identification for thalassemic patients only was 16.3 %. And for SCA patients only was 14.7 %. And screening tests were 91.6% accurate in detecting them.

Table 1: Demographic data of the studied groups.

	Thalassemia	SCA	p
	N (%)	**N (%)**	
Gender: Male Female	 77 (63.1%) 45 (36.9%)	 61 (89.7%) 7 (10.3%)	 <0.001 HS
	Thalassemia	**SCA**	
Age: Mean ± SD Range	 **7.38** ± 3.42 2 – 17	 **7.63** ± 2.84 2 - 15	 **0.418**

Table 2: Comparison of the studied groups regarding complete blood count.

	Thalassemia group	SCA group	p
TLC (10^3/cmm): Mean ± SD Range	 18.63 ± 13.13 4 – 83.4	 19.16 ± 14.7 4.5 - 76	 0.947 NS
Hematocrit (%): Mean ± SD Range	 21.44 ± 5.58 10.1 - 34	 22.17 ± 5.74 10.1 - 34	 0.6 NS
Hemoglobin (g/dl): Mean ± SD Pre-transfusion Post transfusion	 6.75 ± 0.93 8.6 ± 1.06	 6.61 ± 0.94 8.51 ± 1.05	 0.312 0.602

	Thalassemia group	SCA group	p
p	<0.001 (HS)	<0.001 (HS)	
MCV (fl): Mean ± SD Range	 77.38 ± 4.42 70 – 85.8	 77.61 ± 4.27 65.8 – 85.8	 0.730 NS
MCH (pg): Mean ± SD Range	 25.49 ± 2.43 20 – 34	 24.56 ± 2.33 18 - 30	 0.011 S
Reticulocyte count (%): Mean ± SD Range	 7.62 ± 3.81 2 – 22	 7.92 ± 3.74 3 - 22	 0.568 NS
Platelet count: Mean ± SD Range	 400.78 ± 130.06 207 - 680	 381.56 ± 147.58 111 - 680	 0.287 NS

Table 3: Comparison between the studied groups regarding serum alloantibodies by screening and identification.

	Thalassemia	SCA	p
	N (%)	**N (%)**	
By screening: Negative Positive	 89 (73%) 33 (27%)	 55 (80.9%) 13 (19.1%)	 0.221 NS
By identification: Negative Positive	 102 (83.6%) 20 (16.4%)	 58 (85.3%) 10 (14.7%)	 0.760 NS

Table 4: Comparing the frequency of transfusion in positive and negative alloimmunized thalassemic patients.

parameter	Antibody screening and identification [N (%)]			P Value	Sign
	Alloantibodies present	**Alloantibodies absent**	**z**		
Frequency of transfusion (U/year)					
<17	**0 (0%)**	**80 (78.8%)**	**Fisher**	**<0.001**	HS
≥17	**20 (100%)**	**22 (21.2%)**			
HS, highly significant.					

Table 5: Comparing the frequency of transfusion in positive and negative alloimmunized SCA patients**.**

parameter	Antibody screening and identification [N (%)]			P Value	Sign.
	Alloantibodies present	**Alloantibodies absent**	**z**		
Frequency of transfusion (U/year)					
< 6	**1 (10%)**	**50 (86.2%)**	**Fisher**	**<0.001**	HS
≥ 6	**9 (90%)**	**8 (13.8)**			
HS, highly significant.					

DISCUSSION

ß-thalassemia is an inherited disorder of hemoglobin synthesis characterized by deficient synthesis of the ß-globin chain that causes severe anemia. Repeated blood transfusion and chelation therapy have resulted in the prolongation of the life expectancy of these patients, thus changing the course of the disease from a rapidly fatal disease of childhood to a chronic disease compatible with a prolonged life. Anti-RBC antibodies, (alloantibodies and or autoantibodies) can significantly complicate transfusion therapy [7].

Red blood cell transfusion is a key component of therapy in the successful management of sickle cell disease, Transfusion therapy facilitates improved blood and tissue oxygenation reduces the propensity for sickling by diluting the host cells and temporarily suppresses the production of red cells containing HbS. In spite of the beneficial effects of transfusion therapy in sickle cell disease, there are still adverse effects associated with transfusion that can lead to serious short- and long-term morbidity including transmission of infections, transfusion hemosiderosis, alloimmunization among others [8].

Limited data are available regarding alloimmunization in frequently transfused thalassaemic and sickle cell anemic patients in our area. In the present study, we investigated the prevalence of the alloimmunization incidence in frequently transfused children with B-thalassaemia and SCA.

As regarding to genderdifference, for thalassemia, males were 77 (63.1%) and females were 45 (36.9%) & for SCA, males were 61 (89.7%) and females were 7 (10.3%). As regarding age, the mean +SD of age was 7.38 ± 3.42 for thalassemic patients & ranged between 2 - 15 years with mean +SD 7.63 ± 2.84 for SCA. there was a significance increase in Hb level among the studied groups regarding pre and post transfusion, but by comparison the response to blood transfusion in patients with positive alloantibodies there were a statistically significant lowering in Hb level after transfusion among +ve cases compared to -ve cases suggesting hemolytic reaction due to alloantibodies.

The serum ferritin level of the thalassemic patients ranged from 103 to 9314 ng/ml (mean ± SD 1791.09 ± 2090.31 ng/ml), while SCA patients range form 358 - 9870 ng/ml (mean ± SD 2765.75 ± 2702 ng/ml). In our study, the increase in serum ferritin in SCA patients is due to non-compliance to oral chelation therapy while in thalassemic patients, there is continuity in taking the oral chelation therapy. Which is consistent with what reported by Shah et al [8]. Iideal chelation can maintain serum ferritin levels within normal limits irrespective of the total number of transfusions. However, such a uniform maintenance of serum ferritin levels was not found. Thus, indicating irregular and inadequate chelation practices or variable response to chelation therapy.

The incidence of alloimmunization in our study for Thalassemia and SCA was 13.7%, The most common alloantibodies detected were anti-E alone (18.8%), nonspecific (12.5%), inconclusive (12.5%), anti-K (10.4%), and anti-C (6.3%). El-Danasoury et al [9]. reported a slightly lower overall alloimmunization rate of 11.5% in their study that was carried out on 235 Egyptian patients with thalassaemia and SCA. In the study of Hsieh et al [10]. The most frequently encountered alloantibodies in their study were anti-E and anti-C. This predominance is not unexpected given the strong immunogenicity of these antigens.

In our study the prevalence of alloimmunization by identification for thalassemic patients only was 16.3 %. it was similar to several countries namely, 16.32% from Iran Davari et al [11]. and 22% from California Singer et al [12]. and 19% from the CDC data in the USA on Asian and Caucasians patients Vichinsky et al [13] the frequency of alloimmunization is reduced when the patient receives blood from the same ethnic groups like those living in Hong Kong Ho et al [14]. And in Saudi Arabia Abdel Gader et al [15]. Higher incidence of alloantibody formation of 30% was reported by Ameen et al [16]. and was explained by the heterogeneity and the racial diversity in donors and recipients.

In our study the prevalence of alloimmunization by identification for SCA patients only was 14.7 %. Moreira and colleagues reported an RBC alloimmunization rate of 12.9% in Brazilian sickle cell disease (SCD) patients.

There is unknown relation between the number of blood units transfused and antibody formation is in thalassemia, but it is an important factor for increased alloimmunization in patients who receive multiple transfusions Ansari et al [17]. In the current study, there was no statistically significant difference between splenectomized and non-splenectomized patients as regards the alloimmunization rate (15 and 85%, respectively; $P > 0.05$). However, other studies have reported a high incidence of RBC alloimmunization and autoimmunization among patients who underwent splenectomy Singer e al [12]. They reported that the absence of a spleen may further enhance the immune response to the infused foreign antigens, which were not undergone effective filtration.

In our study, there is statistically significant difference between presence of alloantibodies and frequency of blood transfusion in thalassemia and SCA patients. Berentsen et al [18]. Reported that relationship between the number of units transfused and alloimmunization was unknown in thalassemia. Moreover, it was reported that the interval between transfusions did not appear to play a significant role in antibody development as similar interval was observed between all the patients. However, the interval shortened after the development of the antibodies due to decreased survival of foreign RBCs. Also El Sewefy et al [19] found that the frequency of blood

transfusion was more than 17 U/year in all alloimmunized patients. The transfusion index showed high statistical significance with regard to alloimmunization.

Study limitations

Our study limitations were smaller sample size.

CONCLUSIONS

Thalassemic and sickle cell anemic patients are sensitive population need carful follow up for complication of recurrent blood transfusion, Thalassemic and sickle cell anemic patients are at risk of alloantibodies formation and their complications, Thalassemic and sickle cell anemic patients are at risk of alloantibodies formation and their complications.

RECOMMENDATIONS

We recommend that, extended phenotyping must be done for newly diagnosed thalassemia and sickle cell anemia patients before the start of blood transfusion and follow up of newly identified alloantibodies is recommended. Use of leucodepleted blood products and cross-matching for minor red cell antigens is likely improve the rate of alloimmunization. We recommend for the blood transfusion-dependent thalassemic patients to have antibody-screening test performed before each transfusion. Also, blood transfusion-dependent patients must be phenotyped (Rh–Kell) once diagnosed and before their first transfusion to receive Rh–Kell phenotyped blood, thus preventing later complications.

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