Role of D-Dimer Test In β-Thalassemia patients

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Abstract:

Background and aim: β-Thalassemia is hereditary blood disorder that can cause deficient or absent synthesis of beta globin chains, leading to excess alpha chains. Thalassemias are prevalent throughout the Mediterranean region Africa, the Middle East, the Indian subcontinent and South-East Asia**.** The D-dimer is a dependable and sensitive key of fibrin deposition and steadying. In this way, its available in plasma should be reveal of thrombus formation ,There are many circumstances unconnected to thrombosis in which D-dimer level are high, It is oftentimes used in the estimation of acute venous thromboembolism (VTE) , plus in identification of disseminated intravascular coagulation (DIC) .Regular blood transfusion and compliance with iron chelation therapy has markedly improved life expectancy in thalassemia; however, this improvement is accompanied by several complications of this chronic disease including thromboembolic disorders. The objective of this work was planned to estimate the association between level of D-dimer protein in the blood and beta thalassemia. **Materials and Methods:** This retrospective, case-control study was conducted on male patients (βT) (n=90) group at aged (4-29 years) and the patients samples collected according to duration of blood transfusion while the control male(n=45) group at the same aged, This study was conducted in thalassemia center in Babylon province, Iraq . The study extended from the start of October 2018 to the end of February 2019.The activity of D-Dimer protein test , Ferritin and hematological parameter (RBC$_s$ WBC$_s$, HB, PCV and PLT$_s$) were estimated. **Results:** plasma D-Dimer levels were significantly higher in BT patients compared to control groups. There were significantly ($p<0.05$) differences in these biomarkers (D-Dimer , Ferritin and hematological parameters between four age groups of duration of blood transfusion. a positive correlation between D-Dimer levels with PLT$_s$,Ferritin and duration blood transfusion in male patients with β-thalassemia. **Conclusion:-**Our study revealed the role of D-Dimer as biochemical risk factors of thromboembolic disorder as a result of complications related to the disease.

Key Word: D-Dimer, β-Thalassemia, disseminated intravascular coagulation, thrombus formation, VTEs

1. INTRODUCTION

D-dimer testing is important to aid in the exclusion of venous thromboembolic events (VTEs), including deep venous thrombosis and pulmonary embolism, and it may be used to evaluate suspected aortic dissection. D-dimer is produced upon activation of the coagulation system with the generation and subsequent degradation of cross-linked fibrin by plasmin [1].

Beta-thalassemia is caused by the reduced (beta$^+$) or absent beta^0) synthesis of the beta globin chains of the hemoglobin (Hb) tetramer, which is made up of two alpha globin and two beta globin chains (alpha$_2$beta$_2$) [2].

Two main forms have been described **thalassemia major**(severe form of anemia and dependent blood transfusion DBT) **thalassemia intermediate** (have mild to moderate anemia and nondependent blood transfusion NDBT) [3].

Regular transfusions are essential to correct the anemia, suppress erythropoiesis, and inhibit increased gastrointestinal absorption of iron in β-Thalassemia patients which occurs in non-transfused patients as a consequence of increased, although ineffective, erythropoiesis, Several different transfusional regimens have been proposed over the years, but the most widely accepted aims at a pre-transfusion Hb level of 9 to 10 g/dl and a post-transfusion level of 13 to 14 g/dl.5 This prevents growth impairment, organ damage and bone deformities allowing normal activity and quality of life [4].

Patients with thalassemia manifest thrombotic complications. Furthermore the risk of thromboembolic complications appears to be higher following splenectomy due to a wide range of thrombotic risk factors, patients with thalassemia should be monitored by laboratory tests such as D-dimer assay[5].

2. MATERIALS AND METHODES

2.1 Samples collection:

The cohort study was involved 90 BT patients (4-29 years) and 45 healthy as control group age (4-29 years) matched in both groups (patients and controls). The study extended from the start of October 2018 to the end of February 2019. Who were with draw six ml venous blood samples got from all objective.

2.1.1 Blood samples

 (2ml) of blood samples investigated for completely automated hematology analyzer estimated complete blood count (CBC) [6] using Analyzer (Orphée CO., Switzerland).

2.1.2 Serum samples

 (2ml) of blood samples centrifugation for Serum samples were directly frozen and stored at -80°C for measured Serum level of Ferritin [6] using I flash 1800 (Shenzhen YHLO Biotech CO., China)

2.1.3 Plasma samples

 (2ml) of blood samples putting it in tube of sodium citrate and centrifugation to getting of plasma were directly frozen and stored at -80°C for measured level of plasma D-dimer [6] using AFIAS -6 (Boditech Med, Inc, korea).

2.2 Statistical analysis

The garnered data were analyzed using SPSS 24 software and P-value less than 0.05 was considered significant . Kolmogorov Smirnov test was used to analyze whether the data were normally distributed . Independent t-test , Mann Whitney, and Kruskal wallis was applied to compare continuous variables . The results were reported as mean ± SE or median (min-max). categorical variables were compared using a Chi-Square test, with the data presented as numbers (%) . The correlation between parameters determined by Pearson or Spearman correlation test.

3. RESULTS

Table (1), demonstrates the age distribution for patients and control groups of the study (β-thalassemia patients). The ages of patients range between 4-29 years and the range for controls was 4-29 years . No statistically significant difference (P>0.05) was present between the mean age of patients groups (13.56±0.66) when compared with the mean age of healthy groups (15.53±1.02).

The results showed significant difference (P<0.05), decreased mean levels of RBC_S, HB, P.C.V $(3.08±0.08)10^6/mm^3$, $(6.93±0.15)g/dl$, $(23.79±0.49)\%$ were found in β-thalassemia patients as compared to the mean levels of control groups $(5.37±0.10)10^6/mm^3$,$(15.03±0.29)g/dl$, $(48.91±0.95)\%$ respectively . While the mean values of WBCs, PLTs and Ferritin $(9.74±0.26)$ $10^3/mm^3$, $(305.68±14.49)$ $10^3/mm^3$ $(3767.56±316.86)$ ng/ml in the patients group showed highly significant $(P<0.05)$ as compared to the control groups $(6.80±0.26)10^3/mm^3$, $(226.58±11.26)10^3/mm^3$, $(59.90±5.50)$ng/ml (Table 1).

The results of marker D-Dimer showed significant difference (P<0.05) increase mean level of D-Dimer in patients groups (589.47±110.29) ng/ml as compared with control groups (196.97±11.57) ng/ml in Table (1) and Figure (1) , While the Levels in the four groups divided according to duration of blood transfusion are planned in figure (2). The results showed a difference in D-Dimer level among four patients groups, the results shown a significant (P< 0.05) decrease in mean level of D-Dimer $(275.1±29.08)$ ng/ml in age group $(\leq 5$ year) as compared with the mean level of D-Dimer $(342.75±56.02)$ ng/ml , $(1164.7±380.8)$ ng/ml and $(739.12±299.4)$ ng/ml in other age groups (6-10 year) ,(11-15 year) and $(\geq 16$ year) , respectively .The same figure shows a significant increase in the mean level of D-Dimer $(1164.7±380.8)$ng/ml in age group (11-15 year) as compares with the mean level of D-Dimer $(739.12±299.4)$ng/ml in age group duration of blood transfusion $(\geq 16$ year) in male patients with β-Thalassemia . The results of correlation showed the DDimer level were correlated positively with those of PLTS, Ferritin and blood transfusion (Figure 3,4,5)

Table (1): Comparison of the clinical characteristics between patients with β-thalassemia and control groups

Parameters \ Groups	PATIENT n=90		CONTROL n=45		Sig.
	Mean±SE	Median Mim-Max	Mean±SE	Median	
Age (year)	13.56±0.66	12.5 4-32	15.53±1.02	18.0 6-33	0.23
Duration of BT (year)	11.84±0.64	11.15 1-31	0.00±0.00	0.0 0-0	0.0001
RBC $(10^6/mm^3)$	3.08±0.08	3.0 1.40-4.97	5.37±0.10	5.3 4.02-6.78	0.0001
Hb (g/dl)	6.93±0.15	7.0 3.3-9.9	15.03±0.29	14.9 10.9-19.7	0.0001
PCV (%)	23.79±0.49	24.4 11.4-35.2	48.91±0.95	48.9 34.0-58.9	0.0001
WBC $(10^3/mm^3)$	9.74±0.26	9.5 4.0-14.8	6.80±0.26	6.6 4.1-10.9	0.0001
Platelets $(10^3/mm^3)$	305.68±14.49	279.0 121-773	226.58±11.26	221.0 107-407	0.001
Ferritin (ng/ml)	3767.56±316.86	2555.0 429-11680	59.90±5.50	51.8 5-213	0.001

DDimer (ng/ml)	589.47±110.29	278.1 124.07-6213.31	196.97±11.57	163.0 110.38- 378.17	0.001

P< 0.05 statistically significant with control group (Patients (n=90) , Control (n=45))
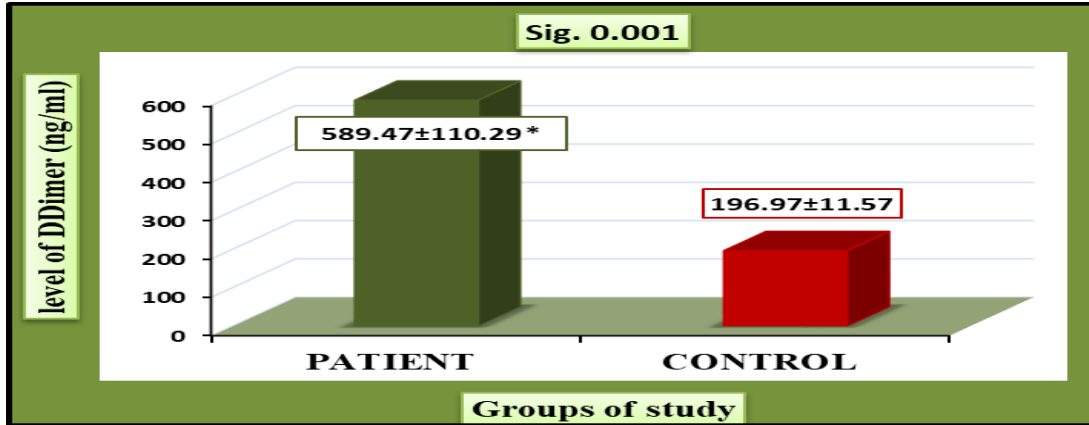
Figure (1): DDimer (ng/ml) level in male patients with β- Thalassemia and control groups *P< 0.05 statistically significant   (Patients n=90 , Control n=45)
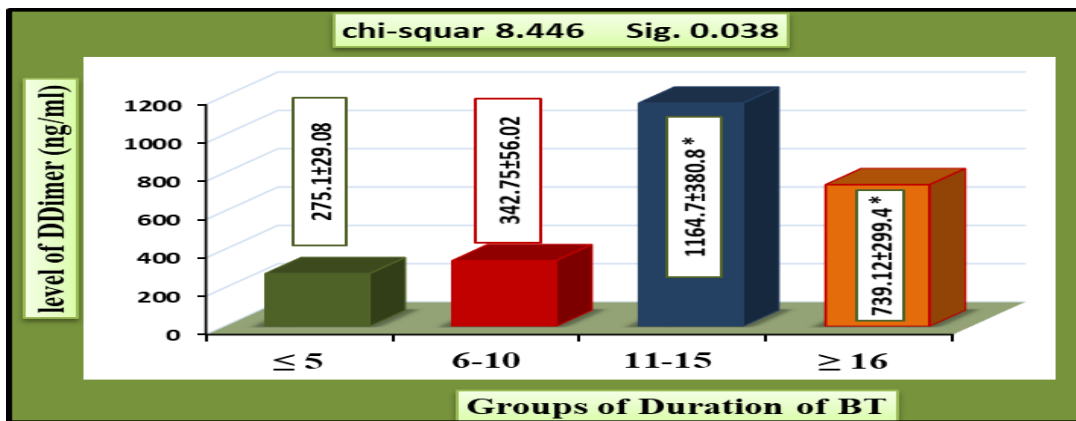

Figure (2) DDimer level (ng/ml) with four age groups in duration of blood transfusion in Male patients with β-thalassemia / *P< 0.05 statistically significant   ≤ 5 year (n=15) , 6-10 year (n=26) , 11-15 year (n=30) , ≥ 16 year (n=19)
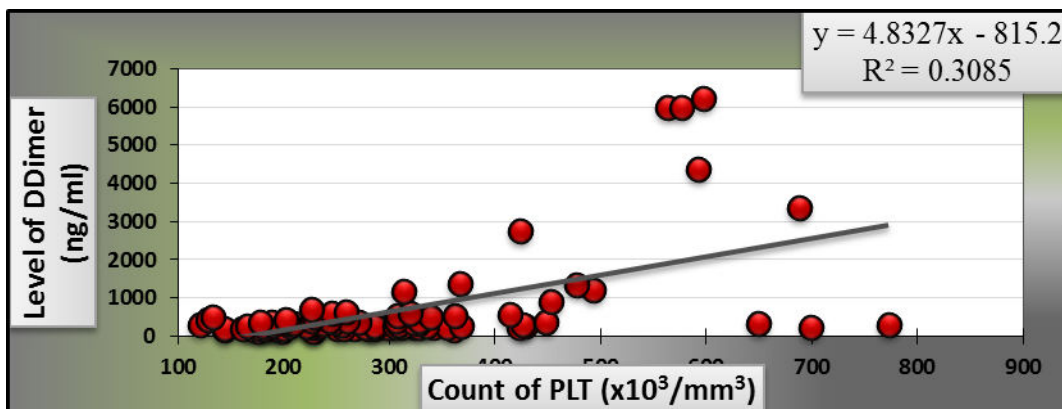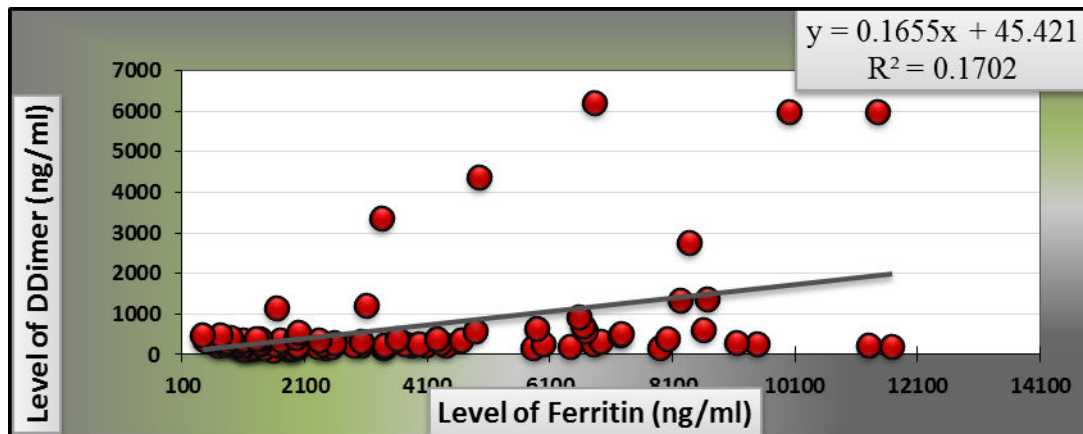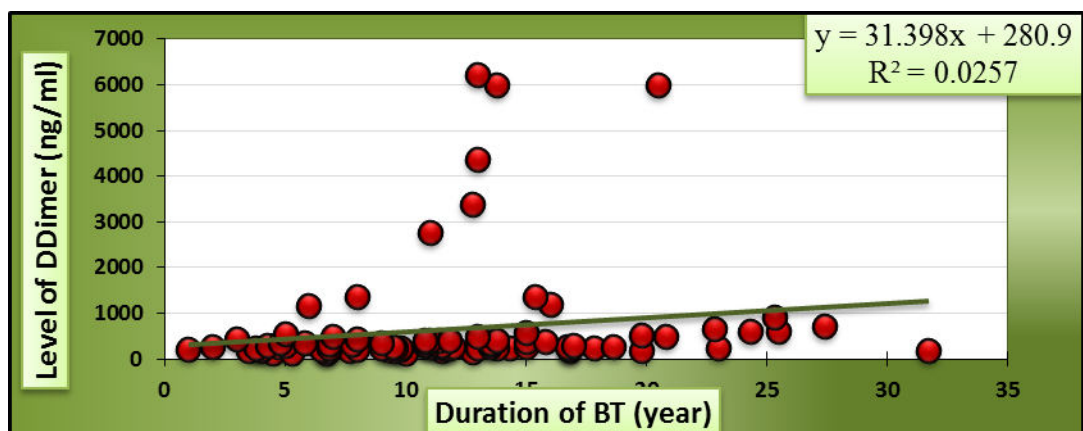

Figure (3) correlation between DDimer (ng/ml) and PLT$_S$ (X10^3/mm^3) in male patients with β-thalassemia

$$y = 0.1655x + 45.421$$
$$R^2 = 0.1702$$

Figure(4) Correlation between D-Dimer (ng/ml) and Ferritin (ng/ml) in male patients with β-thalassemia

$$y = 31.398x + 280.9$$
$$R^2 = 0.0257$$

Figure(5) Correlation between D-Dimer (ng/ml) and Duration of blood transfusion (year) in male patients with β-thalassemia

4. DISCUSSION

As represented in table (1), age distribution showed no significant difference between patients and controls groups in male affected with β-thalassemia , due to effect of matching the rang of age and gender between patients and controls groups.

The results at the same table shows that the hematological parameters significant decrease in the levels of Hb, PCV, RBCs count in male patients groups reflecting the fact that this specific type of blood disease occur mainly due to defective formation of globin chain (Beta chain) of the hemoglobin moiety of the RBC, due to excessive destruction of red blood cells at an early stage which in turn leads to anemia and the present study show similarity with [7] .

Other study decided that in case of beta thalassemia patients, absence of beta globin chains lead to accumulation of unpaired alpha globin chains. Excess presence of the alpha globin chains is a primary reason for the cellular oxidative damage of erythrocyte membranes [8].

A study by [9] concluded that increase of WBCS level (table 1) may be due overall diseased condition and hyper-activity of immune system of the patients receiving blood from different donors .

Moreover, at the same table significant differences in PLT_S level caused by platelet aggregation impressed by transfusion given these changes, thrombotic risk should be considered in beta‑thalassemia patients [10].

In this study have found that the male patients with β-thalassemia had significant increase in serum ferritin level because multiple blood transfusions which believe life-saving therapy for β-thalassemia patients lead to iron overload and deposition of iron in tissue organ like liver caused hepatotoxicity and this results acceptable with the recent study [11].

The male patients group showed significantly higher plasma D-dimers level in comparison to control group which are shown in figure (1) . These result agree with [12] which proved that elevated level of plasma D-Dimer as a result to incidences of several serious complications increase thromboembolic events (TEE) related with optimizing transfusion , iron chelation and splensctomy therapy in male patients with β-thalassemia .

The levels of plasma D-Dimer in present study figure (2) showed significant differences between four age groups divided according to duration of blood transfusion in male patients with β-thalassemia as a result to increase complications of blood transfusion with progressive of age , this result agreed with the recent study [13] that found the incidence of clinical thrombosis is four fold higher in NTDT (non-transfusion dependent thalassemia) compared with TDT (transfusion dependent thalassemia) patients, is mostly venous, and is a leading cause of mortality, The frequency of thrombosis in NTDT is significantly higher in patients older than 35 years.

As was shown in figure (3), a positive correlation was present between D-Dimer and PLT_S . Patients with splenectomized β-thalassemia have a high incidence of thromboembolic events due to a hypercoagulable state, This is a result of a mixture of various abnormalities : activated platelets (as confirmed by the increased expression of CD62P (P-selectin) and CD63 (markers in platelet activation) , variation in red blood cells with the formation of reactive O_2 species and expression of negatively charged phospholipids, expression of endothelial adhesion molecules on endothelial cells , all of them due to elevated plasma D-Dimer level [14;15] .

Figures (4) represented the relationship between D-Dimer with ferritin levels and showed that a positive correlation was present between them. The iron burden on the body can be assessed by means of elevated levels of, serum ferritin, are related to the quantity of iron stored in the body with and without iron overload. Serum ferritin is a useful screening test for the initial diagnosis of thalassemia. Iron overload is responsible for the most damaging effects of the thalassemias, making iron chelation a focal point of the management of this diseases [16] .

Figure (5) demonstrated the relationship between D-Dimer and duration of blood transfusion and a positive correlation was clear between them. When the patient has increased transfusion demand, especially β-thalassemia splenectomized patients cause of a hypercoagulable state due to abnormalities involving platelets aggregation, red blood cells (RBC), endothelial cells and thrombin activation. Other hemostatic changes may include alterations in the levels of procoagulant or anticoagulant factors, and/or chronic activation of endothelial cells or white blood cells, may also increase the risk of thrombosis [17].

5. CONCLUSION

1- Significant differences in biomarker (D-Dimer level) and relationship with platelets, Ferritin and Duration of BT, which may be useful for analysis of thrombosis in β-thalassemia patients.

2- Significant differences in all parameters (RBC_S, HB, PCV, WBC_S, PLT_S, Ferritin) which consider early diagnostics for β-thalassemia disease.

3- Our study revealed the role for D-Dimer as biochemical risk factors of thromboembolic disorder as a result of complications related to the disease.

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