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Original Research

Protein C and protein S levels in patients with major β-thalassemia in Erbil, Kurdistan Region

Tareefa Kakakhan Hadi[1], Nawsherwan Sadiq Mohammad[2*], Saran Abdulqadir Nooruldin[3]

[1] Rizgary Teaching Hospital, Erbil, Kurdistan Region- Iraq
[2] FIBMS (Path), Hawler Medical University, College of Medicine and Nanakaly Teaching Hospital for Blood Diseases, Erbil, Kurdistan Region- Iraq
[3] Ministry of Health, Erbil, Kurdistan Region- Iraq

[*]**Correspondence to:** nawsherwan.sadiq@hmu.edu.krd

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Abstract: Oxygen is transported in the blood through red blood cells and a protein called hemoglobin. The protein consists of two alpha and two beta chains. The lack of any of these chains is caused by the malfunction of the genes that produce them, and can lead to a genetic disease called thalassemia. In β-thalassemia, hemoglobin does not produce enough beta protein. According to mild to severe effects on the body, β-thalassemia is divided into three types minor, interstitial and major thalassemia. There are increasing risks for thrombosis complications in thalassemia major. The purpose of this study was to evaluate protein C and protein S levels in β-thalassemia major and their association to the hypercoagulable state. Seventy patients with β–thalassemia major and 35 apparently healthy subjects as a control group were investigated for protein C and protein S. The mean of protein C (71.31%) and protein S (34.3%) levels were significantly reduced in β-thalassemia major patients in comparison with control subjects (p-value <0.001). Mean of fibrinogen level (2.42) g/l was significantly decreased in β-thalassemia major patients while the mean of D dimer level (0.43) µg/ml was significantly increased in comparison to control subjects (p-value 0.001). This study demonstrates a chronic hypercoagulable state in B- thalassemia major patients.

Key words: Beta Thalassemia Major; Protein C; Protein S; Hypercoagulable State and Thromboembolic event.

Introduction

Thalassemia is a blood disorder inherited from the family, in which the body produces abnormal forms of hemoglobin (1). Beta-thalassemia is characterized by abnormal hemoglobin β-chain synthesis, leading to variable phenotypes, ranging from severe anemia to clinically asymptomatic individuals. Worldwide, the total annual incidence of symptomatic individuals is assessed at 100,000. Three clinical forms have been designated: severe thalassemia (2), moderate thalassemia and minor thalassemia. Patients with major thalassemia usually develop severe anemia within the first two years of life and require regular blood transfusions (3). One of the many types of thalassemia complications is hypercoagulable state (4). In patients with thalassemia, especially those undergoing splenectomy and infrequent blood transfusions, venous and arterial thromboembolism is not uncommon. Abnormal red blood cell membrane contributes to hypercoagulable membrane lipid peroxidation and increases the surface expression of anionic phospholipids (such as phosphatidylserine). The exposure of phosphatidylserine on red blood cells is highly associated with the expression of platelet activation markers. Red blood cells exposed to phosphatidylserine may also directly cause the vascular damage detected in thalassemia (5).

The study of coagulation protein provides substantial evidence for the existence of chronic hypercoagulability in thalassemia. The levels of coagulation factors, coagulation factor inhibitors and fibrinolytic systems vary greatly. Low levels of coagulation inhibitors have been detected in thalassemia patients from different ethnic backgrounds (6). Proteins C and S are two plasma proteins that depend on vitamin K and can work together as a natural anticoagulant system. The anticoagulant activity is expressed by the selective inactivation of factors Va and VIIIa. Various mechanisms and drugs can cause acquired defects in these proteins: oral anticoagulants, DIC, liver disease, and in the case of protein S, nephrotic syndrome, lupus erythematosus, pregnancy, and certain hormones (7).

The beta thalassemia gene cluster is located on chromosome 11. In this cluster, in addition to the β globulin gene, there are also Delta (δ), Gamma (Gg, Ag) and Epsilon (ε) genes. These genes occur at different stages of the fetus. The β gene occurs in adults. Normal people have two versions of the β gene (β β). The severity of the disease depends on the type of mutation. Certain mutations in the β gene (such as gene deletion) cause the protein production to completely disappear (β0), while certain mutations (such as changes in the regulatory region of the gene) cause a decrease in protein production (β +). A secondary carrier called β-thalassemia is

found in both β/β0 and β/β+. Patients with β+/β+ genotype showed moderate phenotype levels. These people usually live a normal life, but depending on the severity of the anemia, blood transfusion may be required in case of illness or pregnancy. People with major β0/β0 and β0/β+ suffer from severe anemia. Their red blood cells are abnormal. These people have increased blood production in their bone marrow, which in the long run will change the shape of the bones, especially the broad ones. Moreover, these patients need to receive blood, which exposes them to complications such as increased iron levels. Heart failure and iron deficiency can also cause heart failure and arrhythmia (inconsistent heart rate). Yellow skin, changes in liver, spleen and bladder (1, 3-6).

There are many patients with thalassemia in Erbil, and the literature does not provide data on their hypercoagulable state. Therefore, this study aims to assess the hypercoagulable state of thalassemia major, and to assess thrombotic events as supportive health improvement risk care can increase life expectancy.

Materials and Methods

This study included seventy β_thalassemia major patients who are attending Thalassemia Center in Erbil, Kurdistan Region and 35 apparently healthy subjects coordinated by age and sex, who were selected as a control group, was conducted in this case-control study. None of the thalassemia patients had liver enzymes more than 5 times normal range or history of thrombosis, new infection or latest management of aspirin or other drugs that affect haemostatic function.

All patients were interviewed at the Thalassemia Center and written consent was acquired from them, or their parents. Demographic data regarding the age, gender, blood group, Rh and residence were recorded. Full clinical history was obtained concerning recent infection, viral disease, splenectomy and history of thrombosis. Hematological and biochemical data were obtained from patients' records. Activated partial thromboplastin time, fibrinogen level, protein C and protein S and D-dimer were done for both cases and control samples. The study has been approved by the Ethical Committee of the College of Medicine, Hawler Medical University. The blood collection was taken immediately from the patients and the control group before blood transfusion. Protein C and protein S assays were measured using Aeskulisa protein C and S kit. Both tests were performed using sandwich ELISA coated with a capture antibody specific for human protein (BioTek ELX800). The protein C concentration in normal human plasma ranges usually between 70% and 140%, and that for protein S concentration range usually between 60 and 150%. Plasma for aPTT, Fibrinogen, and D-dimer assays was measured immediately. Data were analyzed using the statistical package for social science (SPSS, version 22). One-way analysis of variance (ANOVA) was used to measure the difference among means of the parameters of concern. The analyzed data was presented in tables and graphs. The Chi-square test of association was used to compare proportions. Student's t-test for two independent samples was used to compare means of numerical variables (of cases and controls). The correlation coefficient (r) was calculated to assess the strength of the correlation between two numerical variables. A p-value of ≤ 0.05 was considered statistically significant.

Results

Seventy transfusion-dependent β-thalassemia major patients who were attending Thalassemia Center in Erbil, Kurdistan Region and thirty-five healthy control subjects were recruited. Thalassemia group included 58.6% (41) males and 41.4% (29) females. The mean age for β-thalassemia major patients was (19) years old, while the mean age for control was (21.7) years old. Out of (70) β-thalassemia patients, 34 of them had protein C level within the normal range, thirty-five cases their protein C level was less than 70% and one case had protein C more than 140%(Fig 1).

There was no significant correlation between protein C and S with age and gender. Results showed a significantly lower level of protein C and protein S in β- thalassemia major patients in comparison with controls ($P < 0.001$). Mean D-dimer in β-thalassemia patients showed a significant difference compared to the control group (0.43 vs 0.07) µg/l. Mean fibrinogen in thalassemia patients showed a significant difference compared to control group (2.42 vs 2.87)g/l (Table 1).

There were significant correlations between protein C and each of hemoglobin levels and D-dimer in β-thalassemia patients (p-value 0.002 and 0.02 respectively). Protein S showed no significant correlation with the hemoglobin, D-dimer and fibrinogen level in β-thalassemia patientsTable (2).

D-dimer and fibrinogen in splenectomized and none splenectomized patients has been shown in Table (3).

Discussion

Recent treatments have greatly extended the life expectancy of thalassemia patients. The result is new complications. Venous thromboembolic events have been observed, such as pulmonary embolism, deep vein thrombosis and portal vein thrombosis (8). There have

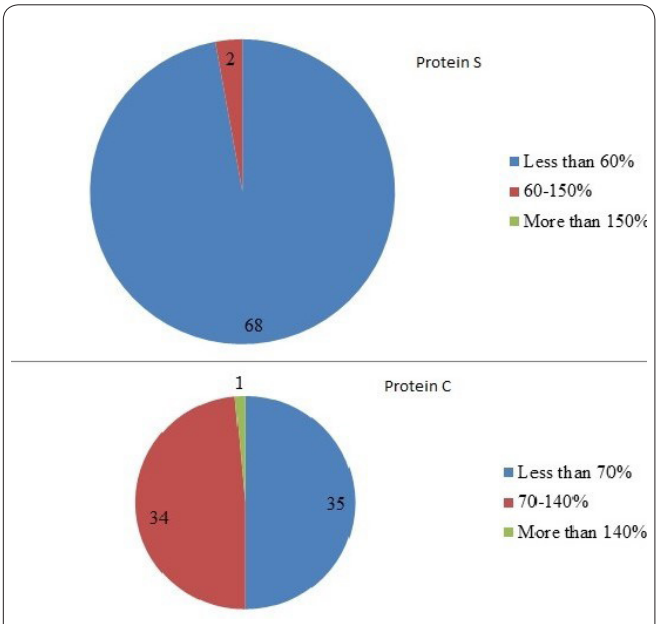

Figure 1. Protein S and Protein C deficiency levels in β-thalassemia major patients.

Table 1. Comparison of mean protein C, S, D-dimer and Fibrinogen level between β- thalassemia patients and control group.

Parameters	Control Mean(SD) NO.(35)	Cases Mean(SD)NO.(70)	P-value
Protein C (%)	104.12 (28.84)	71.31 (28.7)	< 0.001
Protein S (%)	119.98 (34.66)	34.3 (11.5)	<0.001
D-dimer(µg/ml)	0.07(0.05)	0.43 (0.58)	0.001
Fibrinogen (g/L)	2.87 (0.33)	2.42 (0.49)	0.001

Table 2. Correlation of Proteins (C and S) with the level of hemoglobin, D-dimer and fibrinogen of thalassemia patients.

Variables	Hb	P value	D-dimer	P value	Fibrinogen	P value	SGOT	P value	SGPT	P value
Protein C	*r0.357	0.002	*r0.278	0.02	*r0.209	0.083	*r0.184	0.128	*r0.107	0.379
Protein S	*r0.080	0.510	*r0.058	0.63	*r0.122	0,313	*r0.119	0.326	*r0.211	0.080

Table 3. Comparison of Protein C, Protein S, D-dimer and Fibrinogen in Splenectomized and non-Splenectomized patients.

Cases	NO	Protein C Mean (SD)	protein S Mean(SD)	D-dimer Mean (SD)	Fibrinogen Mean (SD)
Splenectomized	43	68.51 %(29.5)	47.41 %(11.5)	0.5121(µg/ml) (0.680)	2.48(g/L) (0.45)
Non splenectomized	27	75.78%(27.5)	45.27 %(7.5)	0.3007(µg/ml) (0.37)	2.33(g/L) (0.53)
P-value		0.3070	0.3953	0.1455	0.2003

been many reports of the hypercoagulable state of thalassemia. The earliest report from Greece showed that of the 138 patients with severe β-thalassemia, two cases had stroke syndrome. An Italian multicenter study of 735 patients with beta thalassemia reported 16 cases of cerebral thrombosis with clinical findings of headache, epilepsy, and hemiplegia (9). The study of coagulation proteins provides strong evidence for the existence of chronic hypercoagulability in thalassemia. Many authors point out that the levels of coagulation factors, coagulation factor inhibitors and fibrinolytic system components have changed significantly (10). Protein C and protein S work together to prevent the coagulation cascade. The levels of protein C and S in the case in this study were lower than those in the control group. A study (11) conducted in Thailand, Oakland, California (12), Italy (13) and Egypt (8) obtained similar results.

In general, the causes of low protein C and protein S in major patients with β-thalassemia include vitamin K deficiency, and iron overload leads to liver dysfunction, because protein C and S are vitamin K-dependent factors, and other causes may be increased consumption of protein C and protein S (14). In addition to the low levels of protein C and protein S in patients with severe thalassemia, this study also showed that compared with the control group, the level of D-dimer was significantly increased and the level of fibrinogen was significantly reduced. Consistent with the research done by Hassan (8) in Egypt.

After chronic activation of the coagulation system, the low content of protein C and protein S in this series may be due to increased consumption. Previous research supports this possibility, which has shown that thrombin-antithrombin (TAT) complex levels in thalassemia critically ill patients are significantly higher than normal subjects (15).

These findings may indicate that thalassemia patients have sustained thrombin generation. However, this explanation does not explain the normal antithrombin levels found in a study conducted by Rosnah (16) targeting severe thalassemia patients. Patients with regular blood transfusions for 2-3 weeks have a protective effect and have a relatively low antithrombin lowering effect. Capellini (13) believes that due to abnormal liver function, low-level natural anticoagulant proteins (such as protein C) found in the study are possible because protein C, protein S and antithrombin are synthesized in the liver, the defect is very sensitive to the liver and even mild.

In this study, serum liver enzymes levels which reflect the function of the liver were moderately elevated but didn't reach statistically significant levels when compared to protein C and protein S. Therefore, the impairment of liver function alone may not explain the significantly low levels of protein C and protein S found in the current study. In addition, the low levels of protein C and protein S may be due to genetic defects of protein C and protein S. However, this is not possible because the prevalence of even more common congenital thrombotic mutations (such as factor V Lieden, MTHFRC677T and prothrombin G20210A mutation) is not increased in thalassemia patients (10).

Shirahata(17) stated that liver impairment was not the only cause of the reduction in natural anticoagulant proteins in β-thalassemia patients. There may be another explanation for the significant decrease in proteins and maybe this type of protein binds to phosphatidylserine, or other negatively charged phospholipids, abnormally exist in the external membrane of the thalassemic erythrocytes(18). This study also showed no significant difference for Protein C, protein S, D-dimer and fibrinogen level found between splenectomized and non-splenectomized patients; the same findings were confirmed by Rosnah(16) in Malaysia, these findings could

be due to small sample size for both groups of patients while other study showed significant difference(19). It was evidenced by clinical history and thorough physical examination that none of our patients has any thromboembolic event.

However, this does not mean that they do not have a hypercoagulable state, because the hypercoagulable state of thalassemia patients may occur in early childhood, and the manifestation of thromboembolic events may occur at a later age. In addition, several studies have shown that the incidence of silent ischemic lesions in patients with moderate beta thalassemia is increased, especially in adults who do not rely on splenectomy for blood transfusion, and some brain magnetic resonance imaging studies have found elevated platelet counts People. The prevalence of people with silent ischemic infarction is as high as 60% (20).

At the same time, today, new technologies such as genome editing technology have been used to help eliminate genetic defects (21). Also, treatments of the epigenetic changes of patients with predisposed to hypercoagulable states have been suggested to reverse destructive gene expression patterns (22). We hope that these technologies will see good results in the treatment of genetic diseases in the future.

Compared with the control group, the levels of protein C and protein S in patients with major β-thalassemia are significantly reduced, and the results of this study show that patients with major β-thalassemia have a chronic hypercoagulable state, which is manifested by a decrease in fibrinogen levels. In addition to the lack of protein C and protein S, the level of D also has dimers.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical Approval

Ethical approval has been done by the ethical committee of the Medical College at Hawler Medical University.

Informed Consent

Informed signed written consent was taken from the patient involved.

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