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EVALUATION OF PROTEIN S ANTIGEN LEVEL IN ADULT PATIENTS WITH CEREBROVASCULAR DISEASE

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ABSTRACT:

Background: Cerebrovascular disease encompasses a group of disorders that affect the brain's blood supply, potentially causing temporary or permanent functional impairments. Stroke is the most common type, often resulting in lasting motor or sensory deficits. Protein S, a vitamin K-dependent protein, acts as a cofactor to activated protein C in the inactivation of clotting factors Va and VIIIa, playing a crucial role in anticoagulation. **Aim of the Study:** This study aimed to evaluate total protein S levels among cerebrovascular disease patients and investigate any association between protein S deficiency and cerebrovascular events. **Patient and Method:** A case-control study was conducted at Al-Imamein Al-Kadhimaen Medical City in Baghdad and Tikrit Teaching Hospital in Iraq, from January to June 2024. A total of 90 subjects were enrolled and divided into two groups: **Patient group:** 60 individuals hospitalized during the acute phase of central nervous system infarction, diagnosed by neurologists and imaging. **Control group:** 30 apparently healthy individuals served as controls. **Results:** Patients with cerebrovascular infarction had significantly lower total protein S levels compared to the control group. A notable difference in protein S levels was observed between patients aged ≥60 years and those <60 years, as well as between male and female patients. Additionally, significant differences were found between patients and controls in terms of family history, smoking, diabetes mellitus, and hypertension prevalence. **Conclusion:** Cerebrovascular infarction patients exhibited lower total protein S levels compared to healthy individuals. Younger and female patients had comparatively lower levels, indicating a higher susceptibility to thrombotic events.

KEYWORDS: protein S antigen, adult, cerebrovascular disease.

INTRODUCTION

Hemostasis is a vital physiological defense mechanism that ensures vascular integrity by maintaining a delicate balance between clot formation and dissolution. Blood coagulation occurs when thrombin proteolyzes soluble fibrinogen into insoluble fibrin, forming a clot. Hemostasis encompasses the regulated initiation and cessation of coagulation, as well as the eventual removal of the clot during vascular remodeling. Effective formation of primary hemostatic plugs is critical in preventing blood loss following vascular injury. Failure in this mechanism may lead to bleeding if plugs are unstable or inadequately formed, or to thrombosis when prothrombotic activity overwhelms anticoagulant regulation.[1] Protein C is a vitamin K-dependent glycoprotein and a natural anticoagulant. It is initially an inactive zymogen in circulation, activated to a serine protease—activated protein C (APC)—upon thrombin binding to thrombomodulin on endothelial cells. APC inactivates coagulation factors Va and VIIIa, thus reducing thrombin formation and preventing excessive clotting.[2,3] Protein S, also vitamin K-dependent but not a serine protease, exists in circulation partially bound to C4b-binding protein and partially in a free form. As a cofactor, protein S enhances the anticoagulant activity of APC, aiding in the inactivation of Va and VIIIa. Beyond hemostasis, protein S is involved in apoptosis, vasculogenesis, inflammation, atherosclerosis, and oncogenesis.[4] Cerebrovascular diseases, including stroke and transient ischemic attack (TIA), affect cerebral blood flow and may lead to transient or permanent neurological impairments. Stroke, the most common type, often causes persistent loss of motor or sensory function.[5] TIA mimics stroke symptoms but resolves within 24 hours.[6] Common stroke risk factors include hypertension, diabetes, hyperlipidemia, heart valve disorders, and smoking. However, in younger patients or in those without these risk factors, underlying

hypercoagulable states should be considered.[7] Up to one-third of ischemic strokes in young adults are cryptogenic, and approximately 8% may have hematologic causes, although this may be underdiagnosed. Inherited thrombophilias such as deficiencies in protein C (PC), protein S (PS), antithrombin III (AT III), and mutations like factor V Leiden are implicated in venous and, more rarely, arterial thrombosis.[8,9] Although protein S deficiency is more often linked to deep vein thrombosis and pulmonary embolism, it may account for up to 10% of unexplained strokes in young adults.[10] Recognizing thrombophilia is crucial, as it influences therapeutic strategies and recurrence prevention. Aim of the Study: This study aims to evaluate total protein S levels in patients with cerebrovascular disease and investigate the potential association between protein S deficiency and the occurrence of such disorders.

Method

This case-control study was conducted from January to June 2024 at Al-Imamein Al-Kadhimaen Medical City in Baghdad and Tikrit Teaching Hospital in Tikrit, Iraq. A total of 90 subjects were enrolled: 60 patients diagnosed with acute-phase cerebrovascular infarction confirmed by neurologists and imaging studies (CT or MRI), and 30 healthy controls randomly selected for comparison.

Inclusion criteria included patients with acute cerebrovascular infarction confirmed clinically and radiologically.

Exclusion criteria involved those on oral anticoagulants, patients with cancer, hematological disorders, thrombosis, disseminated intravascular coagulation (DIC), vitamin K deficiency, renal or liver disease.

Data Collection: Demographic and clinical data were collected, including age, sex, family history, smoking, and comorbidities like diabetes, hypertension, and hyperlipidemia. Laboratory investigations included total protein S (PS) level (measured via ELISA), complete blood count (CBC), prothrombin time (PT), activated partial thromboplastin time (APTT), lipid profile, white blood cell count, and vitamin K level.

Sample Collection: Four mL of venous blood was collected aseptically: 2 mL in K3-EDTA for CBC and 1.8 mL in sodium citrate tubes for coagulation tests. Samples were centrifuged to obtain platelet-poor plasma, and plasma for protein S analysis was stored at -40°C.

Protein S Measurement: A sandwich enzyme-linked immunosorbent assay (ELISA) was used. A pre-coated microplate with monoclonal antibodies captured human PS, followed by detection with biotinylated antibodies and HRP. Color development was measured at 450 nm, and protein S concentration was determined using a reference standard curve.

Other Tests: CBC was performed using Sysmex XN 1000 (Japan). PT and APTT were measured with Stago Compact Max2 (France) using STA® kits. Vitamin K was quantified using HPLC with fluorescence detection after plasma extraction and purification.

Statistical Analysis: SPSS version 25.0 was used. T-tests analyzed numerical data (mean ± SD), while chi-square and Fisher's exact tests analyzed categorical data. Pearson correlation assessed relationships between variables. A P-value ≤ 0.05 was considered statistically significant.

RESULTS

A total number of 90 participants were included in the studied sample (60 patients and 30 controls). Statistical analysis revealed no significant difference between patients with cerebrovascular infarction and controls regarding age and gender; A statistically significant difference was detected between patients with cerebrovascular infarction and controls regarding each of family history of ischemic stroke, smoking history, the prevalence of diabetes mellitus, and hypertension, as shown in table (1).

Table (1): Basic characteristics of the studied sample.

Basic characteristics	Group		P value
	Patients (n=60)	Controls (n=30)	
Age			
<60 years	18	4	
	30%	13.3%	
≥60 years	42	26	
	70.0%	86.7%	
Mean ± SD	65.7 ± 12.5	64.4 ± 7.5	
Sex			
Male	31	13	
	51.7%	43.3%	0.507
Female	29	17	
	48.3%	56.7%	

Basic characteristics	Group		P value
	Patients(n=60)	Controls (n=30)	
Family history			
Positive	26	0	
	43.3%	0.0%	**<0.001**
Negative	34	30	
	56.7%	100.0%	
Smoking history			
Positive	32	0	
	53.3%	0.0%	**<0.001**
Negative	28	30	
	46.7%	100.0%	

Statistical analysis revealed that patients with cerebrovascular infraction had significantly lower PT, PTT, and higher WBC count than controls, whereas controls had significantly higher Hb. No significant difference was detected regarding platelet count or vitamin K levels. A statistically significant difference detected in total protein S level between cerebrovascular infarction patients and controls, as shown in table (2).

Table (2): Laboratory parameters of the studied sample.

Lab parameters (mean ± SD)	Group		P value
	Patients (n=60)	Controls (n=30)	
PT (sec)	**12.3 ± 0.6**	**13.6 ±2.6**	**0.006**
PTT (sec)	**26.9 ± 3.0**	**29.3 ±5.0**	**0.020**
WBC ($X10^9$/L)	**9.9 ± 4.7**	**6.0 ± 1.1**	**<0.001**
Hb (g/dl)	**12.1 ±2.5**	**14.0 ± 1.6**	**<0.001**
Platelet count ($X10^9$/L)	**216.3 ± 82.9**	**239.9 ± 101.8**	**0.241**
Vitamin K (ng/ml)	**2.1 ± 1.0**	**1.9 ± 1.0**	**0.463**

Protein S (pg/ml)	Group		P value
	patients	controls	
Mean ± SD	195.7 ± 108.7	234.9 ± 107.3	**0.037**

No significant difference regarding total protein S was detected between patients with and without DM, no significant difference regarding total protein S was detected between patients with and without hypertension. A significant difference regarding protein S was detected between patients ≥60 and <60 years old, a significant difference regarding total protein S was detected between male and female patients as shown in table (3).

Table (3): Comparison of protein total S in patients with and without DM, Hypertension, among patients ≥60 and <60 years old, male and female patients.

Protein S	Group		P value
	With DM	Without DM	
Mean ± SD	**217.45 ± 115.23**	**204.33 ± 103.71**	**0.580**
Protein S	**Group**		**P value**
	With HTN	**Without HTN**	
Mean ± SD	**217.86 ± 107.48**	**203.06 ± 108.32**	**0.524**
Protein S	**Age**		**P value**
	<60 years	**≥60 years**	
Mean ± SD	**200.99± 112.07**	**221.94 ± 103.81**	**= 0.018**
Protein S	**Sex**		**P value**
	Males	**Females**	
Mean ± SD	**227.24 ± 112.10**	**203.06± 108.32**	**0.007**

Statistical analysis revealed a significant positive correlation between total protein S and each of age (r=0.273, p value= 0.009), PT (r=0.222, p value 0.036), and PTT (r=0.273, p value= 0.009). Moreover, a significant negative correlation was detected between total protein S and Hb (r = -0.275, p-value = 0.009). No significant correlation was detected between total protein S and each of platelet count and vitamin K, as shown in table (4).

Table (4): Correlation between total protein S and each of age, platelet count, PT, vitamin K.

Parameter	Pearson correlation (r)	P value
Age	**0.273**	**0.009**
PT	**0.222**	**0.036**
PTT	**0.273**	**0.009**
WBC	**-0.129**	**0.225**
Hb	**-0.275**	**0.009**
Platelet	**0.061**	**0.568**
Vitamin K	**0.066**	**0.534**

DISCUSSION

This study found that patients with cerebrovascular infarction had significantly lower total protein S levels compared to the control group ($p = 0.037$), consistent with prior findings by Akyol et al. (2006)[11] and Anzola et al. (2016).[12] Studies focused on younger patients, such as those by Sacco et al. (1991)[13] and Nighoghossian et al. (1994)[14], also reported significantly lower protein S levels in cases ($p = 0.0005$), reinforcing the association between protein S deficiency and cerebrovascular infarction. While earlier studies suggested that protein S levels decline mainly in subacute and chronic stages[15], Mayer et al. (1993)[16] observed low levels even during the acute phase, linking this to a reduced ratio of free to bound protein S due to elevated C4b-BP during inflammation.[17] Variability in results across studies may be attributed to genetic, environmental, ethnic, or methodological differences. This study found no significant difference in protein S levels based on diabetes or hypertension status. However, females had significantly lower protein S levels than males ($p = 0.007$), consistent with studies indicating a greater susceptibility to protein S deficiency in women.[18,19] Zhu et al. (2011)[20] highlighted hormonal and genetic factors, such as pregnancy or oral contraceptive use, as contributors to lower protein S levels in women, potentially increasing stroke risk.[21] Protein S levels were also significantly lower in patients aged ≤60 years ($p = 0.018$), aligning with Chiasakul et al. (2019) and Tsalta-Mladenov et al. (2022), who identified protein S deficiency as a prominent risk factor for ischemic stroke in younger populations.[22,23] The study also found that PT, PTT, and hemoglobin (Hb) were significantly lower, while WBC was significantly higher in patients than controls, consistent with findings from Buseri et al. (2019)[24], Tian et al. (2020)[25], Woo et al. (1990)[26], and Akinlua et al. (2019).[27] However, no difference in platelet count was observed, differing from Punekar et al. (2019).[28] No significant vitamin K differences were detected, contrasting with Larson et al. (2018).[29] A significant positive correlation was found between protein S levels and PT/PTT, and a negative correlation with Hb, suggesting broader roles for protein S in coagulation and hematology. Finally, diabetes, hypertension, smoking, and family history were significantly associated with cerebrovascular infarction ($p < 0.001$), echoing findings by Scott et al. (2023)[30], Hankey et al. (2001)[31], and others.[32,33]

CONCLUSION

Patients with cerebrovascular infarction had lower protein S levels than healthy ones. Protein S deficiency is associated with younger illness start, as shown by the considerably lower mean age of cerebrovascular infraction patients with protein S insufficiency compared to those with normal protein S levels. Compared to men, women have lower protein S levels.

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