Original Research Article

Coagulation and Fibrinolysis Dysregulation in β-Thalassemia Major: Potential Impact of Splenectomy and Medications on Thrombotic Risk

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Abstract

Objective: The dysregulation of clotting factors in β-thalassemia major is not fully understood. This case-control study examines the impact of splenectomy and medications on clotting factor dysregulation.

Methods: Lab. tests of coagulation and fibrinolysis were performed.

Results: This study included 60 β-thalassemia major patients of both sexes (7-35 years) and 20 age-matched controls. None of the participants had a previous thrombotic event. No difference existed between control and β-thalassemia groups in all tested parameters except for protein S (PS) which was 26.1% lower in β-thalassemia group compared to the control group ($p = 0.0001$) while D-Dimer, plasminogen activator inhibitor-1 (PAI-1) and platelet count showed an increase in their levels by 53.6%, 93.4% and 112.7%, respectively compared to the control ($p = 0.004$, $p = 0.0001$, $p = 0.0001$, respectively). Notably, a strong positive correlation existed between platelet count and PAI-1 ($r = 0.669$, $p = 0.0001$) in β-thalassemia group. Splenectomized patients had higher platelet count (+45.2%), PAI-1 (+98.1%), fibrinogen (+18.9%) and tPA (+197.2%) compared to the non-splenectomized group (all $p < 0.05$). No significant differences in PS, D-dimer, PT, INR, aPTT, fibrinogen, PC, TF, tPA or ADAMTS13 were found between β-thalassemia major patients taking and not taking aspirin. However, a higher platelet count (+37.1%) and PAI-1 level (+58.9%), along with a lower vWF level (−25.6%), were observed between these two groups (all $p < 0.05$).

Conclusion: Elevation in PAI-1 and platelet count in splenectomized β-thalassemia major could increase the risk of having a thrombotic event. Medications may have significant interactions with blood coagulation in β-thalassemia.

Keywords

aspirin, iron-chelation, plasminogen activator inhibitor-1, protein s, splenectomy, β-thalassemia

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Introduction

β-thalassemia major is a genetic blood disorder characterized by mutations that impair the production of hemoglobin β-globin chains.[1,2] Thus, causing persistent anemia and ineffective erythropoiesis which require urgent management through regular blood transfusion. Unfortunately, this leads to iron overload in many cases.[3] Thalassemia is considered a global economic burden due to the high cost of treatment[4] and its involvement in the development of complications such as the possibility of thrombosis and organ failure.[5]

β-thalassemia major patients require regular blood transfusion.[6] Although there is a marked improvement in

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their life expectancy due to transfusions and iron-chelation therapy, some fatal complications are recognized like thromboembolic events.[7] The pathophysiological mechanisms involved in thrombosis include endothelial activation, alterations in the levels of the body's natural anticoagulants, damage to red blood cell membranes and increased platelet activation.

In thalassemia, endothelial damage is primarily driven by chronic hemolysis, which releases free hemoglobin and arginase. These substances deplete nitric oxide, impairing vasodilation and promoting oxidative stress.[8] Additionally, iron overload—common due to frequent blood transfusions—leads to the generation of reactive oxygen species, which further exacerbates endothelial injury.[9]

One of the most critical pathophysiological mechanisms contributing to thrombosis in thalassemia patients is the alteration in the levels of the body's natural anticoagulants, including protein C (PC), protein S (PS), and antithrombin. In healthy individuals, activated PC exerts its anticoagulant effect by proteolytically inactivating coagulation factors Va and VIIIa. Meanwhile, PS serves as a cofactor for activated PC.[10] Different studies reported variable results concerning the level of anticoagulants in β-thalassemia major. While most studies reported a significant decrease in PC and PS levels,[11–13] others reported no change in these anticoagulants.[14]

Another complication due to repeated blood transfusion is liver damage that may result due to viral hepatitis and/or hemosiderosis. This can result in reduced synthesis of coagulant and anticoagulant factors by the liver.[11] Also, it has been suggested that anticoagulants are consumed in patients with β-thalassemia as a result of long-term asymptomatic activation of coagulation cascade.[15]

In thalassemia, red blood cells exhibit membrane abnormalities, including externalization of phosphatidylserine that facilitates clotting by provides a catalytic surface for coagulation complexes,[16] enhancing thrombin generation. These changes activate endothelial cells and platelets, contributing to a hypercoagulable state. Moreover, abnormal thalassemic red blood cell membrane alterations cause a significant reduction in PC due to its high affinity to bind to phosphatidylserine and other negatively charged phospholipids.[17]

Platelet activation is enhanced in thalassemia due to chronic hemolysis, oxidative stress, and exposure to abnormal red blood cell membranes. Elevated circulating platelet-derived microparticles and increased expression of activation markers contribute to the hypercoagulable state.[18] Furthermore, thalassemia patients may undergo splenectomy to reduce red blood cell destruction, alleviate hypersplenism, and decrease transfusion requirements. However, patients may face increased risks of sepsis and thrombotic events, requiring vaccination and possible prophylactic treatment after splenectomy.[19] Moreover, the spleen's filtering function will be lost, leading to reduced clearance of aged or abnormal platelets from circulation. This leads to the accumulation of reactive platelets that can contribute to a hypercoagulable state, with thrombocytosis commonly reported.[20] Also, splenectomy increases the risk of hypercoagulation in β-thalassemia patients by impairing the clearance of procoagulant red blood cells such as those exposing phosphatidylserine.[16] Other contributing factors to this hypercoagulable state after splenectomy may exist. However, studies failed to find a significant difference in most hemostatic parameters including PC, PS, fibrinogen, and Factor Va.[21]

Plasminogen activator inhibitor-1 (PAI-1) is a key regulator of fibrinolysis. It functions by inhibiting key fibrinolytic enzymes namely: tissue plasminogen activator[22] and urokinase-type plasminogen activator,[23] reducing plasmin generation and slowing clot resolution. PAI-1 overexpression leads to a prothrombotic state, especially when combined with other risk factors like endothelial dysfunction or chronic hemolysis, making it a significant player in the development of thrombosis.[24] In the context of thalassemia, a single study with limited number of patients ($n = 30$) found an elevation in PAI-1 level in thalassemia patients compared to healthy controls.[25] However, no comparison between PAI-1 in splenectomized and non-splenectomized patients was performed.

Given the conflicting findings of previous studies regarding anticoagulant levels, this study aims to investigate the hypercoagulable state in patients with β-thalassemia major who are clinically stable and free of overt thromboembolic events. It also examines the role of PAI-1, which had not been previously compared between splenectomized and non-splenectomized thalassemia patients. Therefore, this study evaluates some alterations in coagulation and fibrinolysis parameters in β-thalassemia major, with particular attention to the impact of splenectomy and commonly used medications on hemostatic profiles. Understanding the various factors contributing to the hypercoagulable state in β-thalassemia major will enable healthcare professionals to enhance preventive and therapeutic strategies and fostering the development of new treatment options.

Materials and Methods

Ethical Approval and Patient Selection Criteria

This study included Jordanian patients of both sexes (7-35 years) admitted to the thalassemia and thrombophilia clinic at Al-Zarqa Public Hospital, Al-Zarqa, Jordan. The study was approved by the Institutional Review Board (IRB) of the Ministry of Health, Amman, Jordan (IRB # 138888). The control group consisted of age-matched healthy subjects (range 10-35 years). A participant written

consent form was signed by adults or children's guardians. Clinical data was extracted from the electronic medical records including information about age, sex, date of diagnosis, thrombotic and bleeding events, medications and blood transfusion. Patients eligible for inclusion in the study were those with a confirmed diagnosis of β-thalassemia major based on hemoglobin electrophoresis, who had been receiving 10–15 mL/kg regular blood transfusions monthly for at least 12 months with periodic testing of ferritin level. Hemoglobin levels of patients were kept ideally above 9 g/dL. However, some patients had hemoglobin level between 7.4 and 9 g/dL. All participants were required to be clinically stable for the preceding four weeks, and had no surgical operation for the removal of spleen in the last 6 months. Exclusion criteria included the presence of other hemoglobinopathies, a history of bone marrow transplantation or gene therapy, significant renal dysfunction, active infections such as hepatitis B, hepatitis C, or HIV and poor adherence to treatment or follow-up protocols. Patients with missing data were also excluded from the study.

Table 1. Demographic Data of the Study Participants. Values Represent Number (Percent) Except for Age Where Numbers Represent Mean ± SD.

Parameter	β-Thalassemia Group	Control Group
Age (years)	20.22 ± 6.87	19.10 ± 7.00
Gender		
Male	30 (50%)	9 (45%)
Female	30 (50%)	11(55%)
Aspirin intake	14 (23%)	0 (0%)
Calcium carbonate intake	20 (33.3%)	0 (0%)
Furosemide intake	59 (98%)	0 (0%)
Deferiprone intake	60 (100%)	0 (0%)
Blood transfusion	60 (100%)	0 (0%)
Splenectomy	44 (73.3%)	0 (0%)
Hepatitis B, C or Human immunodeficiency virus (HIV) virus positive	0 (0%)	0 (0%)
Thrombotic events	0 (0%)	0 (0%)
Bleeding	0 (0%)	0 (0%)

Blood Sample Collection

Blood samples were collected between 10:00 and 12:00 AM, prior to transfusion and four weeks after the previous transfusion. EDTA tubes (AFCO, Amman, Jordan) were used to collect 5 ml of blood for platelet count and were analyzed within 2 h. Also, 1.8 ml blood was collected in 3.2% tri-sodium citrate anticoagulant tubes (AFCO, Amman, Jordan), centrifuged for 10 min at 2500 revolutions per minutes (rpm) and analyzed within 8 h for coagulation profile, prothrombin time (PT), activated partial thromboplastin time (aPTT), D-dimer, fibrinogen, Factor V (FV), tissue factor (TF), tPa, PAI-1, Von Willebrand factor (vWF), A disintegrin-like metalloproteinase with thrombospondin motif type 1 member (ADAMTS13), PC and PS.

Laboratory Tests

Platelet count was performed using Sysmex model XN-1000 hematology analyzer (Sysmex, Japan). While Stago STA Compact Max coagulation analyzer (Stago, UK) was used to measure PT, aPTT, D-dimer, and fibrinogen. Enzyme-Linked Immunosorbent Assay[26] technique was performed using HiPo MPP-96 microplate reader (Biosan, Latvia) and using the following kits: Human Factor V (ab137976, Abcam, UK), tPA (BMS258-2, Invitrogen, Thermo Fisher Scientific, USA), PAI-1 (BMS2033, Invitrogen, Thermo Fisher Scientific, USA), Tissue Factor (ab220653, Abcam, UK), Von Willebrand factor (vWF) (ab223864, Abcam, UK), Human Protein C (ab137987, Abcam, UK), Human ADAMTS13 (ab234559, Abcam, UK) and Human Protein S (ab125969, Abcam, UK). Reference ranges and sensitivity of the ELISA kits are illustrated in Table 1 Supplementary. INR: international normalized ratio for PT was automatically calculated by the Stago machine. The international sensitivity index (ISI) is furnished by the manufacturer in the package insert and is entered in the calibration page for PT along with the geometric mean (reference time).

$$\mathrm{INR} = \left(\frac{\text{Patient's PT}}{\text{Reference plasma mean PT}}\right)\mathrm{ISI}$$

Statistical Analysis

Data analysis was performed using SPSS version 26 (SPSS Inc., Chicago, USA) for Windows. Categorical variables were summarized as frequencies and percentages, while numerical variables were expressed as means ± standard deviations.[27] The Kolmogorov-Smirnov test was used to assess the normality of variable distributions, revealing that INR, PT, aPTT, fibrinogen, PS, and tissue factor were the only variables following a normal distribution. Normally distributed variables were analyzed using the independent sample t-test, whereas the Mann-Whitney test was applied to compare non-normally distributed variables.

Spearman's correlation coefficient was determined through a two-tailed bivariate correlation analysis. The chi-square test was used to assess significant differences in sex and age between the β-thalassemia major group and the control group. Receiver operating characteristic (ROC) curve analysis was conducted to determine the area under the curve (AUC), which reflects the diagnostic performance of each test. A higher AUC value indicates greater diagnostic accuracy in terms of sensitivity and specificity.

Table 2. Hemostatic Profile among Control and β-Thalassemia Groups.

	Variable	Reference Range	Control (Mean ± SD)	β-Thalassemia (Mean ± SD)	*p*-Value
	Platelet count (10^3/ μL)	150–440	291.05 ± 78.94	618.92 ± 247.41	**0.0001**
Coagulation tests	aPTT (seconds)	24–40	30.32 ± 2.27	29.29 ± 3.31	0.126
	PT (seconds)	11.5–15.3	14.14 ± 3.39	14.68 ± 1.66	0.201
	INR	0.8–1.2	1.05 ± 0.09	1.09 ± 0.12	0.168
	Fibrinogen (g/L)	2.0–4.0	2.70 ± 0.73	2.41 ± 0.58	0.073
	FV (ng/mL)	0.6–1.81	0.73 ± 0.48	0.75 ± 0.48	0.855
	TF (pg/mL)	52–215	59.92 ± 46.37	59.68 ± 35.06	0.983
	D-Dimer (μg/mL)	<0.5	0.28 ± 0.10	0.43 ± 0.23	**0.004**
	vWF (ng/mL)	5000–20,000	7780 ± 5870	6640 ± 4550	0.494
	ADAMTS13 (ng/mL)	500–2800	2726.60 ± 1105.23	2484.89 ± 1097.87	0.224
Natural anti-coagulants	PC (μg/mL)	5–100	74.04 ± 15.32	70.76 ± 29.62	0.104
	PS (μg/ mL)	15–40	39.51 ± 6.98	29.21 ± 7.14	**0.0001**
Fibrinolytic parameters	tPA (pg/mL)	2–8	1.3 ± 0.70	2.00 ± 3.01	0.903
	PAI-1 (pg/mL)	78–5000	2429.64 ± 812.34	4699.32 ± 2469.30	**0.0001**

Abbreviations: ADAMTS13, disintegrin-like metalloproteinase with thrombospondin motif type 1 member; PT, prothrombin time; aPTT, activated partial thromboplastin time; INR, international normalized ratio; FV, Factor V; vWF, Von Willebrand Factor; tPA, tissue plasminogen activator; PAI-1, Plasminogen activator inhibitor-1; TF, Tissue factor. Numeric variables are summarized as mean ± standard deviation.[27]

Results

Demographic and Clinical Characteristics

This study included 60 β-thalassemia major patients (1:1 male to female ratio) with an average age of 20.22 ± 6.87 years. The control group included 20 healthy volunteers with an average age of 19.10 ± 7.00. No difference in age or sex between β-thalassemia major and control groups was found ($p = 0.939$ and 0.689, respectively) and none of the participants had a previous thrombotic event.

All β-thalassemia major patients were taking iron chelation therapy, deferiprone, and 98% of them take furosemide, a diuretic. About 73% of β-thalassemia major patients underwent splenectomy and all of them had regular monthly blood transfusions. About 23% of β-thalassemia patients were taking prophylactic aspirin and about 33% of them take calcium carbonate, as a calcium supplement. None of the participants tested positive for human immunodeficiency virus (HIV), hepatitis B or hepatitis C virus (Table 1).

Hemostatic Profile among Control and β-Thalassemia Groups

Laboratory tests of the hemostatic profile revealed no differences between control and β-thalassemia major group in all tested parameters except for D-dimer, PAI-1 and platelet count ($p = 0.004$, $p = 0.0001$, $p = 0.0001$, respectively) in which β-thalassemia group had higher D-dimer, PAI-1 and platelet counts by 53.6%, 93.4% and 112.7%, respectively compared to controls. On the other hand, PS was 26.1% lower in β-thalassemia major group compared to the control group ($p = 0.0001$) (Table 2, Figure 1). Medians, interquartile ranges and number of outliers for platelet count, PAI-1, D-dimer and PS in control and β-thalassemia major groups are summarized in Table 2 Supplementary.

Correlation Analysis Between Coagulation and Fibrinolytic Parameters in β-Thalassemia

Significant weak correlations existed between coagulation and fibrinolytic parameters (Table 3). Notably, a strong positive correlation existed between platelet count and PAI-1 ($r = 0.669$, $p = 0.0001$) (Table 3). Other correlations not listed in Table 3 were insignificant.

Differences in Hemostatic Profiles Between Splenectomized and Non-Splenectomized Groups

Splenectomized patients had higher platelet count (+45.2%), PAI-1 (+98.1%), fibrinogen (+18.9%) and tPA (+197.2%) compared to the non-splenectomized group (all $p < 0.05$) (Figure 2) while no significant difference was found in other hemostatic parameters.

Utility of Hemostatic Parameters as Diagnostic Biomarker in β-Thalassemia Major

Receiver operating curve (ROC) analysis was used to calculate the area under the curve for the studied parameters. The area under the curve (AUC) values for PT, aPTT, fibrinogen, FV, tPA, vWF, PC, and INR were either poor or no better than random chance. While, the AUCs for PAI-1 (0.815), platelet count (0.887), and PS (0.846) were considered good, while the AUC for D-dimer was fair (0.715) (Figure 3, Table 3 Supplementary).

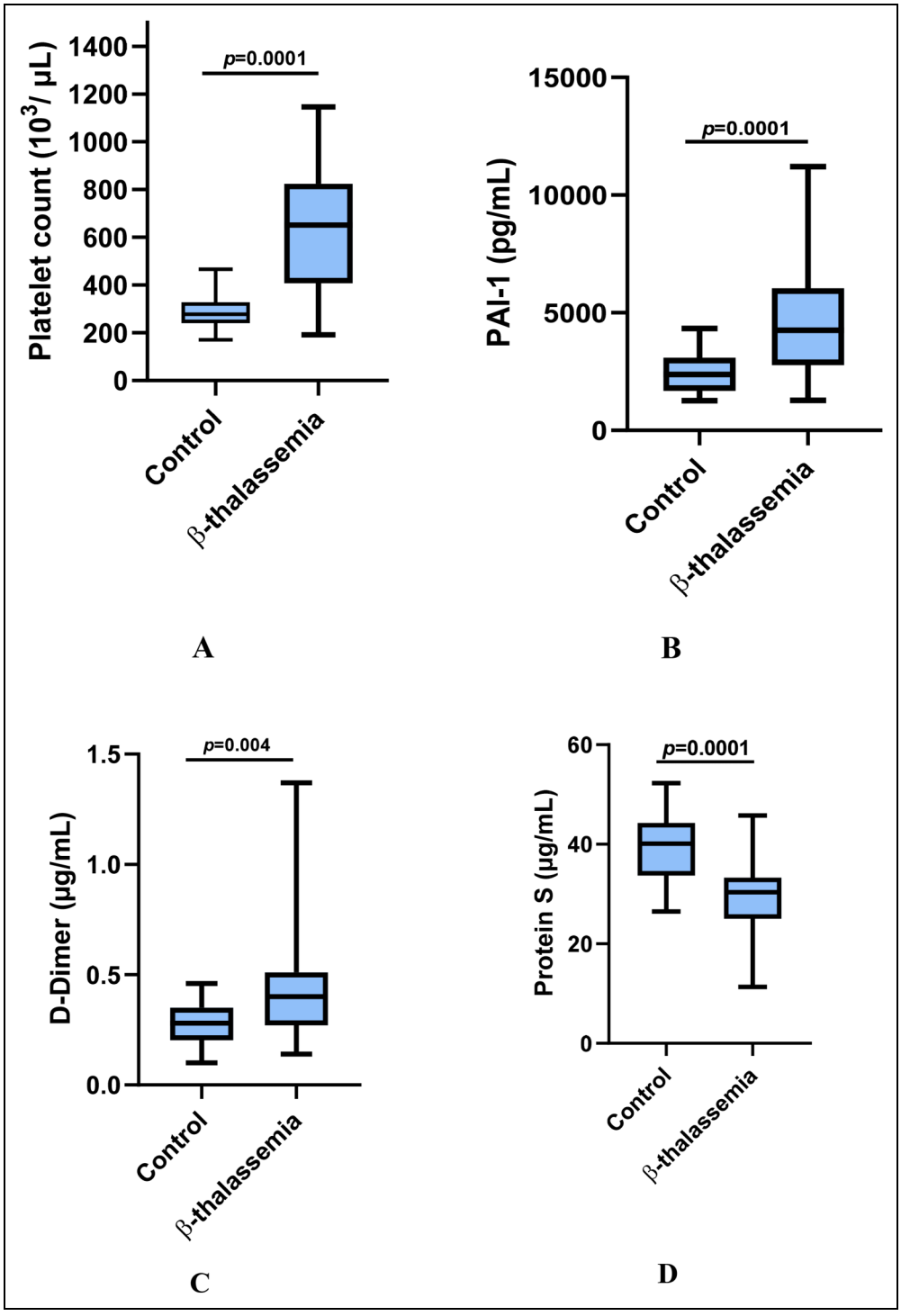

Figure 1. Box-plot representation of platelet count, PAI-1 (Plasminogen activator inhibitor-1), D-dimer and PS in control and β-thalassemia major groups.

Comparison of Hemostatic Profiles in Thalassemia Patients with and Without Aspirin Use

No significant differences in PS, D-dimer, PT, INR, aPTT, fibrinogen, PC, TF, tPA and ADAMTS13 were found between β-thalassemia major patients taking aspirin and those who does not take aspirin. However, a higher platelet count (+37.1%) and PAI-1 level (+58.9%), along with a lower vWF level (−25.6%), were observed between these two groups (Figure 4).

Table 3. Significant Correlations Between Platelet Count, Coagulation and Fibrinolysis Parameters.

Correlation Between	Spearman Correlation Coefficient (r)	P Value	Correlation Between	Spearman Correlation Coefficient (r)	P Value
PAI-1 & platelet count	0.669	0.0001	PC & PT	0.256	0.049
PAI-1 & vWF	−0.264	0.042	vWF & tPA	0.351	0.006
PS & D-dimer	0.291	0.024	D-dimer & vWF	0.397	0.002
PS & vWF	0.276	0.033	D-dimer & tPA	0.315	0.014
PS & fibrinogen	0.274	0.034	Fibrinogen & INR	−0.302	0.019
PC & tPA	0.283	0.028	Fibrinogen & PT	−0.307	0.017
PC & INR	0.255	0.049			

Discussion

Thrombosis is a significant complication in patients with β-thalassemia. An epidemiological study conducted in Iran and the Mediterranean region reported thrombotic events in 1.65% of 6670 β-thalassemia major patients[7] while a more recent study found a 9% incidence, particularly in older and post-splenectomy patients.[16] Another study reported a 1.5% incidence of thrombosis after splenectomy for hematological disorders.[28] Moreover, silent cerebral ischemia was detected in 37.5% of 40 β-thalassemia major patients.[29] Unlike the above investigations, a study reported no evidence of thrombotic complications in patients with β-thalassemia major.[30] This agrees with the findings of the present study in which no thrombotic event was reported in any of the patients possibly due to regular patient follow-up, use of iron chelation therapy, and routine monitoring of coagulation parameters. Additionally, some patients were receiving prophylactic aspirin therapy, which may have contributed to the prevention of thrombosis. Surprisingly, thromboembolic events were more frequent in thalassemia intermedia than in thalassemia major, likely due to the protective effect of regular transfusions in the latter. Iron chelation therapy may further reduce thrombotic risk by preventing hepatic hemosiderosis from iron overload.[31]

The current study investigated the deregulation of hemostasis in β-thalassemia major patients, taking into consideration splenectomy and the intake of medications. The findings of the present study suggest that patients with β-thalassemia major have significant thrombocytosis compared to the control group, consistent with the findings of previous studies.[32] A cohort study conducted in Pakistan aimed to identify the main cause of thrombocytosis in children with thalassemia major. The study involved 41 children who had persistent platelet counts of $\geq 1000 \times 10^9$/l for at least three months. These children were treated with a modified transfusion protocol and iron overload chelation. After the changes in their treatment, 73% of the children's platelet counts returned to normal, along with significant improvements in their hemoglobin levels.[33] Furthermore, the present study demonstrated that splenectomized patients exhibit a higher platelet count compared to non-splenectomized patients. Loss of the spleen's filtering function reduces clearance of abnormal platelets, promoting their accumulation and contributing to thrombocytosis and a hypercoagulable state.[20]

This study demonstrated a significant increase in PAI-1 levels in patients with β-thalassemia major, consistent with findings from a previous study involving a small sample of thalassemia patients ($n = 30$).[25] Additionally, the present study provided new insight by highlighting higher PAI-1 levels in splenectomized β-thalassemia patients compared to those who have not undergone splenectomy.

Elevated PAI-1 levels may significantly contribute to a prothrombotic state by inhibiting fibrinolysis, especially when combined with other risk factors like endothelial dysfunction, increased platelet counts, or chronic hemolysis, making it a significant player in the development of thrombosis.[24] Importantly, a strong positive correlation was found between PAI-1 and platelet count in thalassemia patients. This may indicate that the elevation in PAI-1 levels is attributed, at least partially, to thrombocytosis. As a result, careful monthly monitoring of PAI-1 and platelet count, especially in splenectomized β-thalassemia patients, is required. Consideration of prophylactic anticoagulant or antiplatelet therapy may be warranted in this patient population to reduce the risk of potentially life-threatening thrombotic events.

In the current study, PS was 26.1% lower in β-thalassemia major group compared to the control group. This may increase the risk of hypercoagulability and thromboembolic complications. Our findings agree with the results of previous studies that reported lower PS in β-thalassemia patients.[11,12] Also, our ROC analysis showed that PS has a good diagnostic accuracy in terms of specificity and sensitivity in distinguishing thalassemic from non-thalassemic subjects. The effect of splenectomy on PS levels remains controversial. Some studies report decreased levels in splenectomized thalassemic patients[11] while our findings showed no difference between groups, consistent with previous studies.[14,34]

In the present study, PC level was not statistically different between control and β-thalassemia groups. Previous

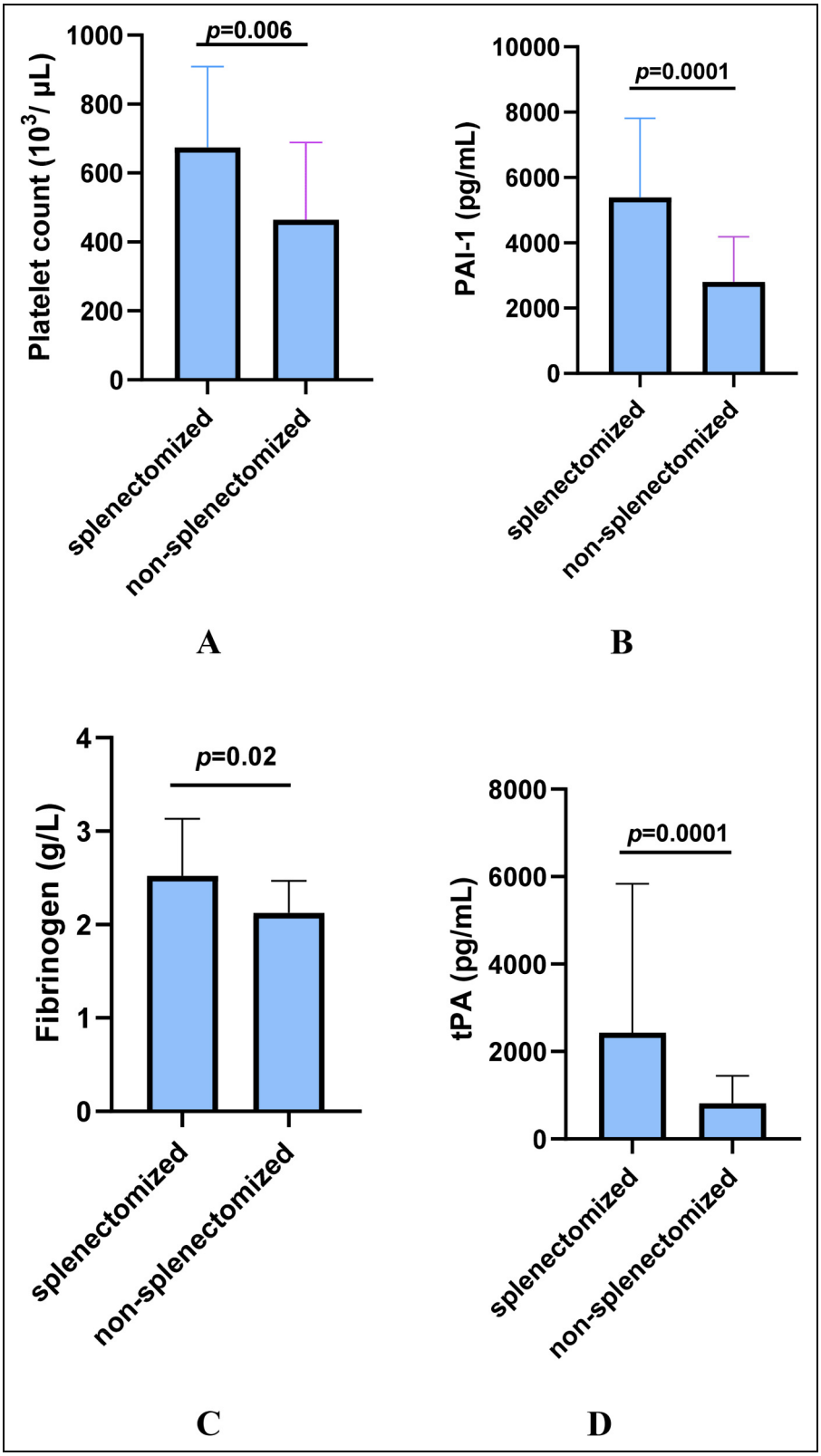

Figure 2. A-D Platelet count, PAI-1, fibrinogen and tPA in splenectomized and non splenectomized patients.

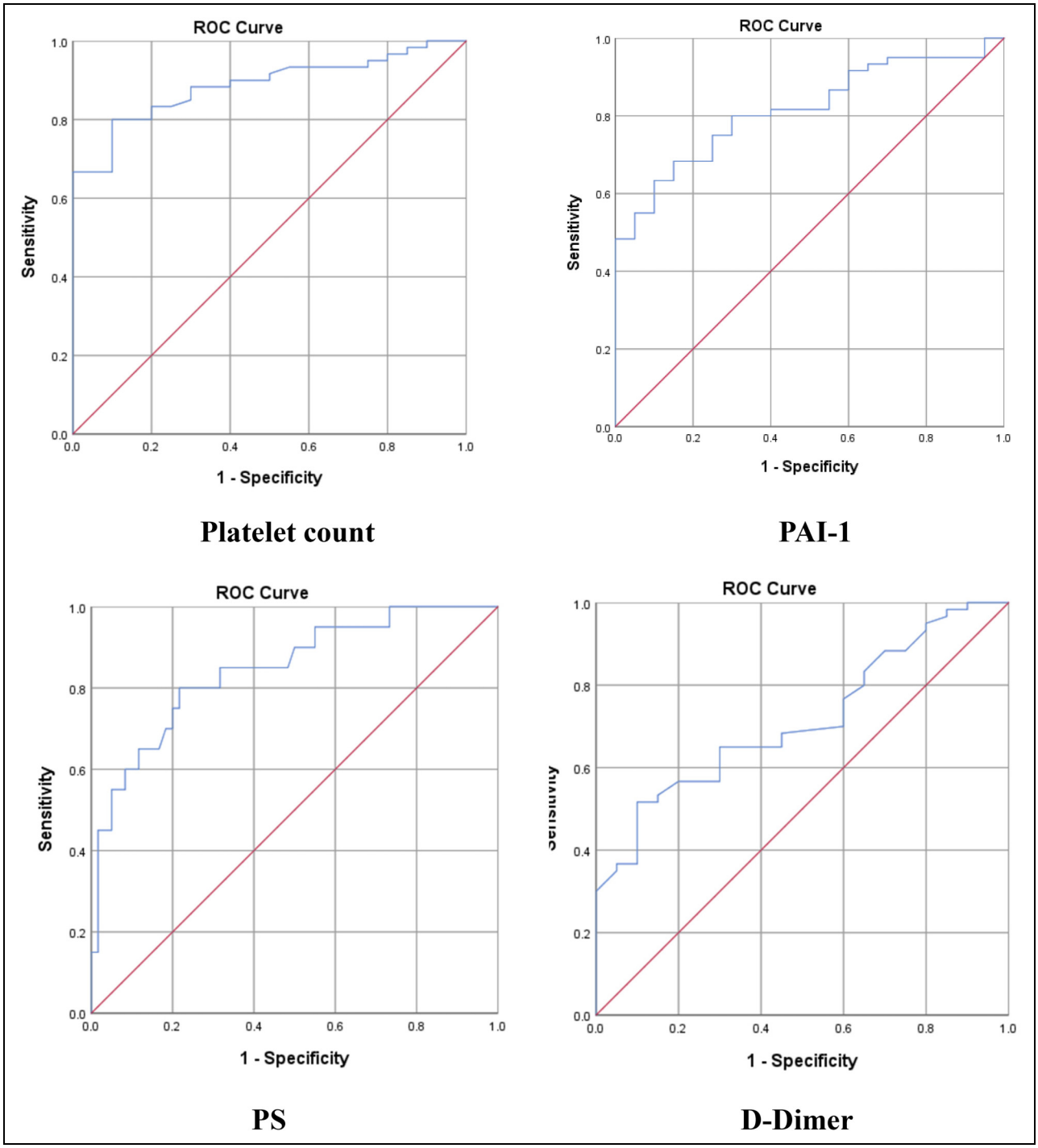

Figure 3. ROC curves for platelet count, PAI-1, PS and D-Dimer.

studies reported contradicting results in which a lower PC level was observed in β-thalassemia major compared to the control group.[11–14,35] In addition, it was reported that splenectomized β-thalassemia major patients have decreased PC activity as compared to non–splenectomized.[11,34,35] One explanation for the lower levels of PC and PS in thalassemic patients is that these proteins are highly sensitive to even mild liver dysfunction, which is commonly observed in thalassemia due to factors such as hepatic hemosiderosis, viral infections, and deficiencies in vitamins and proteins.[11] It has also been suggested that the more significant drops in PC and PS levels in splenectomized thalassemic patients may be linked to procoagulants found on the surface of red blood cells and abnormal

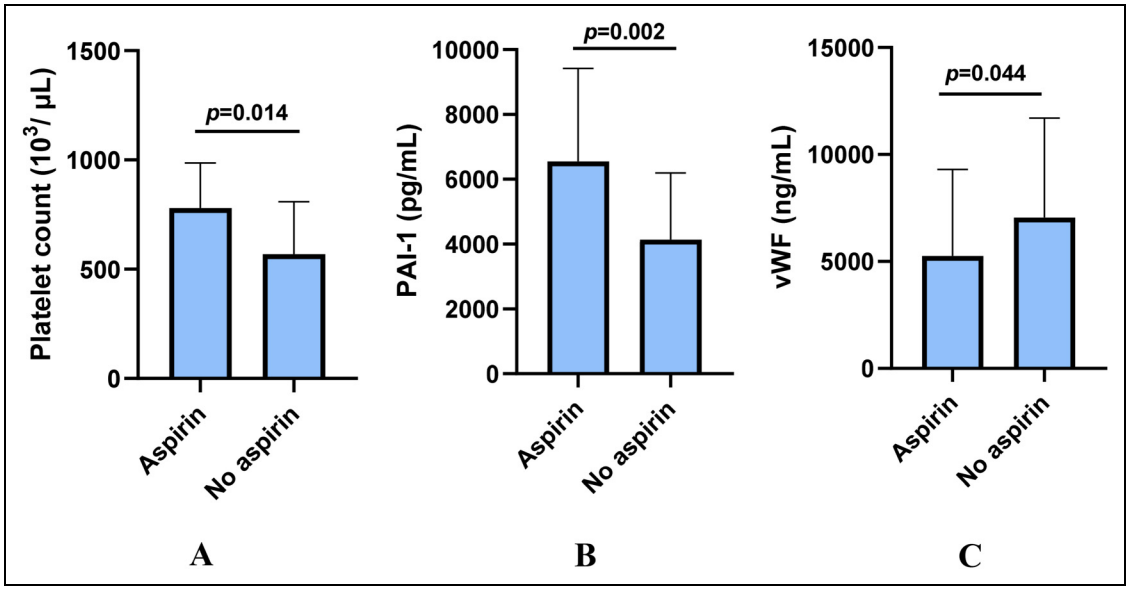

Figure 4. Comparison of hemostatic profiles in thalassemia patients with and without aspirin use.

platelets. Normally, the spleen would help remove these from circulation, but after a splenectomy, they remain in the bloodstream, leading to the consumption of PC and PS as the body tries to manage the increased clotting tendency.[11]

In transfusion-dependent β-thalassemia patients, prolonged coagulation times are often linked to iron overload from frequent blood transfusions. The excess iron accumulates in the liver, which can impair its function and affect the production of coagulation factors, resulting in prolonged PT and aPTT.[36] The present study reported no significant variation in PT and aPTT. These findings agree with a previous study reporting normal PT value in 95.5% participants and normal aPTT value in 87.5% participants.[37] On the other hand, other studies reported higher PT and aPTT in thalassemia patients compared to controls.[13] It has been suggested that liver capacity to synthesize clotting factors in thalassemia is decreased.[38] However, the findings of the current study showed that the levels of Factor V were within the normal range in the thalassemia major group. This agrees with previous studies that reported no change in the level of these factors.[12]

The D-dimer level was 53.6% higher in β-thalassemia patients compared to controls in the present study, despite being within the normal range. However, its level was not different between splenectomized and non-splenectomized groups. This agrees with previous studies that reported significantly higher in D-dimers in thalassemic children.[11,16] High D-dimer levels may indicate increased fibrin formation and degradation, suggesting activation of the coagulation and fibrinolytic systems. Therefore, monitoring of D-dimer levels in β-thalassemia patients cannot be neglected. Prophylactic anticoagulants or antiplatelet drugs may be recommended in such cases.

Although platelet count, PAI-1, PS, and D-dimer levels differed significantly between thalassemia patients and controls, none proved to be a reliable standalone marker of thrombotic risk based on ROC analysis. However, combining these variables into a composite thrombotic risk score may offer improved predictive value for thrombotic complications in thalassemia patients.

The interaction of deferiprone and furosemide with coagulation is clinically significant in β-thalassemia patients, especially in those who underwent splenectomy. In the current study, all patients were on deferiprone, and 98% received furosemide, preventing a comparison between users and non-users of these drugs. Chelation therapy with deferiprone has been shown to reduce platelet hyperactivity in patients with β-thalassemia. This effect may be due to both the direct inhibition of COX-1 activity and an indirect reduction in oxidative stress.[39] On the other hand, furosemide is clinically useful for preventing volume overload in transfusion-dependent thalassemia. However, it may lead to hemoconcentration, potentially increasing thrombotic risk. Therefore, further studies are necessary to evaluate its safety and optimize its use in this patient population.[40] Therefore, careful monitoring and individualized treatment strategies are essential for optimal patient management.

Antithrombotic prophylaxis in thalassemia major includes low-dose aspirin for high-risk patients, especially post-splenectomy. Anticoagulants like low-molecular-weight heparins are used selectively.[41] Furthermore, iron chelation and hydroxyurea may reduce thrombosis risk by improving oxidative balance and lowering cell counts.[39] The findings of this study revealed that β-thalassemia patients taking aspirin have higher platelet count and PAI-1

levels. Concerns regarding the effectiveness of aspirin as a preventive strategy in thalassemia patients need further investigation. The use of alternative approaches to better protect these patients from thrombotic complications may be necessary.

In the current investigation, 33% of thalassemia patients were taking calcium carbonate to prevent bone complications and mineral imbalances. Calcium carbonate intake correlated weakly, but significantly, with PT ($r = -0.265$, $p = 0.041$). This highlights the possible pro-thrombotic risk of this medication. Standardization of calcium carbonate dosage and risk-benefit evaluation in thalassemia patients is needed.

Conclusion

This study demonstrated that splenectomized patients with β-thalassemia major exhibited elevated PAI-1 levels and increased platelet counts. Though a previous small study highlighted that PAI-1 is higher in thalassemia patients compared to control, our study reports a novel finding that PAI-1 is also higher in splenectomized patients compared to non-splenectomized patients. Additionally, our study highlights that PS is lower in β-thalassemia patients compared to controls, underscoring a subclinical hypercoagulable state. The use of a well-characterized cohort with no history of thrombosis further strengthens the novelty of linking these coagulation changes to splenectomy status and medication use.

One limitation of this single-center study is the small sample size. Larger multicenter studies are needed. Another limitation is the lack of advanced assays to assess the full scope of coagulation and fibrinolysis. Techniques such as thrombin generation assays and evaluations of plasma fibrin formation or whole blood clot dynamics could offer a more comprehensive understanding of overall hemostatic function. Another limitation is that all patients were using iron chelation therapy and 98% of them were using a diuretic. Therefore, we were unable to compare users versus non users of these drugs.

Thrombotic risk in β-thalassemia major should be monitored regularly, especially in postsplenectomy patients and those with elevated D-dimer, PAI-1, and platelet counts, as well as low PS levels. Chelation therapy with agents like deferiprone may help reduce platelet activation, while caution is advised with diuretics like furosemide due to potential hemoconcentration. Choice of calcium carbonate dose must be selected carefully to prevent a pro-thrombotic effect. Preventive measures include maintaining hydration, encouraging physical activity, and considering low-dose aspirin in high-risk patients. Anticoagulants may be used selectively.

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Ethical Considerations

Ethical approval to report this case was obtained from the Institutional Review Board (IRB) of the Ministry of Health, Amman, Jordan (IRB # 138888).

Consent to Participate

Written informed consent was obtained from the patient(s) for their anonymized information to be published in this article.

Author Contributions

O.M.S conceptualized and designed the study. A.K.T, W.J.H and A.A.H: Acquired the data. M.A.A. and O.M.S analyzed and interpreted the data and M.A.A. drafted the paper. All authors revised the manuscript critically, approved its final version, and agree to be accountable for all aspects of the presented work.

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Declaration of Conflicting Interests

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Data Availability Statement

Written informed consent was obtained from the patient(s) for their anonymized information to be published in this article.

Supplemental Material

Supplemental material for this article is available online.

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