

Evaluation of Thrombin Activatable Fibrinolysis Inhibitor (TAFI) in Patients with β -Thalassemia

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Abstract

Background: Thalassemia is an inherited disorder of hemoglobin (Hb) synthesis that results in reduced or absent production of globin chain. Hypercoagulable state in thalassemia is a well-recognized event. Thrombin activatable fibrinolysis inhibitor (TAFI) is an enzyme considered to play an important role in the regulation of fibrinolysis by the coagulation system. With this background, the present study was conducted to know the role of TAFI as a contributing factor in hemostatic alteration state in adults with β -thalassemia. **Aim:** The aim of this article is to evaluate the level of TAFI and hematological parameters in splenectomized and non-splenectomized β -thalassemia patients and to compare their levels with normal subjects. **Materials and Methods:** This case-control study was conducted in 56 adult thalassemic patients. The plasma TAFI level was evaluated by the ELISA technique. **Results:** There was a significant reduction in TAFI levels in all thalassemic patients when compared with thalassemia minor patients and controls ($P < 0.0001$). The TAFI level was significantly lower in the splenectomized group when compared with the non-splenectomized group ($P < 0.0001$), and the TAFI level was significantly lower in the thalassemia major group when compared with the thalassemia intermediate group ($P < 0.0001$). There was a significantly higher prothrombin time and activated partial thromboplastin time level in thalassemic major and intermediate patients when compared with thalassemia minor patients and controls ($P < 0.001$). **Conclusion:** The TAFI enzyme level could be an important predictor for hemostatic alteration. Its level can be considered as a helpful marker for monitoring and follow-up of thalassemic patients for early and proper intervention to minimize complications.

Keywords: Beta-thalassemia, enzyme-linked immunosorbent assay, thrombin activatable fibrinolysis inhibitor

INTRODUCTION

Thalassemia is a congenital hemolytic disorder caused due to partial/complete deficiency of α - or β -globin chain synthesis.^[1] The two major categories are the α - and β -thalassemias, whereas the rare forms include the γ -, δ -, and $\epsilon\gamma\delta\beta$ -thalassemias. Most thalassemias are inherited in a Mendelian recessive fashion.^[2] The result is imbalanced globin chain production, ineffective erythropoiesis, hemolysis, and a variable degree of anemia.^[3]

Clinically, the thalassemias are classified according to their severity into major, intermediate, and minor forms. Thalassemia major is a severe and transfusion-dependent disorder. Thalassemia minor is the symptomless trait or carrier state. Thalassemia intermediate is characterized by anemia (with or without splenomegaly), though not of such severity as to require regular transfusion.^[2]

A chronic subclinical hypercoagulable state already exists in thalassemia. The various factors are involved in its pathogenesis. Also a few studies have described bleeding tendencies in these patients in the form of epistaxis. Thus these patients can have thrombosis/bleeding depending on the balance between coagulation cascade and fibrinolytic system. Thrombin activatable fibrinolysis inhibitor (TAFI) is a recently discovered zymogen. It is synthesized in the liver and megakaryocytes and circulates in blood in complex with plasminogen. Increased TAFI levels have

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been correlated with increased clot lysis time in normal individuals. The role of TAFI has been studied in various conditions such as stroke, deep vein thrombosis, and gastric carcinomas. Increased TAFI levels were positively correlated in patients with deep vein thrombosis. TAFI thus serves as a link between the coagulation cascade and the fibrinolytic system.^[1] With this background, the present study was conducted to understand the role of TAFI as a contributing factor in hemostatic alteration state in adults with β -thalassemia.

MATERIALS AND METHODS

Fifty-six adult thalassemic patients were included in this cross-sectional study performed at the Thalassemia Center, Al Karamah Teaching Hospital, Baghdad, Iraq during the period from July to November 2018. All cases were diagnosed by a consultant hematologist based on clinical and laboratory evaluation including peripheral blood evaluation and hemoglobin electrophoresis from early years of life by reviewing medical records.

Criteria for exclusion of the patients

This includes

1. Patients under 15 years old;
2. Patients who had previous blood transfusion, use of salicylates, or anticoagulants in the preceding 4 weeks;
3. Patients with positive hepatitis viral screening;
4. Any evidence of acute infection or other hemoglobinopathies.

Proper history with a special emphasis on bleeding or any symptoms suggestive of thromboembolic events and physical examination for any signs of thrombosis were performed, and laboratory evaluation in forms of complete blood count (CBC), prothrombin time (PT), and activated partial thromboplastin time (APTT) was done for all patients. A peripheral venous blood sample was withdrawn from each patient and control subject at early morning: 4 mL of whole blood was collected in two tubes: 2 mL on EDTA for CBC and 2 mL of blood was collected on sodium citrate for immediate assay of PT and APTT; part of citrated plasma was stored at -40°C until assay

of TAFI. CBC was performed using a Sysmex XP-300 Automated Hematology Analyzer and PT and PTT using a coagulometer.

Assessment of plasma TAFI level was done by using the ELISA technique, using a MyBioSource TAFI, ELISA Kit.^[4] All other laboratory investigations were done in the Laboratory of Al-Imamein Kadhimein Medical City.

Statistical analyses were performed using SPSS Statistical Package for Social Sciences (version 17.0 for Windows, SPSS, Chicago, IL, USA). Data are presented as mean $\pm$ standard deviation for quantitative variables and as number and percentage for qualitative variables. Differences between groups were evaluated with the analysis of variance test for quantitative data, followed by the least significant difference (LSD) test or individual group differences. Pearson's correlation test was used to test the relation between different variables. Qualitative data relations were analyzed by the χ^2 test. *P*-value of less than 0.05 was considered statistically significant.

RESULTS

This study was conducted on 56 patients with a known history of beta-thalassemia. The patients were subdivided into four groups: group I were 17 splenectomized thalassemia major patients, group II 19 non-splenectomized thalassemia major, group III 10 non-splenectomized thalassemia intermediate, and group IV 10 thalassemia minor patients. Nineteen healthy individuals were served as controls.

Demographic and clinical characteristics of the study population

The mean age was significantly higher in groups I, IV, and controls compared with groups II and III. Likewise, there was no significant difference in the frequency of bleeding between groups I and II. However, the last transfusion was significantly farther in groups I and II than group II [Table 1].

Laboratory characteristics of the study population

Groups I–III showed a significantly higher PT and APTT than group IV and controls. Mean serum ferritin decreased

Table 1: Demographic and clinical characteristics of the study population

Variables	Major with splenectomy (<i>n</i> =17)	Major without splenectomy (<i>n</i> =19)	Intermediate (<i>n</i> =10)	Minor (<i>n</i> =10)	Controls (<i>n</i> =19)
Age	30.8 $\pm$ 6.9 ^a	17.9 $\pm$ 2.3 ^b	19.8 $\pm$ 6.7 ^b	29.2 $\pm$ 6.2 ^{ac}	25.7 $\pm$ 7.8 ^c
Sex					
Male	12 (70.59%)	10 (52.63%)	3 (30%)	4 (40%)	10 (52.63%)
Female	5 (29.41%)	9 (47.37%)	7 (70%)	6 (60%)	9 (47.37%)
Last transfusion (days)	34.1 $\pm$ 4.4 ^a	34.2 $\pm$ 6.3 ^a	27.1 $\pm$ 7.1 ^b	—	—
Epistaxis					
Yes	4 (23.53%)	3 (15.79%)	0 (0%)	0 (0%)	0 (0%)
No	13 (76.47%)	16 (84.21%)	10 (100%)	10 (100%)	10 (100%)

Different small letters indicate significant differences

gradually from groups I to IV with a significant difference between all these groups. Mean corpuscular volume and mean corpuscular hemoglobin were significantly lower in patients when compared with controls. Finally, group I showed a significantly higher neutrophils and platelets count than the other groups of patients and controls [Table 2].

Serum levels of TAFI

Values of serum level of TAFI show an ascending trend from group I to group IV and then controls (7.2 ± 1.6 , 9.3 ± 1.9 , 11.4 ± 1.3 , 13.8 ± 1.6 , and 14.3 ± 2.1 ng/mL, respectively). All these groups differed significantly from each other, except minor thalassemia and controls with no significant difference [Figure 1].

Association of epistaxis with TAFI concentration

In group I, patients with epistaxis showed lower serum level of TAFI than those with no epistaxis (6.0 ± 0.69 vs. 7.5 ± 1.6 ng/mL); however, the difference did not reach a significant level [Figure 2].

Correlation of TAFI concentration with the other variables

Serum ferritin

Using Spearman's correlation test between serum levels of TAFI in patients from all groups with serum ferritin revealed a strong negative highly significant correlation ($r = -0.765$, $P < 0.001$), as shown in Figure 3.

Platelet count

Similarly, there was a negative moderate significant correlation between TAFI in patients from all groups with platelet count ($r = -0.416$, $P = 0.001$), as shown in Figure 4.

DISCUSSION

Thalassemia is an inherited disorder of hemoglobin synthesis that results in reduced or absent production of globin chain.^[5] Hypercoagulable state in thalassemia is a

well-recognized event. The incidence of thromboembolic events in β -thalassemia major varied from 0.9% to 4.0% in various studies,^[1] although bleeding tendencies had also been reported.^[6,7]

TAFI is an enzyme considered to play an important role in the regulation of fibrinolysis. Increased TAFI levels had been reported in association with thromboembolic disease.^[6]

In the present study, none of the patients presented with clinical evidence of thrombosis, but 7 out of 56 thalassemic (12.5%) patients had been suffered from epistaxis. This observation is in congruence with another study performed by Mokhtar *et al.*,^[6] who studied 51 thalassemic patients having bleeding manifestation, particularly in the form of epistaxis, and this was observed in 17.5% of the thalassemic patients.

The present work showed a significantly higher PT and APTT in thalassemic major and intermediate patients when compared with thalassemia minor patients and controls ($P < 0.001$); this is in agreement with other studies.^[6,7] The total WBC count, neutrophil count, and platelet count were significantly higher in splenectomized thalassemic patients compared with non-splenectomized patients, which is due to the pooling effect on WBC and platelets in a large spleen, and this was similarly achieved by the study of Mokhtar *et al.*^[6,8] The present work revealed elevated mean serum ferritin, particularly in group I which is compatible with other reports.^[6,9,10]

In the present work, highly significant reduction in TAFI levels was observed in all thalassemic patients compared with thalassemia minor patients and controls ($P < 0.0001$). The TAFI level was significantly lower in the splenectomized group compared with the non-splenectomized group ($P < 0.0001$). The TAFI level was significantly lower in the thalassemia major group when compared with the thalassemia intermediate group ($P < 0.0001$). These findings were in agreement with other studies performed by Mokhtar *et al.*^[6] and

Table 2: Laboratory characteristics of the study population

Variables	Major with splenectomy (n=17)	Major without splenectomy (n=19)	Intermediate (n=10)	Minor (n=10)	Controls (n=19)	P-value
PT (s)	16.7 $\pm$ 1.7 ^a	17.1 $\pm$ 2.8 ^a	16.1 $\pm$ 1.5 ^a	14.3 $\pm$ 0.5 ^b	14.4 $\pm$ 1.3 ^b	<0.001
APTT (s)	35.5 $\pm$ 3.5 ^a	37.4 $\pm$ 4.8 ^a	35.9 $\pm$ 7.2 ^a	30.2 $\pm$ 1.1 ^b	30.7 $\pm$ 1.5 ^b	<0.001
Hb (g/dL)	8.3 $\pm$ 0.6 ^a	8.5 $\pm$ 0.8 ^a	8.5 $\pm$ 1.5 ^a	10.8 $\pm$ 0.9 ^b	13.6 $\pm$ 1.3 ^c	<0.001
Ferritin (ng/mL)	2172 $\pm$ 362.9 ^a	1322 $\pm$ 115.5 ^b	988.3 $\pm$ 193.5 ^c	185.8 $\pm$ 40.8 ^d	116.5 $\pm$ 19.2 ^d	<0.001
WBC ($\times 10^3$)	10.7 $\pm$ 1.3 ^a	6.3 $\pm$ 1.4 ^b	7.5 $\pm$ 2.8 ^b	7.4 $\pm$ 1.5 ^b	9.8 $\pm$ 1.7 ^a	<0.001
MCV (fL)	75.6 $\pm$ 2.1 ^a	76.0 $\pm$ 2.0 ^a	74.0 $\pm$ 4.8 ^a	68.1 $\pm$ 4.5 ^b	88.2 $\pm$ 3.0 ^c	<0.001
MCH (pg)	23.8 $\pm$ 1.2 ^a	24.2 $\pm$ 1.3 ^a	23.3 $\pm$ 1.6 ^a	20.3 $\pm$ 2.1 ^b	28.9 $\pm$ 2.1 ^c	<0.001
Neutrophil $\times 10^3$	7.3 $\pm$ 0.8 ^a	3.8 $\pm$ 1.0 ^b	4.4 $\pm$ 1.7 ^{bc}	5.1 $\pm$ 0.9 ^c	6.8 $\pm$ 1.0 ^c	<0.001
Platelets $\times 10^3$	500.8 $\pm$ 96.3 ^a	257.1 $\pm$ 88.1 ^b	367.6 $\pm$ 177.0 ^c	318.6 $\pm$ 46.5 ^{bc}	326.5 $\pm$ 109.8 ^c	<0.001

PT = prothrombin time; APTT = activated partial thromboplastin time; Hb = hemoglobin; WBC = white blood cell; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin

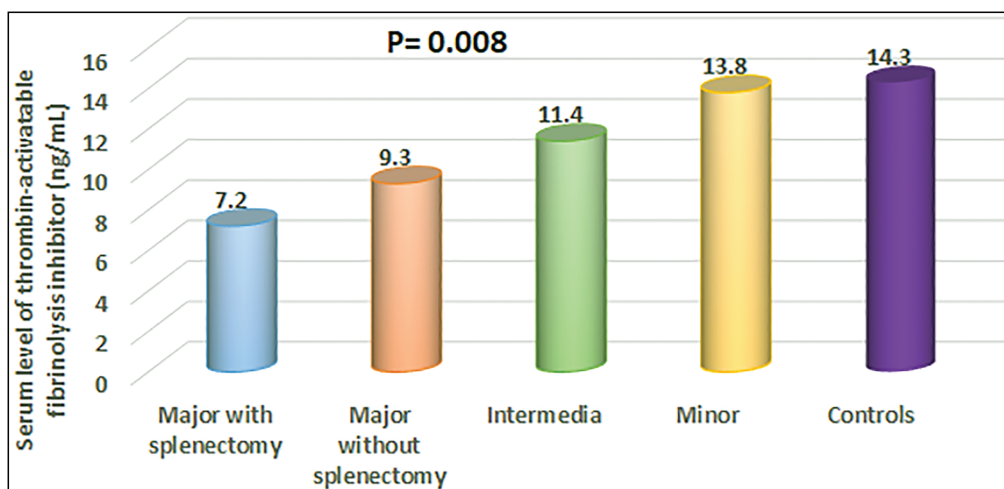


Figure 1: Serum level of TAFI in different groups

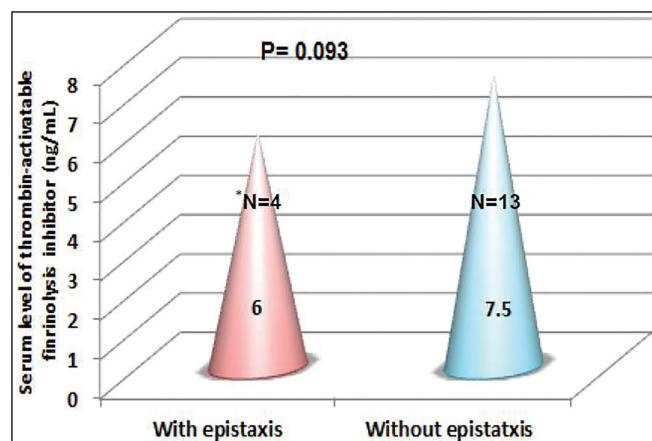


Figure 2: TAFI in splenectomized thalassemic major group patients with and without epistaxis

Tripatara *et al.*,^[10] although they disagreed with the study of Chhikara *et al.*^[11]

The low level of TAFI could point to the fact that the enzyme had been used to inhibit fibrinolysis as a result of the existence of low grade of consumptive coagulopathy among blood transfused patients as was proposed by the study of Mokhtar *et al.*^[6] The study of Bouma and Meijers^[11] emphasized that there are polymorphic variations in the TAFI gene expression, which had its effect on the TAFI level in the blood which in turn may partially explain the variation of its level among different individuals.

In the present study, patients with epistaxis showed lower mean serum level of TAFI than those with no epistaxis, a finding that is in correspondence with other studies.^[1,6,11] This finding confirms that the concept of the lower level of TAFI enzyme is associated with increased fibrinolysis; this will result in increased bleeding tendency.

The present study revealed that the TAFI enzyme level was significantly lower in splenectomized thalassemic

patients when compared with that of non-splenectomized thalassemic patients, which may be partially explained by the decreased TAFI enzyme synthesis in affected liver in those patients, as splenectomized thalassemic patients had higher serum ferritin level and increased chance of hemochromatosis.^[10] Variable results regarding correlation between TAFI level and serum ferritin and platelets had been reported.^[1,6]

The current work illustrated a significant negative correlation between TAFI level and serum ferritin ($P < 0.001$) and TAFI level and platelets ($P = 0.001$); these findings agree with those reported by Mokhtar *et al.*^[6] and there was a significant negative correlation between TAFI level and serum ferritin levels and platelets. This negative correlation of TAFI level with serum ferritin may reflect the toxic effect of iron on hepatic cells, leading to decreased synthesis of TAFI enzyme in the liver.^[12]

In conclusion, the TAFI enzyme level could be an important predictor and valuable investigation for hemostatic alteration, whether in the form of thrombosis or bleeding. Its level can be considered as a helpful marker for monitoring and follow-up of thalassemic patients for early and proper intervention to minimize complication.

CONCLUSION

The TAFI level was low in thalassemia major and intermediate patients when compared with thalassemia minor patients and controls, and it is lower in the thalassemia major than the thalassemia intermediate. As TAFI had a direct effect on fibrinolytic system, the low level of TAFI in thalassemic patients can be considered as a marker to assess the hemostatic status of those patients, especially if they had bleeding tendency. Also, the TAFI level was lower in splenectomized thalassemic patients than that in non-splenectomized thalassemic patients, which may reflect the toxic effect of high ferritin level

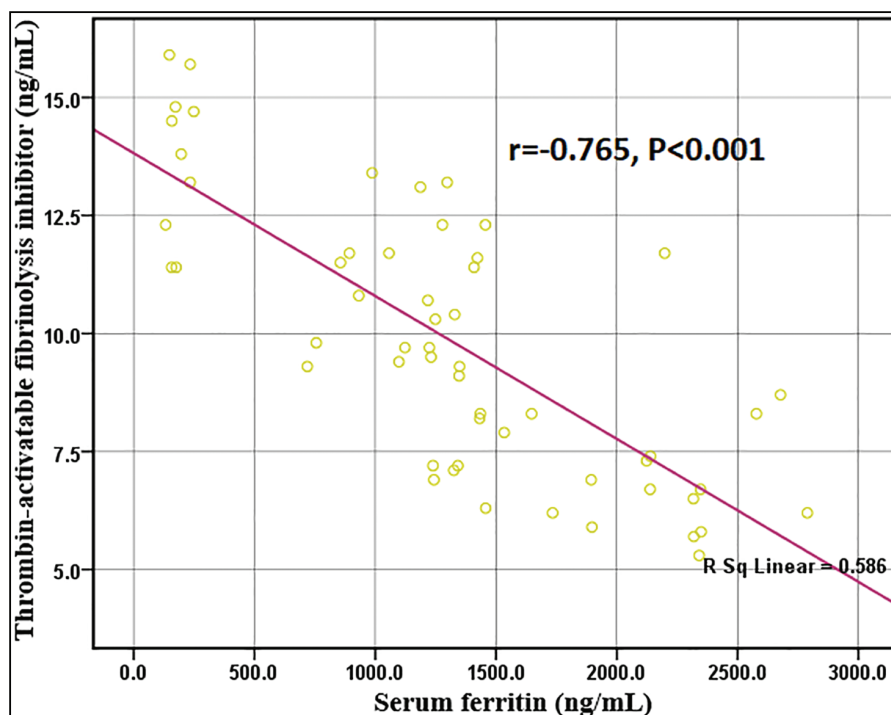


Figure 3: Correlation between TAFI and serum ferritin in 56 thalassemic patients

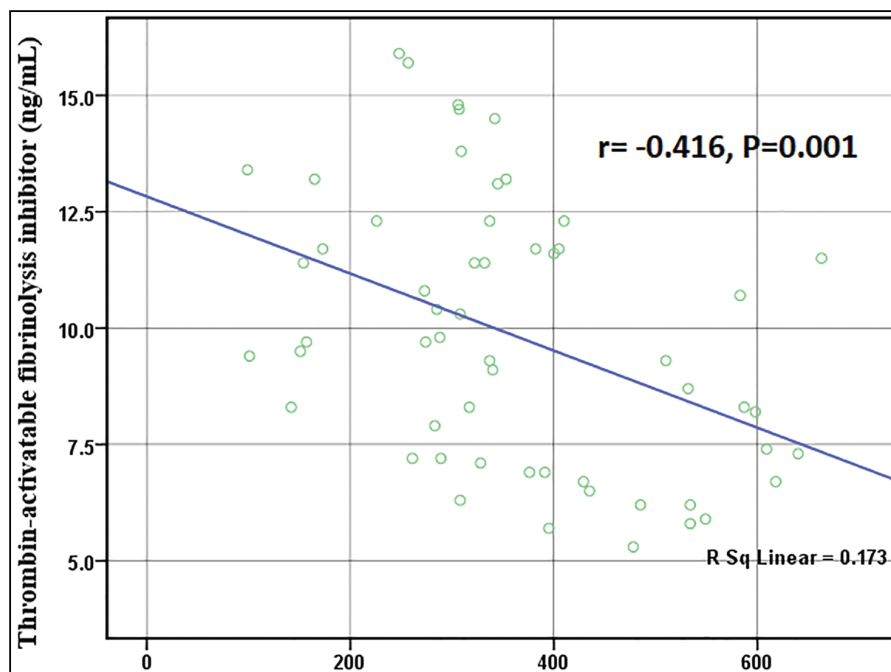


Figure 4: Correlation between TAFI and platelet count in 56 thalassemic patients

observed in splenectomized than in non-splenectomized. The normal level of TAFI in the thalassemia minor is possibly due to the benign course of the disease with less derangement of hemostatic parameters.

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Conflicts of interest

There are no conflicts of interest.

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