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Natural anticoagulant protein levels in patients with transfusion-dependent thalassemia in Nineveh province

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Abstract:

BACKGROUND: Protein C (PC), protein S (PS), and antithrombin levels dramatically reduced in thalassemic patients, particularly those with splenectomies and few transfusions, indicating a significant involvement in hypercoagulability. These findings question the cost-effectiveness of recommending prophylactic antithrombotic treatment for high-risk thalassemic patients.

OBJECTIVES: The objectives of this study are to assess PC, PS, and antithrombin levels in transfusion-dependent β- thalassemic patients and determine their association with clinical, biochemical, and hematological parameters.

MATERIALS AND METHODS: A study of 50 B-thalassemia major patients under 18 years old who received regular blood transfusions at the thalassemia center at Al Hadba Teaching Hospital in Mosul from January to February 2025. Blood samples were taken for pack cell volume, antithrombin, ferritin, serum alanine aminotransferase, serum aspartate aminotransferase, and viral hepatitis screening. Enzyme-linked immunosorbent assay was used to estimate PC, and free PS in plasma, and radioimmunodiffusion was used to measure antithrombin. The data were analyzed using mean, mean and standard deviation, percentages, Pearson's correlation, and independent *t*-test.

RESULTS: There was a significant reduction of PC, PS, and antithrombin in thalassemia patients than the controls. Splenectomized thalassemic individuals show significant decreases in PC, PS, and antithrombin levels.

CONCLUSION: Low amounts of PC and free PS in thalassemic individuals may be acquired early in life. How much the imbalance between coagulation inhibitors and clotting factors contributes to hypercoagulability in thalassemia is unknown.

Keywords:

Antithrombin, protein C, protein S, thalassemia

Introduction

Protein C (PC) and protein S (PS) are Vitamin K-dependent serine proteases that regulate hemostasis and act as natural anticoagulants. PS, both free and in association with C4 binding protein, collaborates with activated (PC) to degrade activated coagulation of FV mean factor five and FVIII mean factor eight in the coagulation cascade. Human PC circulates as a 2-chain zymogen and converts to active PC via limited proteolysis with thrombin and thrombomodulin at the endothelial and platelet surfaces.[1] The glycoprotein antithrombin is found in human plasma at a concentration of around 2.6 μM. Active coagulation factors including thrombin, factor IXa, and factor Xa are inhibited. The inhibitory process involves a stable 1:1 combination between antithrombin and the protease. Despite treatment challenges, thalassemic patients' life expectancy has nearly doubled due to increased care standards.[1-3]

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Table 1: Patients characteristics (*n*=50)

Parameter	Mean±SD	Range
Age (years)	7.6±3.8	2–16
Age at diagnosis (years)	1.2±0.6	0.19–2.3
Age at starting blood transfusion (years)	1.2±0.6	0.19–2.3
Number of blood transfusion	83.6±70.6	8–150

SD=Standard deviation

Several mechanisms have been proposed for this hypercoagulable state in thalassemia, including platelet activation, hyperaggregability, reduced platelet survival, elevated urinary excretion of prostacyclin and thromboxane A2 metabolites, reduced natural anticoagulants, and increased plasma thrombin–antithrombin complex.[2] Moreover, newly discovered complications have emerged. Hemostatic changes in individuals with beta-thalassemia major, particularly following splenectomy, are quite distinct. The high incidence of thromboembolic events in thalassemic individuals and prothrombotic hemostatic abnormalities at even very young age suggests a persistently hypercoagulable state.[3-5]

Liver injury was not the sole source of reduced anticoagulant proteins.[6] One theory suggests that thalassemic erythroid cells lose membrane phospholipid asymmetry, exposing procoagulant phosphatidylserine in the outer leaflet of the red cell membrane.[7] Low PC and PS levels in thalassemic individuals may result from Vitamin K deficiency, hepatic malfunction from hemosiderosis, and higher turnover rates. Shirahata *et al*. discovered that liver injury was not the sole source of reduced anticoagulant proteins.[1,8,9]

The objectives of this study were to assess PC, PS, and antithrombin levels in transfusion-dependent β-thalassemic patients and determine their association with clinical, biochemical, and hematological parameters.

Patients and Methods

A case–control study was conducted on 50 patients with B-thalassemia major who received regular blood transfusions at the Thalassemic Centre in Al-Hadbaa Teaching Hospital in Mosul city from January to February 2025. A control group of 50 healthy individuals matched by sex and age were also selected. Exclusions criteria included age 18 years and more, hepatitis C and/or B positivity, other hemostasis affecting disorders like liver diseases, recent severe infection, or recent hemostatic drug administration. No one in the study group developed thromboembolic diseases. A complete and detailed clinical history was obtained from each patient along with the corresponding fetal hemoglobin (Hb F) results at time of diagnosis. The following blood samples were taken before transfusion: Plasma was collected from the blood tubes with 3.2% sodium citrate (9:1 ratio) and centrifuged at 2000 g for 15 min. Samples were kept at − 8°C until testing. PC and PS assays were conducted utilizing a SKEULAB's Sandwich enzyme-linked immunosorbent assay method. Plasma antithrombin levels were determined using radio immunodiffusion. Serum alanine aminotransferase (S. ALT) measured by unit/milliliter (u/ml) and serum aspartate aminotransferase (S. AST) levels measured by u/ml were measured using an automated enzymatic colorimetric method, following the manufacturer's instructions. Serum ferritin also measured by nanogram/milliliter (ng/ml) using a chemiluminescent immunoassay technique, performed on an automated analyzer in accordance with the manufacturer's protocol. D-dimer levels were assessed using VIDAS D-dimer (bioMérieux Inc. France); then, all patients were divided into two groups: Splenectomized and nonsplenectomized.

Statistical analysis

The data were analyzed using SPSS version 21 (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL, USA), including mean, standard deviation, percentages, Pearson's correlation, and independent *t*-test.

Ethical statement

This study received ethical approval from the Medical Ethics Committee of the College of Medicine, University of Mosul, the Nineveh Health Directorate, and the Iraqi Ministry of Health with code number (UOM/COM/MREC/24-25/FEB8). Written informed consent was obtained from all participants or their legal guardians, in the case of children, prior to their inclusion in the study, in accordance with ethical research standards.

Results

The patients characteristic like age, age of diagnosis, age of starting transfusion and number of blood transfusion per year were shown in table 1.

The 30 nonspenectomized patients (60% of all patients) and 20 splenectomized patients (40% of all patients) distributed according to age and sex as shown in Figure 1. The mean and standard deviation of the ages of all patients was 7.6 ± 3.8, and the mean age at the start of the blood transfusion was 1.2 ± 0.6 years. Table 2 displays hematological and biochemical data for B-thalassemia major patients. Table 3 shows a substantial positive correlation between serum ferritin and S. ALT and S. AST (P = 0.002 and 0.002), but a negative correlation with PC, free PS, and antithrombin (P = 0.001, 0.001, and 0.05). Table 4 reveals substantial differences in PC, free PS, and antithrombin levels between patients and controls (P < 0.001). Table 5 shows substantial

Table 2: Hematological and biochemical data in B-thalassemia major individuals (*n*=50)

Parameters	Mean±SD	Range
Hb F (% at diagnosis)	65.5±38.2	42–100
Serum ferritin (ng/ml)	1941±1220	420–7865
Serum ALT (U/ml)	20.2±16.2	5–95
Serum AST (U/ml)	24±19.2	8–970
Protein C (%)	58±12.8	42–78
Free protein S (%)	62±32	47–112
Antithrombin (µg/dl)	6.3±2.9	2.7–9.4

SD=Standard deviation, Hb.F=Fetal hemoglobin, ALT=Alanine aminotransferase, AST=Aspartate aminotransferase

Table 3: Correlation between serum ferritin and different parameters (*n*=50)

Parameter	Mean	*R*	*P*
Hb F (%)	65.5	0.124	Not significant
Serum ALT (U/l)	20.2	0.285	0.002*
Serum AST (U/l)	24	0.3	0.002*
Protein C (%)	58	−1.2	0.001*
Free protein S (%)	62	−1.4	0.001*
Antithrombin (µg/dl)	6.3	−0.5	0.05*

*Significant (≤0.05). Serum ferritin=1941±1220 (ng/ml). Hb.F=Fetal hemoglobin, ALT=Alanine aminotransferase, AST=Aspartate aminotransferase

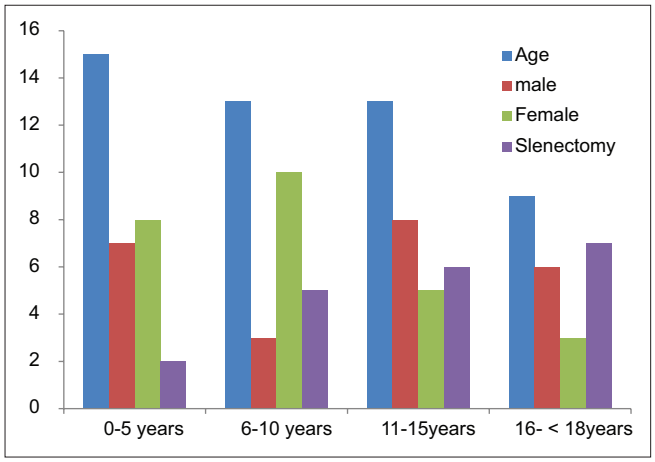

Figure 1: Age, sex, and splenectomy distributions of thalassemia patients

differences in PC, free PS, antithrombin, and S. ferritin levels between plenectomized and nonsplenectomized patients ($P < 0.001$).

Discussion

In this case–control study, 24 (48%) were male and 26 (52%) were female. The percentage of patients with splenectomy increased with age, only 2 patients (4%) were between the age group 0-5 years and 7 patients (14%) were between the age group 16 to less than 18 years old as shown in Figure 1, as spleen enlarged, anemia worsened and abdominal discomfort increased. The average age was 7.6 years, ranging from 2 to 16 years. The median age at diagnosis was 14.4 months, with a range of 3–27.6 months, similar to the mean of 10.9 months reported by Abdul-Zahra *et al*. in 2016.

Table 4: Comparison of protein C, free protein S, and antithrombin between patients and controls

Parameter	Control (*n*=50), mean±SD	Patient (*n*=50), mean±SD	*P*
Protein C (%)	109±11.2	58±12.8	0.001*
Free protein S (%)	75±17.2	62±32	0.001*
Antithrombin (µg/dl)	18.9±6.2	6.3±2.9	0.001*

*Significant (≤0.05). SD=Standard deviation

Table 5: Protein C, free protein S, antithrombin, and serum ferritin comparison between splenectomized and nonsplenectomized patients

Parameter	Splenectomized (*n*=20), mean±SD	Nonsplenectomized (*n*=30), mean±SD	*P*
Protein C (%)	42±18	57.5±10	0.001*
Free protein S (%)	48±12	65±8.5	0.05*
Antithrombin (µg/dl)	2.5±1.4	8.5±2	0.05*
Serum ferritin (ng/ml)	1230±400	1520±250	0.01*

*Significant (≤0.05). SD=Standard deviation

Research on coagulation proteins strongly suggests a chronic hypercoagulable condition in thalassemia. Numerous studies have observed significant alterations in coagulation factors, inhibitors, and fibrinolytic system components.[2-5]

Hb F% did not correlate with serum ferritin in this study, although ALT and AST revealed a strong positive correlation. PC, PS, and antithrombin inversely correlated with serum ferritin, as demonstrated by Huang *et al*.[2]

The current study found considerably decreased PC and PS in all patients compared to controls ($P < 0.001$), as reported by Rosnah *et al*.,[1] Ali *et al*.,[10] and Abd El Mabooda *et al*.,[3] while Kareem *et al*.[7] found no significant difference in PC and free PS with the control group. Patients had considerably lower antithrombin levels than the controls ($P < 0.001$), although Rasnah *et al*.[1] found no significant difference, possibly due to regular blood transfusions.

Ahmadi *et al*.[5] found significant differences in PC and antithrombin, but not in PS between patients and controls. Low amounts of natural anticoagulant proteins in thalassemia patients are caused by various factors, including liver synthetic defects like hemosiderosis and viral hepatitis.

Splenectomized individuals had significant reductions in PC and free PS compared to nonsplenectomized individuals, but PS was not significant.

Splenectomy affects activated PC and PS clearance, increasing thrombosis risk.

Abd El Mabooda *et al*.[3] found that splenectomized patients had significantly lower antithrombin levels than nonsplenectomized patients, while Rosnah *et al*.[1] found no significant reduction in antithrombin levels after splenectomy.

A comparison with nonsplenectomized patients supports Kareem *et al*.[7] and Rosnah *et al*.[1]

Limitations

While the findings of this study are valuable, several limitations should be noted. The relatively small sample size may limit the accuracy and generalizability of the results. In addition, recruiting participants from multiple centers, rather than a single location, could have improved the representativeness of the sample and enhanced the study's external validity.

Conclusion

In thalassemic patients, especially those with splenectomies and infrequent transfusions, PC levels were significantly lower, indicating a major role in hypercoagulability. These results make us question whether giving anticoagulant medications to high risk group of thalassemia patients is really helpful or rather costly without benefit. To identify the precise conditions and degree of PC deficiency that call for preventative antithrombotic medication, a more extensive prospective study is required.

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Conflicts of interest

There are no conflicts of interest.

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