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Plasma level of soluble glycoprotein IIb/IIIa in Beta-thalassemia major patients in relation to thrombotic risk score.

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ABSTRACT:

Background: Multifactorial mechanisms are tangled in the pathogenesis of Thromboembolic events (TEE) in thalassemia major(TM) including the combination of the components of the hemostatic process together with the disease-specific features. GpIIb/IIIa is an integrin complex found on surface of platelets. It is a receptor for fibrinogen and prothrombin. Its activation leads to platelet aggregation.

Aim: Estimate the plasma level of s GpIIb/IIIa in β- TM Subjects and methods: The study was conducted on 67 individuals divided into 2groups. The first group included 47 TM patients, while the second group included 20 healthy control subjects. complete physical examination , CBC, serum ferritin, CRP, and s GpIIb/IIIa by ELISA were done.

Results The serum level of GpIIb/IIIa was significantly higher in the TM group with a p value <0.001. , there was a significant relation between patients with high and intermediate risk TRT – RSS and Gp IIb/IIIa with ap value of <0.001.

Conclusion: TM are characterized by stronger platelet aggregation as reflected by increased GpIIb/IIIa which was associated with high , and intermediate TRT-RSS.

Keywords: hypercoagulability, glycoprotein (Gp IIb/IIIa), Thalassemia-related thrombosis risk scoring system (TRT-RSS)

INTRODUCTION

βeta-Thalassemia is a worldwide genetic disorder characterized by excess unpaired alpha globulin chains and deficient or absent beta globulin chains. The mainstay of treatment is blood transfusion and iron chelation therapy . The resulting hemosiderosis cause various endocrine disorders such as diabetes mellitus, hypothyroidism, hypopituitarism, hemochromatosis, cirrhosis, and cardiomyopathy (Lei et al., 2019).

A hypercoagulable state has been recently observed in thalassemic patients (Chanpeng et al., 2019). It manifests as pulmonary embolism , venous thromboembolism (VTE), and recurrent arterial occlusion. These microthrombi might be circulating platelet aggregates attributed to increased expression platelet-selectin (P-selectin) and a decreased platelet lifespan because of increased platelet consumption indicating activation of platelets

One of the important confounding causes of increased coagulability was excessive iron deposition resulting in reactive oxygen species which cause further platelet hyperaggregation in transfusion dependent thalassemia (TDT)patients (Vasilopoulou et al., 2022).

Various risk assessment models, such as Geneva , Padua prediction score risk , and International Medical Prevention Registry on VTE (IMPROVE), are in use to stratify the risk of VTE. Thus, the proper selection of the suitable risk score for a specific population is crucial (Woller et al., 2011; Mehta & Bhave, 2023).

A. Taher et al., proposed Thalassemia-related thrombosis risk scoring system (TRT-RSS), which include laboratory and clinical parameters related to the hypercoagulable state of TDT (such as hemoglobin level, age , transfusions rate, serum ferritin, and splenectomy) and can be used for patients' re-evaluation during their life and long-term assessment of thrombotic risk (Taher et al., 2019).

Glycoprotein IIb/IIIa (GpIIb/IIIa, also known as CD41/ CD61) is found on platelet surface. It is an integrin act as receptor for fibronectin, plasminogen, prothrombin, thrombospondin and fibrinogen. Its activation leads to platelet to platelet interaction via soluble

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fibrinogen binding
consequently causing prompt platelet aggregation. Von Willebrand factor promote and
Fibrinogen further cause platelet activation (Fullard, 2004).
The role of GpIIb/IIIa in controlling adhesion , aggregation of platelet which initiated by activation of platelet through an agonist leading to initiating a signaling process known as "inside-out" signaling, causing conformational changes within GpIIb/IIIa resulting in binding sites exposure which facilitate soluble fibrinogen recognition. This leads to increasing its affinity for fibrinogen, which by its turn operates as a linking factor accelerating the adjacent platelets interaction . The binding of GpIIb/IIIa to fibrinogen to causes another conformational change termed the "outside-in" signaling which ultimately ended in aggregation process acceleration (Duan et al., 2012).
The hypercoagulable state observed in TDT patients needs to be further studied and due to unavailable literature regarding the role of GP IIb/IIIa in inducing thromboembolic events in TDT, this encouraged us to further investigate its role in β-thalassemia induced hypercoagulability

Aim of the study

The present work aimed at estimating plasma level of soluble glycoprotein IIb/IIIa (GpIIb/IIIa) in β- thalassemia major patients in relation to thrombotic risk score

Subjects

This study was conducted on forty seven Egyptian TDT patients who are followed up in Hematology department, Medical Research Institute during the period from September 2022 to May 2023. Twenty normal sex and age matched individuals were included as controls.
An informed consent was collected from each participant and the approval of Ethical Committee of Medical Research Institute, Alexandria University, for the planned research was obtained.
Samples were obtained from thalassemic patients just prior to blood transfusion.

Exclusion criteria

Patients with other forms of thalassemia were excluded, as well as those with infection especially COVID-19.

Methods

All patients in this study were subjected to detailed history taking, clinical examination with special emphasis on spleen and liver, Complete blood count (CBC) (Bain et al., 2017), Serum Ferritin (Bain et al., 2017), C-reactive protein (CRP) (Burtis et al., 2015), Calculation of VTE risk assessment scores (Taher et al., 2019; Mehta & Bhave, 2023), and Plasma level of GpIIb/IIIa by ELISA (Fadel et al., 2022).

Statistical analysis

IBM SPSS software package version 20.0 was used . Qualitative data were described using percent and number .
The quantitative variables distributions were tested for normality through Shapiro-Wilk and D'Agstino test, also Histogram and QQ plot were used for vision test. If it reveals normal data distribution, parametric tests was applied. Quantitative data were described using mean and standard deviation, median, minimum and maximum.

Results

The patients group consisted of 47 TDT patients , 17 (36.2%) patients were males, and 30 patients (63.8%) were females. The median age of the patients group was 20 years and ranged from 8 to 40 years.
The control group consisted of 20 healthy subjects, 7 (35%) subjects were males, and 13 (65%) were females. The median age of the control group was 30.5 years and ranged from 10 to 41 years.
Thirteen patients (27.7%) suffered from splenomegaly, while 25 patients underwent splenectomy (53.2%) and the rest 9 patients (19.1%) had normal sized spleen.
Serum levels of CRP and ferritin were significantly higher among TDT patients than control subjects with a p value of <0.001. Table (1)

Table (1) Comparison between TDT and control groups according to serum levels of CRP and Ferritin.

Parameter	Patients (n = 47)	Control (n = 20)	P
CRP mg/dL			
Min. – Max.	1.32 - 4.30	1.00 - 2.90	<0.001*
Mean ± SD.	2.79 ± .80	1.92 ± 0.53	
Median (IQR)	2.90 (2.02 - 3.35)	1.95 (1.55 - 2.30)	
Ferritin ng/mL			
Min. – Max.	600.0 -11192.0	30.0 - 80.0	<0.001*
Mean ± SD.	2967.9 ± 2072.6	51.0 ± 14.35	
Median (IQR)	2500.0 (1751.5 - 3421.0)	50.0 (39.0 - 59.50)	

IQR: Inter quartile range SD: Standard deviation.
p: p value for comparing between TDT and control group.
*: Statistically significant at p ≤0.05

The serum level of GpIIb/IIIa was significantly higher in TDT patients than control subjects with a p value <0.001.Table (2)

Table (2) Comparison between the TDT and control groups according to GpIIb/IIIa.

Parameter	Patients (n = 47)	Control (n = 20)	Test of sig.	P
GpIIb/IIIa ng/mL				
Min. – Max.	**2.94 – 21.11**	**0.04 – 3.92**		
Mean ± SD.	**7.35 ± 3.96**	**2.30 ± 1.05**	**U = 20.0***	**<0.001***
Median (IQR)	**5.88 (4.90 – 8.64)**	**2.16 (1.43 – 3.25)**		

IQR: Inter quartile range SD: Standard deviation U: Test Mann Whitney.
p: p value for the 2 studied groups comparison.
*: Statistically significant p ≤0.05

The TRT-RSS score was significantly higher among the splenectomised group than the non splenectomised one with a p value <0.001. Table (3) figure (1)

Table (3) Comparison between splenectomised and non splenectomised groups according to TRT- RSS grade and TRT score.

	Cases				Test of Sig.	p
	Non-splenectomised (n = 22)		Splenectomised (n = 25)			
	No.	%	No.	%		
TRT- RSS grade						
Low risk	22	100.0	1	4.0	χ^2= 50.420*	MCp <0.001*
Int. risk	0	0.0	22	88.0		
High risk	0	0.0	2	8.0		
TRT Score						
Min. – Max.	0.0 – 6.0		8.50 – 17.0		U= 0.000*	<0.001*
Mean ± SD.	3.66 ± 1.59		11.88 ± 1.71			
Median (IQR)	4.50 (2.0 – 4.50)		11.0 (11.0 – 13.50)			

IQR: Inter quartile range SD: Standard deviation U: Test Mann Whitney *: Statistically significant p ≤0.05
χ2: Test Chi square MC: Monte Carlo p: p value for the 2 studied groups comparison.

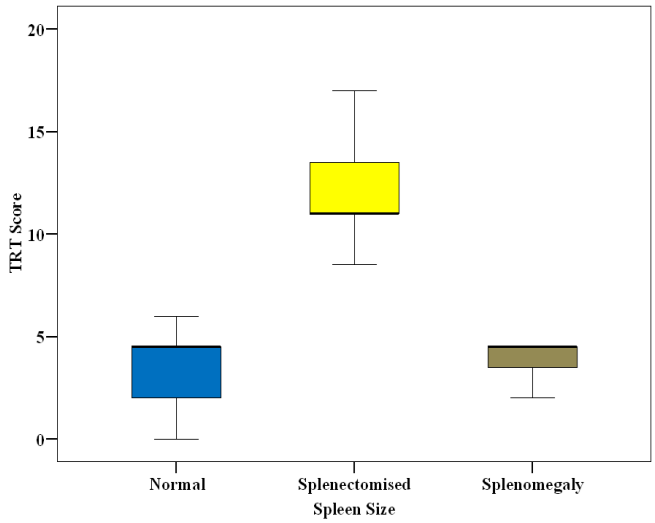

Figure (1): Relation between TRT Score and Spleen Size in cases group (n = 47).

There was a significant relation between glycoprotein IIb/IIIa and splenectomised patient as the median concentration in these patients was significantly higher in the splenectomised patients than those with splenomegaly and those with normal spleen size with a p value <0.001.

Also, there was a significant relation between TDT patients with high and intermediate risk TRT – RSS grade and glycoprotein IIb/IIIa as the median concentration of glycoprotein IIb/IIIa in these patients was significantly higher than in low risk patients with ap value of <0.001. Table(4) figure (2),(3)

Table (4): Relation between GpIIb/IIIa and different parameters in TDT patients group (n = 47).

Parameter	No.	GpIIb/IIIa ng/mL		Test of Sig.	P
		Mean ± SD.	Median (Min. – Max.)		
Spleen Size					
Normal	**9**	5.29 ± 1.78	4.88 (3.55 – 9.52)	H = 15.909*	<0.001*
Splenectomised	**25**	9.20 ± 4.33	8.05 (3.63 – 21.11)		
Splenomegaly	**13**	5.20 ± 2.28	5.04 (2.94 – 11.88)		
TRT- RSS grade					
Low risk	**23**	5.41 ± 2.16	5.04 (2.94 – 11.88)	H = 14.242*	0.001*
Int. risk	**22**	9.02 ± 4.42	8.04 (3.63 – 21.11)		
High risk	**2**	11.24 ± 5.45	11.24 (7.39 – 15.1)		

SD: Standard deviation H: H for Kruskal Wallis test
No: Number p: p value for Relation between GpIIb/IIIa and different parameters
*: Statistically significant p ≤0.05

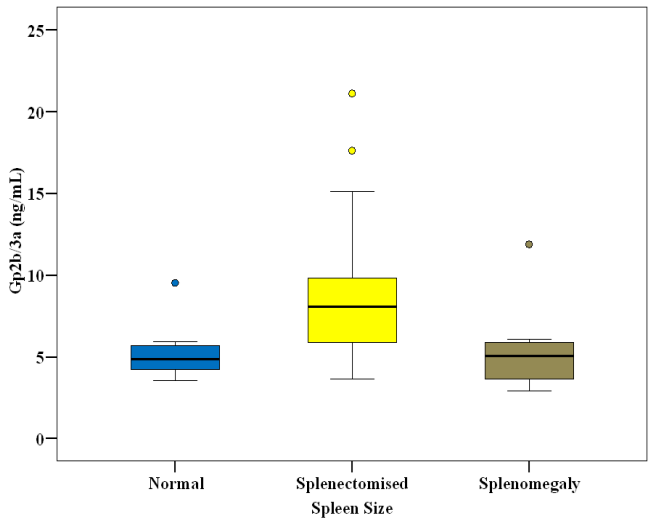

Figure (2): Relation between GpIIb/IIIa and spleen size in cases group (n = 47).

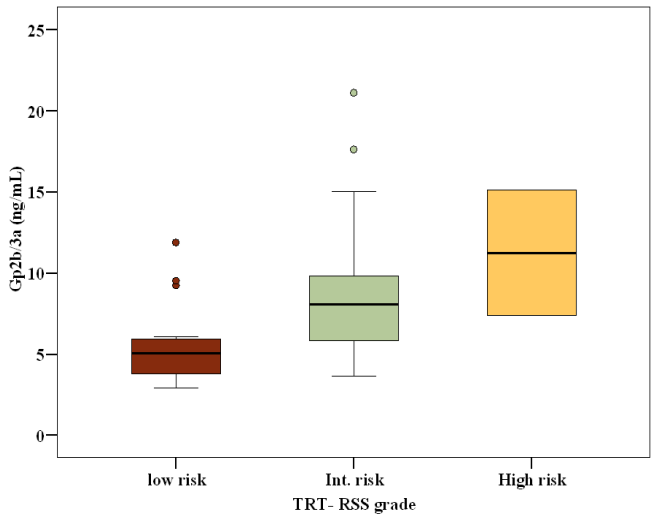

Figure (3): Relation between GpIIb/IIIa and TRT- RSS grade in cases group (n = 47).

There was a significant relation between TRT Score and splenectomised patients as they were associated with higher risk scores than the rest of the patients, having a p value of <0.001

Glycoprotein IIb/IIIa was positively correlated with high TRT Score with a p value of <0.001.

the frequency of high risk of VTE in TDT according to TRT-RSS was 4.3 %, while the intermediate risk was 46.8 % and the low risk was 48.9 %.(figure 4)

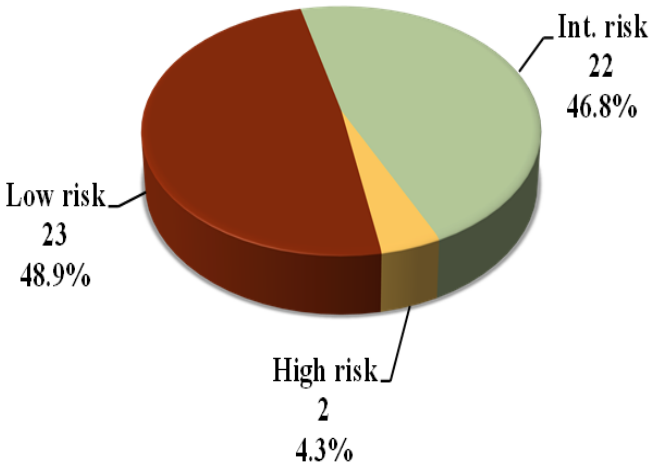

Figure (4): Distribution of the studied cases according to Thalassemia related thrombosis risk scoring system (TRT-RSS) grade in cases group (n = 47).

Discussion

Frequent blood transfusion is life saving for thalassemia major patients; however, it comes with risks of infection with blood born viruses such as hepatitis C. Until recently, it was a highly prevalent disease in Egypt (Kouyoumjian et al., 2018), as well as iron overload reflected on their serum ferritin levels (Ben Salah et al., 2017). We found that Serum levels of ferritin was significantly higher among TDT patients than control subjects with a p value of <0.001.The increased ferritin level in thalassemic patients especially the TDT was related to increased transfusion frequency with increased ineffective erythropoiesis, Hemoglobin instability and elevated the gastrointestinal tract iron absorption are

consider important causes of hemosidrosis in thalassemia. This is consistent with other researchers which showed increased ferritin serum level and iron stores in TDT patients than the control subjects with p value <0.001 (Mohammed & Abd-El Rasoul, 2020; Zeidan et al., 2022).

The serum level of C-reactive protein was significantly higher among TDTpatients than control subjects with a p value of <0.001 despite being both within the normal reference range. Our result agreed with AbdulJabbar and Mehmetçik who also reported significantly higher serum CRP level among the thalassemic group yet not exceeding the physiological limit.

Moftah et al., and Abduljabbar et al., reported similar results and they declared that CRP and other inflammatory markers should be used in the assessment of TDT patients follow up for early complications detection (Abduljabbar & Mehmetcik, 2019; Moftah et al., 2020).

Iron homeostasis is associated with inflammation and oxidative stress. Chronic hemolysis , iron overload , globin chain imbalance, and antioxidant defenses deficiency leads to formation of oxidative stress. All these factors lead to cell death, anemia, organ damage, inflammation, and hypoxia (Jokhio et al., 2009; Zeidan et al., 2022).

To our best of knowledge this is the first study to assess the level of Gp IIb/IIIa in TDT patients. The present study revealed that the serum level of GpIIb/IIIa was significantly higher in the TDT patients as compared to the control subjects and in the splenectomised group as compared to the non splenectomised one with a p <0.001 and p = 0.002 respectively.

Fiodorenko-Dumas et al., revealed that the GpIIb/IIIa expression receptors on the activated platelets surface was much increased in patients with vascular complications, than in the control subjects (Fiodorenko-Dumas et al., 2019). Guo et al. (2015) reported that on the atherosclerotic endothelium, the GpIIb/IIIa expression on activated platelets surface is a biomarker for detecting high-risk vascular calcifications impeding to rupture and thus preventing acute cardiovascular events (Guo et al., 2015).

Moreover, our study showed a significant relation between increased glycoprotein IIb/IIIa level and splenectomised patients as the median concentration was significantly higher in the splenectomised patients than those with splenomegaly and those with normal spleen size with a p value <0.001. Also there was a significant high TRT-RSS score in splenectomized patients than those with either splenomegaly or normal spleen with p value <0.001. This can be explained as Splenectomy is often accompanied with a subsequent rise in platelet count (PLTs), known as postsplenectomy reactive thrombocytosis potentially exposing these patients to an increased risk for VTE development (P.N. Khan et al.,2009).

Also there was a significant relation between increased glycoprotein IIb/IIIa level and high TRT-RSS with a p value 0.001.

In the current study the frequency of high risk of VTE in TDT according to TRT-RSS was 4.3 %, while the intermediate risk was 46.8 % and the low risk was 48.9 %. Taher et al , found that VTE incidence in TDT patients is between 1.7 and 9.2%, therefore, approximately this incidence is ten times higher than normal population (Taher et al., 2006).

Splenectomy and blood transfusion induce the vascular complications in TDT patients as VTE events due to hypercoagulability. The VTE pathophysiological mechanisms include damaged abnormal erythrocyte membranes with phosphatidylserine exposure, abnormal activation and aggregation of platelet, endothelial activation, low nitric oxide due to hemolysis, and finally organ dysfunction (Taher et al., 2018; Taher et al., 2021).

Early identification and diagnosis can effectively reduce the risk of venous thromboembolism (VTE). Several risk assessment models (RAMs) of VTE have already been established and evaluated in various populations (Lian et al., 2023).

Different models use clinical data from the disease history and clinical examination to identify subjects with high risk of developing VTE in clinical diseases. Fortunately, Taher et al. (2019) introduced a new VTE risk assessment model that was designed specifically for thalassemic patients. TRT-RSS helps physicians in classifying patients for thrombosis risk according to the basic nature of the disease, and thus assists in treatment decisions. The TRT-RSS is intended to predict initial thrombotic events in patients lacking previous events and offers continuing risk assessment taking into account the chronicity, lifetime characteristic of the disease (Taher et al., 2019; García & Molina, 2020).

Conclusion

GpIIb/IIIa is considered as a significant indicator of platelet activation and increasesd thrombotic events. There is increased platelets activation in thalassemic patients even in absence of active thromboembolism.The TRT-RSS is a useful risk assessment tool intended to predict initial thrombotic events in patients lacking previous events.

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