Original Article

Hemostatic parameters in Thalassemia patients; a single institute experience

Safa A. Faraj* **FICMS (Ped.), FICMS (Hem.), DCH**

Abstract:

Background: Changes of coagulation profile have been described in patients with B-Thalassemia major. Prolongation of prothrombin time and partial thromboplastin time and reduced levels of coagulation factors and natural anticoagulants have been variously described though the mechanisms involved in the thrombotic tendency seen in some Thalassemia patients have not been fully clarified.

Objectives: To investigate changes in the coagulation profile in patients with B-Thalassemia according to the available hemostatic parameters in Thalassemia patients in Al-Karama Teaching Hospital in Wasit governorate.

Methods: Hemostatic variables were studied in pre-transfusion blood samples from 50 transfusion-dependent children with Beta Thalassemia (mean age 13.2 years) and from 20 healthy controls.

Results: Laboratory evaluation showed thrombocytopenia in 43.5%, prolongation of prothrombin time (PT) in 54% and prolongation of activated partial thromboplastin time (aPTT) in 56% of the patients. All measured coagulation factors level were low in activity as compared with control group. Serum ferritin had positive correlation with PTT and PT (r=0.12 and r=0.11 respectively) and significant negative correlation with platelet count (r=-0.3).

Conclusions: Changes in the hemostasis in Thalassemia patients is notifiable.These laboratory finding may be subclinical, but play important role in anticipation of future hemorrhagic manifestation and thrombotic events.

Keywords: Bleeding, Thalassemia, hemostasis.

Fac Med Baghdad 2016; Vol.58, No.2
Received: Jan, 2016
Accepted:April.2016

Introduction:

Thalassemia refers to a various group of genetic disorders of hemoglobin production, which consequences from lack or reduced rate of production of one or further of globin chains of hemoglobin. (1) Thalassemia is principally divided into alpha - Thalassemia and Beta - Thalassemia, depending upon reduced or absent-minded synthesis of a-globin chain or B-globin chain of hemoglobin, separately. Clinically, B - Thalassemia is divided into major, intermediate, and minor forms. (2)

Survival of patients with Beta-Thalassemia major had become better with the progress of comprehensive Thalassemia care facilities. With sustained improvement in survival, the development of a number of late effects has become increasingly apparent. These include the effect on somatic growth, pubertal development, heart, lungs and musculoskeletal role, psychosocial alteration and neuro-cognitive abilities. The acknowledgement of these effects has led to the understanding of the need to develop treatments to reduce the late effects without compromising the survival rates. The adverse effects on heart, liver and the endocrine system have been known for over four decades but focus on hemostasis is relatively recent. The frequently seen clinical manifestations are epistaxis. (3) Prolongation of prothrombin time and partial thromboplastin time, reduced levels of coagulation factors, natural anticoagulants like protein C, protein S and Antithrombin III (AT III) have been described though the mechanisms involved in the thrombotic tendency seen in some Thalassemia patients and bleeding indices in others have not been fully clarified. (4).

**Dept. of pediatrics, College of Medicine, Wasit University.*
E Mail: safafaraj@yahoo.com

Aims of study:

To investigate changes in the coagulation profile in patients with B-Thalassemia according to the available hemostatic parameters in Thalassemia patients in Al-Karama Teaching Hospital in Wasit governorate.

Patient and methods:

This is a cross sectional study conducted for patients with Thalassemia in the center of Hereditary Anemia in Wasit Governorate in period from 1st of April to 31 of May 2015. Fifty transfusion dependent Thalassemia patients and twenty normal health control persons were involved in this study. All history and clinical data were obtained from files of the patients. Hematological parameters were studied before transfusion. The average of pre transfusion period is 18-21 days. Blood samples for both groups (patients and control) were drawn under aseptic technique. Two milliliter of whole blood was collected from clean venipuncture in a (K2 EDTA) coated tube for Complete Blood Count using hematology auto analyzer CELC-DYN Ruby. Blood samples for coagulation assays were collected in 3.2% trisodium citrate tube (1:10 v/v). Coagulation factors assay was carried out using Automate Diagnostica Stago (France). Prothrombin

time (PT) and activated partial thromboplastin time (aPTT) via 3.2% trisodium citrate were estimated using "Liquicellin" supplied by Tulip Diagnostica. Liver function test was done by enzymetic methods Cobas C111 (Germany). Serum iron was measured by automated Cobas C111 (Germany). Serum ferritin estimation was done by automated Minividas using Vidas ferritin biometrica (France). A platelet count less than 150,000/mm3 was labelled as thrombocytopenia. The cardinal parameters of hemostasis; prothrombin time (normal 11–15 seconds), activated partial thromboplastin time (normal 27–40 seconds).(5) Patients with suspected liver impairment are prone to have coagulation abnormalities and were excluded from analysis. Patients with splenectomy usually have platelets abnormalities and were exempted from the further analysis of platelets count and correlation.

Statistical analysis

The data were admitted, tabulated, processed and statistically analyzed by using SPSS-13. Results were calculated and reported as mean, frequency, percentages, and standard deviation. Student's t-test was used to compare parameters in different subgroups. P-value of 0.05 or less was considered significant. Pearson's correlation coefficient (r) was used to test the correlation between continuous variables.

Results:

Among 50 patients with Thalassemia, the mean age was 13.2 years, range (4-31 years ± 6.6) and median 12 years. Male to female ratio was 1.5:1. The majority of the patients had Thalassemia major (44 patients, 88%), six patients had Thalassemia intermedia (12%). Hepatitis C infection was reported in 10 patients (28%), and one patient had hepatitis B infection. Four patients were splenectomised. All patients were receiving iron chelating therapy, seventy percent of patients were receiving Deferasirox (Exjade), twenty percent of patients were receiving Desferrioxamine (Desferal), and ten percent were receiving both. Table (1) shows demographics data of the patients.

Table (1): Demographic data of the patients with Thalassemia

Item	No	Percent
Sex		
Male	**30**	**60**
Female	**20**	**40**
Thalassemia type		
Major	**44**	**88**
Intermedia	**6**	**12**
Hepatitis infection		
Hepatitis C	**10**	**20**
Hepatitis B	**1**	**2**
Splenectomy	**4**	**8**
Iron chelation therapy		
Deferasirox (Exjade)	**35**	**70**
Desferrioxamine (Desferal)	**10**	**20**
Both	**5**	**10**

Table (2) shows the laboratory data of the patients and controls, serum iron and serum ferritin were highly elevated in the patients with Thalassemia. Mean of serum iron was 250 ± (90) mg /ml, for controls was 72 ± (20) mg/ml, which is statistically highly significant, mean serum ferritin for the patients was 4750 ± (2700) ng/dl which is higher than what reported in controls group, 130 ± (100) ng/dl. Although all the patients clinically had no jaundice, parameters of liver function were higher than what reported in controls group. Pre transfusion Hb level mean was 8.92 ± (.992) g/dl, the number of WBC count was equal for both groups (patients and controls), while the mean of platelet count in the patients group was lower than controls group. Thrombocytopenia (platelet count less than 150 ×1012) was reported in twenty patients (43.5%), four patients (8%) with splenectomy were excluded.

Table (2): Liver enzymes, iron status and blood parameters differences between patients and control

Item	Patients/ Mean (SD)	Range	Controls/ Mean (SD)	P value
Serum iron (mg /ml)	**250 ± (90)**	**193-286**	**72 ± (20)**	**0.0001**
Serum ferritin (ng/ml)	**4750 ± (2700)**	**1096-9950**	**130 ± (100)**	**0.0001**
AST (IU/L)	**62.2 ± (22)**	**54-68**	**30 ± (5)**	**0.0001**
ALT (IU/L)	**79.1 ± (61.6)**	**65-81**	**32 ± (6)**	**0.001**
Serum Bilirubin mg/dl	**0.92. ± (0.4)**	**0.3-0.9**	**0.7 ± (0.2)**	**0.03**
Hb (g/dl)	**8.92 ± (.992)**	**7-9**	**13.0 ± (0.6)**	**0.001**
WBC (×10 9)	**8830 ± (9700)**	**1110-69000**	**8677 ± (8200)**	**0.8**
Platelet (×1012)	**199 ± (128)**	**24-760**	**256 ± (52)**	**0.056**

Table (3) describes coagulation parameters for patients and controls; twenty-seven (54%) patients with Thalassemia had abnormal PT, twenty-eight (56%) patients had abnormal aPTT. All coagulation factors measured were low in activities in the patients with Thalassemia if compared with controls group with statistically significance, except factor V, which was reported as low activity in Thalassemia but no statistical significance.

Table (3): Coagulation parameters differences between patients and controls.

Item	Patients mean ±SD	Controls ±SD	P value
PT sec.	16.22±(2.5)	12±(0.6)	0.0001
PTT sec	49.8±(9.6)	31±(1.7)	0.0001
FII (%)	79.9±(12.4)	94±(16)	0.0001
FV (%)	92±(21.2)	98.9±(8.7)	0.16
FVII (%)	72±(15.3)	106±(24)	0.0001
FVIII (%)	83.4±(28)	103±(25)	0.006
F IX (%)	77.2±(21)	97±(24)	0.001
FX (%)	69.2±(16.9)	94±(15)	0.0001
FXI (%)	66±(23)	97±(17)	0.0001
FXII (%)	75±(27)	102±(28)	0.0001

Further analysis of the data to look for the correlation between serum ferritin and aPTT, PT and platelets was carried out. Serum ferritin showed positive correlation with PTT, (r = 0.12), as shown in figure (1). Positive correlation of serum ferritin and PT was another finding in this study (r= 0.11) as figure (2). Figure (3) shows significant negative correlation between serum ferritin and platelets count, yet the result was statistically significant (r=0.3, p value 0.01).

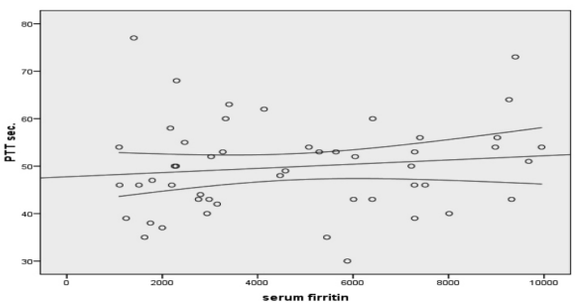

Figure (1): Correlation between serum ferritin and PTT

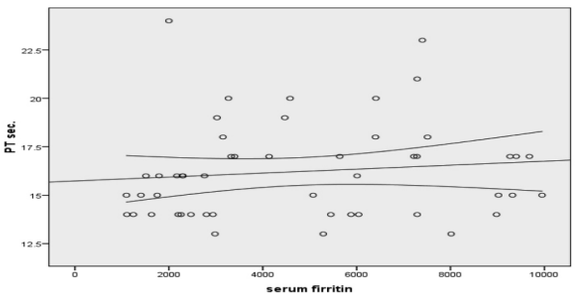

Figure (2): Correlation between serum ferritin and PT

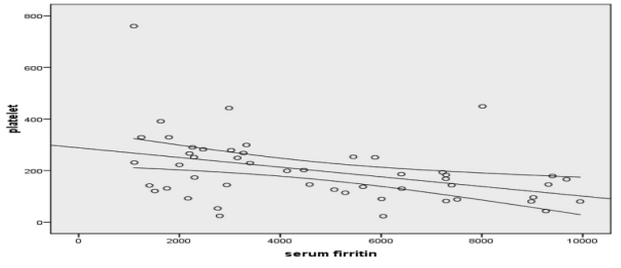

Figure (3): Correlation between serum ferritin and platelets

Discussion:

The aim of this analysis is to look for the hemostatic profile among hematological variables in Thalassemia patients. Thrombocytopenia was reported in 20 (43.5%) of patients who were not splenectomised by the time of data collection. This finding is higher than that reported in Naithani study (33.3%) and Ibrahim study (30.7%). (6,3) The figure is nearly equal to what is reported in Abhishek Marti study (40%). (7) All patients with splenectomy have normal platelet count which is similar to what reported in Caocci et al study. (8) Thrombocytopenia can be found in patients with Thalassemia as was reported by many articles about hemostatic changes in Thalassemia patients, this is mainly because of hypersplenism, iron chelating therapy and serum ferritin. All these factors May play role in developing thrombocytopenia. Current study showed negative correlation between platelet count and serum ferritin (r = -0.3). The correlation was statistically significant, which was similar to that mentioned by Naithani study. (6) Thrombocytopenia is explained by high serum ferritin and chelating therapy. Caocci et al study reported normal platelets count for all patients as nobody used iron chelating therapy. (8) PT and PTT assessment were done for the patients and control group, and there was difference in the value which is statistically significant (p value is 0.0001), this finding is similar to that mentioned in Abhishek study. (7) Prolongation of the PT was reported in 54% of the patients which is higher than what was reported in karami study (3%), Abhishek study (12%) and Naithani study (40%). Prolongation of PT demonstrated positive correlation with serum ferritin (r = 0.1). which is compatible with that mentioned in Naithani study. (6) Prolongation of aPTT was reported in 56% of the patients, this figure is higher than what is reported in karami study (13%), and higher than figure mentioned in Naithani study (48%) and Abhishek (6%). (10,8 7) Prolongation of aPTT was positively correlated with serum ferritin (r = 0.12), this finding was also reported in Naithani study. (6) Reduction in PT and aPTT activities is noticeable in Thalassemia patients receiving blood transfusion, this is explained by parenchymal liver damage due to iron overload and/or circulating hemolysates which may lead to prolongation of both PT and aPTT. (8,3) Multiple transfusion indeed play role in activation of intrinsic coagulation pathway as well as intravascular hemolysis. There is a correlation

between hemolysates infusion and hyper coagulable state. (8) Iron overload lead to kallikrein like protease activity which is released from tissue. (10,11) The below table is set to see the profile of difference between this study and other comparative studies, results were higher in current study which is possibly due to poor control or compliance of the patients to iron chelating therapy that lead to iron over load and high serum ferritin, which cause alteration in coagulation process Table (4).

Table (4): Comparison of bleeding profiles according to different studies

Finding	Current study (50 patients)	Naithani (6) (50 patients)	Abhishek (7) (54 patients)
Thrombocytopenia	43.5%	33.3%	40%
Prolonged PT	54%	40.7%	12%
Prolonged aPTT	56%	46.3%	6%

Current analysis had shown significant statistical difference between coagulation factor activities of patient with Thalassemia and control groups. Assessment of Intrinsic pathway (factor VII) in this analysis showed that there was difference in activity of this factor with p value (0.0001). This issue is compatible with what is reported in Romcai study and Caocci study. (12,8)

Vitamin K dependent factors (II, VII, IX and X) were markedly reduced in activities. This finding were also reported in Romcai and Caocci studies. (12,8)

Factor V activity was decreased but with out statistical significance. Normal factor V activity was reported in Caocci study (8), but low activity was reported in Romcai study. (12)

Factor VIII activity was low in this study, same to what is reported in Romcai study. (12) Yet, factor VIII activity was normal in Caocci study. (8)

Factor XI and XII were markedly depressed in Thalassemia patients with p value (0.0001), these results are similar to those in Romcai and Caocci studies. (12,8)

Liver impairment can not explain the low activities of factor XI and XII. The explanation was mentioned by Caocci (8) as it is mainly due to activation of the intrinsic coagulation and/or kallikrein systems following intravascular hemolysis and multiple blood transfusions.

Measurement of level of protein C, protein S, Antithrombin III are very important to explain the reduction in coagulation factors activities, most of studies used to measure these factors to identify the patients at risk of coagulopathy and possible development of thrombotic complications. Unfortunately, because of lack of facilities, assessment of these factors was not done in this study.

Conclusions: Significant alterations in the hemostatic system already exist in polytransfused children with beta-thalassemia.

Recommendation:

Current study highly recommends regular screening of Thalassemia patients for any possible hemostatic changes and institution of medical intervention in notified cases.

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