DISCUSSION

Thalassemias are a heterogeneous group of genetic disorders that result from a reduced rate of synthesis of *a* or β chains of hemoglobin.[78] The β Thalassemia pose, by far, a major public health problem because they are common and usually produce severe anemia in their homozygous and compound heterozygous states.[79] In Egypt the carrier rate varies between 5.5 and 9%; it was estimated that 1:1500 live births per year have β-thalassemia.[3]

The molecular defects in β thalassemia result in absent or reduced β chain production while α chain synthesis is unaffected. The excess of α-chains are highly unstable and precipitate in red cell precursors, forming intracellular inclusions leading to ineffective erythropoiesis and also interfere with their passage through the microcirculation, particularly in the spleen. Thus, the anemia of β Thalassaemia results from a combination of ineffective erythropoiesis and peripheral hemolysis.[80]The previous pathophysiology of β Thalassaemia made it one of the most important genetic diseases that depends on regular blood transfusion in its management.[22]

Blood transfusion carries many hazards which can be classified as immunological such as AHTR, DHTR, FNHTR, allergic reactions and anaphylaxis or infectious as bacterial, viral, parasitic transmission, and others complications as iron over load, dilution of coagulation factors and hyperkalemia.[81]

Febrile non hemolytic transfusion reactions are considered the most common adverse effects of blood or blood components transfusion. It is caused by the interaction between transfused leucocytes and recipients cytotoxic antibodies. However, many patients who experience FNHTRs do not possess leukocyte antibodies and other factors such as release of cytokines by WBCs during the storage of blood components and the presence of recipient antibodies (most likely IgE) to donor plasma proteins are also involved. These reactions are generally not life threatening, but they are expensive in their management, evaluation, and associated blood-product wastage.[82]

Febrile non hemolytic transfusion reactions can be prevented by leucoreduction which may be done pre-storage (laboratory) or post- storage (bed-side filtration) but as proportion of reactions are mediated by biological response modifiers released by WBCs and accumulate in the blood component over the period of storage so the washed RBCs will be more effective in preventing these reactions in those patients.[43]

The goal of the present study was to determine the prevalence of HLA alloimmunization among the studied thalassemic patients, assess the relation between HLA alloimmunization and the occurrence of FNHTRs and observing whether the alloimmunization is affected by age, gender, age of starting transfusion or frequency of transfusions .We also studied the effect of splenectomy on the frequency of the FNTHRs, frequency of transfusions and HLA alloimmunization.

In this study washed RBCs was used to prevent FNHTRs for its low cost compared to the expensive filtered RBCs which are used worldwide for this purpose taking into consideration the Hb rise after transfusion by both methods.

This study was conducted on sixty five thalassemic patients admitted to the Hematology Clinic of Alexandria University Children's Hospital and twenty healthy volunteers matched for age and sex served as a control group. The patients were further subdivided into forty five chronically transfused β thalassemia major children and twenty splenectomised ones. All patients were maintained on regular blood transfusion program according to their needs and most of them were suffering from FNHTRs.

Comparing between both the positive and negative ALA patients regarding age and gender, we did not find statistically significant difference (p=0.746, p=0.137 respectively) which means that both factors did not have an effect on HLA alloimmunization rate.

McPherson et al.,[83] observed that children with HLA alloimmunization tend to be older than non alloimmunized children, they attributed this finding to the increased number of RBCs transfusions especially the non leucoreduced RBCs.

In the present study no statistically significant difference between the positive and negative ALA groups as regards age of starting transfusion (p=0.338). This finding is consistent with the study performed by Ahmed et al.,[84] who studied alloimmunization in Egyptian transfusion dependant thalassemic patients and reported no significant association between alloantibody formation and age at start of transfusion (p = 0.3). Similarly, in a Malaysian study done by Noor Haslina.,[85] no relation between alloantibody development and age at start of transfusion (p = 0.500) was reported.

On the contrary, Singer et al.,[86] who aimed at studying alloimmunization in transfusion dependant thalassemia patients of predominately Asian descent, observed that the immune response was affected by the age at the start of transfusion, their study concluded that transfusion at an early age (less than 1-3 years) may offer some immune tolerance and protection against alloimmunization in thalassemic patients. We can explain this finding by the polymorphic difference in the immunogenic RBCs and WBC antigens between the predominately white general blood donors and patients of predominately Asian descent.

A similar result to the previous study was obtained by Sood et al.,[87] who observed that an earlier start of transfusion may impart some immune tolerance in thalassemic patients. They reported that only one patient developed alloimmunization after starting transfusion at an early age.

In the present work, no statistically significant relation was found between the frequency of transfusions and the development of ALA (p=0.362). Sood et al.,[87] confirmed our results by observing that the risk of developing alloimmunization was not associated with the number or frequency of transfusions received.

This result were also supported by Schiffer [88] who studied the development of HLA antibodies in acute myeloid leukemia (AML) patients receiving multiple transfusions and observed that there was no relation between the number of transfusions and the development of these antibodies.

Another study, done by Bajpai et al.,[89] aimed at studying the factors influencing alloimmunization in fifty patients receiving multiple transfusions for different

hematological disorders, reported that there was no definite association between the frequency of transfusions and the development of HLA antibodies.

As regards the frequency of FNHTRs, no significant relation was found with the development of ALA. Accordingly, it is suggested that HLA alloimmunization does not constitute the main cause of FNHTRs occurring to the thalassemic patients and other factors such as the presence of recipient antibodies to several blood components as plasma proteins and release of cytokines during blood storage are thought to be the main cause of the FNHTRs. [82]

The low percentage (13%) of the ALA positive thalassemic patients in the present study is attributed to an important factor which is the absence of ethnical differences between the donors and recipients as all the donors were Egyptian like the recipients who were exposed to minimal variable patterns of leucocytic antigens. A similar low percentage of alloantibodies (11.3%) was reported in a study done by Ahmed et al.[83] who also studied Egyptian thalassemic patients explaining this low percentage by the ethnical similarity between the donor and the recipients in Egypt .

The conclusion (HLA alloimmunization was not the main cause of FNHTRs) of the present study was confirmed by transfusing 30 negative ALA patients by washed RBCs which is so efficient in removing most of the cytokines and plasma proteins which are thought to be the cause of these reactions and we found that all the patients did not suffer any FNHTRs which confirms our results.

On the contrary, Yazdanbakhsh et al.,[90] who studied the risk factors affecting alloimmunization, observed the presence of high alloimmunization rate (20%-50%) among the transfused population in the US and it was explained by the polymorphic differences in the immunogenic RBC and WBC antigens between the predominately white general blood donors and patients of predominately African descent, whereas the rate of alloimmunization was only 6.1% and 2.6% in Uganda and Jamaica respectively were racial homogenicity between the donors and recipients are found. A similar result was also obtained by Singer et al., [85] who studied the transfusion dependant thalassemic patients of Asian descent and observed high alloimmunization rate (22%) among Asian patients where most of the donors were white as compared to the low rate of alloimmunization (5-10%) observed in Greece and Italy were the homogenous population are present.

Spleen involvement is known to occur in thalassemia. The spleen is most commonly affected in the form of splenomegaly because of excessive destruction of abnormal RBCs (extravascular haemolysis), extra- medullary hematopoiesis and transfusional overload. Splenomegaly further increases transfusional requirement as it sequesters even normal and transfused RBCs. So these patients benefit from splenectomy. [91]

This fact was noticed in the present study as a statistically significant increase in the frequency of requiring transfusion in non splenectomized patients as compared to the splenectomised ones denoting that splenectomy has an effect on reducing the demand for transfusion (p=0.036).This was also in agreement with the study conducted by Porecha et al.,[91] who studied 50 splenectomised thalassemic patients aimed at determining the efficacy of splenectomy in reducing post-operative blood requirements. They observed that most of their patients (96%) had a decrease in blood transfusion requirements (less than 150 ml/kg/year) compared with pre-operative requirement (250-300ml/kg/year).

The previous finding was also consistent with Easa et al.,[92] who studied 140 thalassemic patients; dividing them into 70 non splenectomised and 70 splenectomised patients aiming at determining the effect of splenectomy on transfusion requirements. They reported that splenectomy was beneficial in reducing frequency of blood transfusions in 77.1 % of the splenectomised group.

Confirming our results, Bader-Meunier et al.,[93] observed that the overall need for blood transfusions in hereditary spherocytosis patients decreased dramatically after subtotal splenectomy. They reported that Patients with hereditary spherocytosis received, on the average, 0.32 units of blood per year of life before surgery but only 0.02 units of blood per year of life after surgery.

On the contrary, a study done by Salih et al.,[94] aimed at evaluating some consequences of thalassemia major in splenectomised and non splenectomised Iraqy patients who were subdivided into 22 non splenectomised and 18 splenectomised patients, observed that there was no significant difference between the two groups regarding frequency of transfusions. We can explain this contrasting result by the low sample size of this study.

As regards the frequency of FNHTRs among group I and II, there was no statistically significant difference (p=0.463), denoting that splenectomy did not have an effect on frequency of these reactions.

In the present study, ALA development was statistically higher in groups I than group II (100% of splenectomised patients were negative for HLA antibodies) (p=0.048), denoting that splenectomy had a role in the reduction of HLA alloimmunization rate. This finding is not unexpected since spleen is primarily responsible for stimulating immunological antibody response to blood stream antigen.[83] Similar to our study, Macpherson et al.[83] who studied the risk factors affecting HLA alloimmunization rate in pediatric thalassemic patients observed an obvious trend toward lowering alloimmunization among splenectomised patients.

To prove that HLA antibodies are alloantibodies that rise due to exposure of thalassemic patients to foreign antigens of the donors through blood transfusion, the patients were compared with twenty healthy children as a control group who did not receive any blood transfusions for any reason ,finding a statistical significant difference (p=0.048) which confirms this fact.

In an attempt to determine the efficacy of washed RBCs as compared to filtered RBCs in the prevention of FNHTRs. A trial was done by giving all HLA antibody positive thalassemic patients one session of filtered RBCs and another one of washed RBCs then comparing the occurrence of FNHTRs after transfusion by both blood products, we observed that all positive HLA antibody thalassemic patients did not develop any FNHTRs by both methods except one patient who was improved only by filtered RBCs but suffered from FNHTRs after receiving the washed RBCs. Statistical analysis of this test showed that there was no significant difference between both products regarding their ability to prevent FNHTRs (p=1.000). So, we can conclude that washed RBCs are as efficient as filtered RBCs and can replace it as a method for prevention of these reaction.

Confirming our trial, a study done by Sharma et al.,[82] aimed at knowing the efficacy of pre-storage universal leucoreduction in controlling FNHTRs, was performed on two groups; control and study group. Control group constituted of 14,292 blood transfusions from the 1st of January 2009 to the 31st of December 2009 (one year) where non leucoreduced whole blood (WB) and blood components were supplied to the recipients. In the study group 45,064 transfusions were performed with leucoreduced blood components during the period of 1st January 2010 to 31st December 2013 (4 years), observed that rate of FNHTRs came down significantly in the study group (0.84%) over control group (4.26%) with the reduction of 3.42%. Significant reduction of FNHTRs in study group over control group was reported in WB and other blood products except saline washed RBCs because of the fact that saline washed procedure reduced the contaminating WBCs transfused in both studied groups.

The previous study confirms our conclusion regarding the efficiency of the washed RBCs in prevention of FNHTRs as it was the only product capable of decreasing FNHTRs in the control and study group equally giving no statistically significant difference comparable to other blood products which were given in leucoreduced form only to the study group and in non leucoreduced form to the control group making a statistically significant difference between both groups regarding the decrease of FNHTRs.

Our result was also consistent with King et al.,[95] who select two time periods for direct comparison, July to December 1994 which represents the time period before the initiation of an increase in leucoreduction and July to December 2001 which represents a time period when almost complete leucoreduction (99.5%) had been achieved. They observed that the incidence of FNHTRs decreased from 0.37 % to 0.19 % ($p = 0.0008$). The trend over the entire 7.5 year study period confirms the decrease in FNHTRs as the percentage of leucoreduced RBCs increased.

In the present study the hemoglobin rise was compared after transfusion by both washed and filtered RBCs in every ALA positive thalassemic patient but no statistically significant difference was noticed ($p=0.409$). Although, it is expected that the process of washing blood units may exposes them to loss of 10% of RBCs while discarding the supernatant compared to filtered RBCs in which there are no loss of RBCs at all [27], so we can conclude that washed RBCs gives the same hemoglobin rise as the filtered RBCs.

Among the major limitation of this study are the relatively small sample size and the lack of exact quantitation of ALA in patients and control.

SUMMARY

Thalassemia is among the most common genetic disorders worldwide, particularly in the Mediterranean region, Africa, the Middle East and Southeast Asia. Thalassemia is a hereditary anemia resulting from defects in haemoglobin production. β- Thalassemia, which is caused by a decrease in the production of β globin chains, affects multiple organs and is associated with considerable morbidity and mortality. Accordingly, lifelong care is required and financial expenditures for proper treatment are substantial. The recommended treatment for thalassemia major involves regular blood transfusions, usually administered every 2 to 5 weeks to maintain a pre-transfusion haemoglobin level between 9-10.5 g/dl.

Thalassemic children experience various problems if the transfusion is inadequate but at the same time repeated blood transfusions are associated with hazards like iron overload and risk of acquiring transfusion-transmitted infections (TTIs). Iron overload can lead to endocrinal dysfunction in the form of growth retardation and diabetes mellitus. Transfusion-transmitted infections such as HIV (with risk of progression to AIDS), HBsAg, and HCV (with high risk of developing chronic hepatitis, liver cirrhosis and hepatocellular carcinoma) can also occur. Thus, chronic blood transfusion in thalassemic patients is a double-edged sword. Ultimately thalassemic patients die either due to transfusion complications or due to lack of it, with the result that they seldom survive beyond the age of 25 years.

Packed RBCs were usually used for thalassemic transfusion until recently when little attention had been paid to the contaminating leukocytes present in various blood components and thought to be responsible for many immunological complications as HLA alloimmunization which is considered the main cause of FNHTRs and platelet refractoriness observed in multi-transfused patients, but nowadays those adverse reactions reduced dramatically after the usage of the leucoreduced blood products.

The aim of this study was to detect the rate of HLA alloimmunization in pediatric chronically transfused thalassemic patients in the hematology clinic of Alexandria University Children's Hospital who were transfused by non leucoreduced PRBCs, to determine the relation between HLA alloimmunization and the occurrence of FNHTRs and to study the effect of splenectomy on these adverse reactions and their relation to HLA alloimmunization.

We also compared between transfusing washed and filtered RBCs regarding their ability to prevent the occurrence of FNHTRs taking into consideration the Hb rise after transfusion by both products.

This study was conducted on sixty five thalassemic patients, selected from the hematology clinic of Alexandria University Children's Hospital and twenty healthy volunteers who served as a control group. The patients were further subdivided into forty five non splenectomised chronically transfused β thalassemia major children and twenty splenectomised ones. All patients were maintained on regular blood transfusion program according to their needs and most of them were suffering from FNHTRs.

All patients (n=65) included in the present study were subjected to detailed history of blood transfusions regarding the age of starting transfusion, frequency of transfusions and frequency of febrile reactions then 2 ml of venous blood were drawn from every patient for qualitative estimation of human leucocytic antibodies by anti- lymphocytotoxic antibody (ALA/LCA) ELISA kit.

All thalassemic patients with positive HLA antibody were subjected to 2 sessions of blood transfusion; one by bed-side leucofiltration and the other by washed RBCs, then observing the occurrence of FNHTRs and estimating the hemoglobin rise after transfusion by both products.

Results showed low rate of HLA alloimmunization among studied thalassemic patients (13%), and there was no clear association between HLA alloimmunization and the occurrence of FNHTRs (p=0.887). No relation between frequency of transfusions and the rate of HLA alloimmunization (p=0.362) was noticed.

Regarding splenectomy, we noticed that splenectomised thalassemic patients had fewer need for blood transfusion than non splenectomised patients (p=0.036), there was also a significant relation between splenectomy and the absence of HLA alloimmunization (p=0.048), but there was no relation between splenectomy and the Frequency of FNHTRs (p=0.463).

The comparison between the washed RBCs and filtered RBCs showed no difference regarding their efficiency in prevention of the FNHTRs (p=1.000), there was also no difference in the Hb rise after transfusion by both blood products (p=0.409).

CONCLUSIONS

The following data could be concluded from the present study:

- There is low rate of HLA alloimmunization among thalassemic patients.
- HLA alloimmunization is not the main cause of FNHTRs among thalassemic patient.
- No statistically significant relation between HLA alloimmunization and age of starting transfusion.
- No statistically significant relation between HLA alloimmunization and frequency of transfusions.
- Splenectomy significally decreases the demand for blood transfusions among thalassemic patients.
- Splenectomy has no effect on frequency of FNHTRs.
- Splenectomy signifcally decreases the rate of HLA alloimmunization.
- Washed RBCs have the same efficiency in preventing FNHTRs as filtered RBCs.
- There is no difference in the Hb rise after transfusion whether washed or filtered RBCs are used.