Evaluation of Protein C and Protein S in Pregnant Females with Preeclampsia

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ABSTRACT:

BACKGROUND:

Preeclampsia (PE) is a multisystem progressive disorder characterized by the new onset of hypertension and proteinuria. Women with PE have been shown to be at increased risk of venous thromboembolism (VTE).

OBJECTIVE:

To evaluate the levels of protein C and protein S among females with PE**,** and to determine whether there is relationship between deficiencies of these proteins and PE severity.

SUBJECTS AND METHODS:

A total of 30 pregnant women with PE and other 30 normotensive age-matched healthy pregnant women in their third trimester of pregnancy were enrolled in this case-control study. Beside blood pressure and general physical examination of each participant, peripheral venous blood sample were tested for complete blood count (CBC) prothrombin time (PT) activated partial thromboplastin time (APTT), protein C and protein S.

RESULTS:

Out of 30 patients, there were 8 patients 26.67%) who had protein C deficiency compared to only 1 pregnant woman (3.33%) among the control group with a significant difference. Likewise, the frequency of protein S deficiency was 20% among patients, while none among controls had such deficiency. Mean plasma level of protein C and protein S in preeclmaptic women was 79.07±21.94% and 68.73±13.19%, respectively which was significantly lower than that of women with normal pregnancy (90.0± 14.96% and 78.67±10.17%, respectively). Furthermore, 50% of women suffering from protein C or protein S deficiency experienced severe PE while 95.45% and 91.67% of women with sufficient protein C and protein S, respectively displayed mild form of the disease.

CONCLUSION:

Both protein C and protein S significantly reduced in pregnant women with PE and are associated with the severity of the disease.

KEYWORDS: Preeclampsia, protein C, protein S.

INTRODUCTION:

Preeclampsia is a multisystem progressive disorder characterized by sudden onset of hypertension and proteinuria, or hypertension and significant end-organ dysfunction with or without proteinuria in the last half of pregnancy or postpartum. It typically affects 2%–5% of pregnant women and is one of the leading causes of maternal and perinatal morbidity and mortality [(1)].

Preeclampsia presents a great challenge to obstetricians because of its unknown etiology, complex pathophysiology, and the fact that the only definitive cure is delivery of the fetus and the placenta. If PE is not controlled, it can progress to HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets) or can also present with hematologic abnormalities like disseminated intravascular coagulopathy (DIC) [(2)].

Protein C and protein S are two proteins in the blood that participating in the regulation of blood clot formation. Homozygous deficiency of either protein C or protein S is presenting as purpura fulminans and massive thrombosis. By contrast, warfarin inducing skin necrosis, deep and superficial venous thrombosis (VTE) are features of heterozygous deficiency [(3)]. Another manifestations of deficiencies of these proteins are adverse pregnancy outcomes, such as recurrent

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fetal loss, pre-eclampsia, and worse outcomes among patients with pre-eclampsia than among those without thrombophilia [4].

The present study aimed to evaluate the levels of protein C and protein S among females with PE. Determine whether there is relationship between deficiencies of these proteins and PE severity.

SUBJECTS AND METHODS:

The Study Population

A total of 60 women were included in this case control study that was performed at Al-Imamein Al- Kadhimein Medical City/ Baghdad, during the period from the 1st of January till the end of march 2019. Those patients comprised 30 pregnant women with PE, who were diagnosed by consultant gynecologist based on clinical and laboratory evaluation (blood pressure>140/90 and proteinuria>300 mg/24hr), and 30 age-matched normotensive healthy pregnant women in their third trimester of pregnancy to be served as control group.

Data including name, age, past obstetric history (gravidity, parity, previous abortion, history of still birth, previous history of gestational hypertension or PE) past medical, surgical and drug history, blood pressure and urine analysis were collected by direct interview or from the hospital records.

When any of the following diseases or conditions was encountered, a woman was excluded from the study: liver, renal and vascular diseases, sickle cell anemia, current thrombosis or DIC, within the last 3–6 months, any evidence of active infection, acute illness or history of inherited coagulopathy, women on anticoagulant therapy within the last 2 weeks or having recent surgery.

For all participant a written consent was obtained. The study was also approved by the Iraqi Board for Medical Specializations.

Blood sampling and Investigations

A Peripheral venous blood sample (from the antecubital fossa) was withdrawn from all participants under aseptic technique. About 4 milliliters of whole blood were collected and separated into 2 tubes; 2 mL were poured into EDTA tube for complete blood count (CBC) and 2 mL were poured into sodium citrate tube for immediate assay of prothrombin time (PT) and activated partial thromboplastin time (PTT). Part of citrated plasma was stored at −40◦C until protein C and protein S assay, which were performed within a maximum of one month of deep freezing.

Complete Blood Count

Blood sample from each patient were examined for complete blood indices including Hb, WBC count and differential, platelet count by Automated Hematology Analyzer (Cell-DYN, RUBY Abbott Diagnostics, Germany).

Prothrombin Time and Activated Partial Thromboplastin Time

Both PT and PTT were performed using BIO-TP Prothrombin Time (PT) kit from BIOLABO French manufacturer of Reagents for Medical Biology following the manufacturer's instructions.

Protein C and Protein S Assays

Plasma level protein C and protein S was measured based on enzyme-linked immuno sorbent assay (ELISA) using ready commercial kits (AESKULISA Protein C/ Protein S, ELISA Kit, AESKU Diagnostics, Wendelsheim, Germany). The manufacturer's protocols were followed precisely. Plasma levels of these protein were expressed as percentage. According to these instructions, the normal value of protein C is ≥70%, while that of protein S is ≥ 60%).

Statistical analysis

Statistical package for social science (SPSS) software (version 25.0, IBM, USA) were used for data analysis and graph construction. Numeric variables were expressed as mean ±standard deviation (SD), and analyzed with student t-test. Categorical variables were expressed as frequency and percentages and analyzed with Chi square/exact Fisher test. A p-value of 0.05 or less was considered significant.

RESULTS:

Demographic, Reproductive and Clinical characteristics of Mothers

Mean age of patients and control were 30.17±5.27 and 29.4±5.78 respectively with no significant differences (Table 1). Likewise, there were no significant differences between the two groups regarding Hb concentration, platelet count, PT and PTT. Three women among patients had a history of PE toxemia versus none of controls had such a history; however, this difference did not reach a significant level.

Systolic blood pressure and diastolic blood pressure, *per se*, were much higher in patients than in controls with a highly significant differences. Although women with PE showed higher frequency of gravida, parity and previous abortion than controls, the differences were not significant.

Table 1: Demographic, reproductive and clinical characteristics of the study population.

Variables	Patients N=30	Controls N=30	P-value
Age, years	30.17±5.27	29.4±5.78	0.234
Hemoglobin (g/dL)	10.45±1.67	10.6±1.29	0.705
Platelets count ($\times 10^3$/ml)	245.1±67	268.8±69.5	0.184
SBP, mmHg	149.5±8.34	113.73±6.91	<0.001
DBP, mmHg	98.33±7.12	73.33±7.23	<0.001
Prothrombin time (sec)	13.37±0.67	13.4±0.62	0.842
PTT (sec)	32.1±2.53	32.2± 2.4	0.876
Gravida ≤3 >3	 18(60%) 12(40%)	 22(73.33%) 8(26.67%)	0.273
Parity ≤2 >2	 21(70%) 9(30%)	 25(83.33%) 5(16.67%)	0.222
Previous abortion No Yes(one or two)	 15(50%) 15(50%)	 22(73.33%) 8(26.67%)	0.063
Previous PE toxemia No Yes	 27(90%) 3(10%)	 30(100%) 0(0%)	0.076
Severity Mild Severe	 25(83.33%) 5(16.67%)	 …….	

SBP: Systolic blood pressure, DBP: Diastolic blood pressure

Plasma levels protein C and protein S

Mean plasma level of protein C in PE women was 79.07±21.94% which was significantly lower than that of women with normal pregnancy (90.0± 14.96%). Similarly, control group showed higher level of protein S than PE women in PE women and controls were and (78.6±10.17% vs. 68.73±13.19%) a highly significant difference.

Frequency of Protein C and Protein S Deficiency in Patients and Controls

Out of 30 patients, there were 8 (26.67%) who had protein C deficiency compared to only 1 (3.33%) among controls with a significant difference. Likewise, the frequency of protein S deficiency was 20% among patients, while none among controls had such a deficiency. Chi square test revealed a highly significant difference between the two groups (Table 2).

Table 2: Frequency of protein C and protein S deficiency in patients and controls. The nor level of protein C and S is ≤ 70% and ≥ 60% respectively.

Variable	Patients (n=30)	Controls (n=30)	p-value
Protein C Normal deficient	 22(73.33%) 8(26.67%)	 29(96.67%) 1(3.33%)	0.011
Protein S Normal deficient	 24(80%) 6(20%)	 30(100%) 0 (0%)	0.010

Correlation Between Different Variables

Pearson's correlation test was used to explore the possible correlation between continuous variables with protein C and protein S in patients (Table 3). There were four significant correlations, all of which related to protein S. This protein showed a negative correlation with each of age (r= -0.386, p= 0.035) and proteinuria (r= -0.407, p= 0.026). On the other hand, protein S positively correlated with each of platelet count (r= 0.362, p= 0.049) and protein C (r= 0.823, p< 0.001).

Table 3: Correlation of the correlation between continuous variables with protein C and protein S in PE patients.

Variables	Protein C		Protein S	
	Correlation	p-value	Correlation	p-value
Age	-0.220	0.242	-0.386	0.035
Hb	-0.132	0.486	-0.125	0.509
Platelet	0.183	0.332	0.362	0.049
PT	-0.293	0.116	-0.051	0.789
PTT	-0.041	0.831	0.082	0.668
Proteinuria	-0.152	0.423	-0.407	0.026
Protein C			0.823	<0.001

PT: prothrombin time, PTT: partial thromboplastin time

Associations of Protein C Deficiency with the Reproductive Characteristics of PE Women

Protein C deficiency demonstrated a significant association with almost all included reproductive characteristics of PE women (Table 4). Protein C deficiency was reported in all women with previous history of PE toxemia but in none among women with no such a history. Similarly, 75% of patients suffering from protein C deficiency had >3 gravida while most patients (72.73%) with ≤3 gravida had normal concentration this protein. Also 62.5% of women with protein C deficiency had frequent parity (> 2) while 81.82% of women with normal protein C had less frequent parity (≤ 2). Finally, 50% of women suffering from protein C deficiency experienced severe PE while 95.45% of women with sufficient protein C displayed mild PE. Of note, mean plasma level of protein C in patients with severe and mild PE (among protein C deficient group) was 52.0± 10.7% and 50.25± 12.01% respectively with no significant difference.

Table 4: Associations of protein C deficiency with the reproductive characteristics of PE women.

Characteristics	Protein C deficiency (n=8)	Normal protein C (n=22)	p-value‡
Previous PE No Yes	5(62.5%) 3(37.5%)	22(100%) 0(0%)	0.014
Gravida ≤3 >3	2(25%) 6(75%)	16(72.73%) 6(27.27%)	0.027
Parity ≤2 >2	3(37.5%) 5(62.5%)	18(81.82%) 4(18.18%)	0.032
Previous abortion No Yes(one or two)	2(25%) 6(75%)	13(59.1%) 9(40.9%)	0.215
Severity Mild Severe	4(50%) 4(50%)	21(95.45%) 1(4.55%)	0.011

‡: Fisher's exact test

Associations of Protein S Deficiency with the Reproductive Characteristics of PE Women

Protein S deficiency significantly associated with three reproductive characteristics in women with PE (Table 5). The vast majority of women (83.33%) with protein S deficiency had >3 gravida compared with over 70% of women with sufficient protein S had ≤ 3 gravid. Almost, the same scenario could be applied for parity. Also,50% of women with protein S deficiency were suffering from severe PE, while mild PE was observed in 91.67% of women with normal protein S. Of note, mean plasma level of protein S in patients with severe and mild PE (among protein C deficient group) was 45.0± 3.0% and 54.0± 7.8 respectively with no significant difference.

Table 5: Associations of protein S deficiency with the reproductive characteristics of PE women.

Characteristics	Protein S deficiency (n=6)	Normal protein S (n=24)	p-value‡
Previous PE No Yes	4(66.67%) 2(33.33%)	23(95.83%) 1(4.17%)	0.094
Gravida ≤3 >3	1(16.67%) 5(83.33%)	17(70.83%) 7(29.17%)	0.026
Parity ≤2 >2	2(25%) 4(75%)	19(79.17%) 5(20.83%)	0.049
Previous abortion No Yes(one or two)	1(16.67%) 5(83.33%)	14(58.33%) 10(41.67%)	0.169
Severity Mild Severe	3(50%) 3(50%)	22(91.67%) 2(8.33%)	0.041

DISCUSSION:

Protein C and protein S levels were estimated in two groups, patients with PE and age matched normotensive pregnant women to clarify if there is any relationship between these proteins deficiencies and PE occurrence and severity.

Hb concentration, platelet count, PT and APTT were done to all patients included in this study and showed no significant difference from control group.

Regarding protein C 8 out of total 30 patients were deficient for protein C compared to only 1 out of 30 among normal pregnant females. This percent was lower than the frequency of protein C deficiency found in other studies, in Nigeria by Onakewhor et al. (2008) [5]. In another study, Okoye et al. (2017) [6] reported that 37% of the test population were protein C deficient. In contrast to another study carried out in India, Sooraj et al. (2018) [7] that found much lower frequency of protein C deficiency than the present study.

However, regarding protein S, 6 out of total 30 (20%) preeclamptic patients were deficient for protein S compared to none of the normotensive control group, those results were higher than protein S deficient patients in Philipp et al study, 2014 with (15.2%) frequency [8]. Whereas the percentage of women with protein S deficiency in the present study was lower than that seen by Hossain et al. (2005) [4] who recorded an incidence of (45%) in Pakistan.

These differences in the incidence of protein C and protein S deficiencies among different studies and populations may be due to ethnic and racial differences in addition to the differences in the sample size that may over or under estimate the actual frequencies.

So, the incidence of both protein C and protein S deficiencies were more in preeclamptic patients compared to control group. These findings agree with other two studies, Alfirevic et al. (2002)[9] who found that women with pre-eclampsia are more likely to have protein C and protein S deficiency. Similarly, Kupferminc et al. (1999) [10] noted that these proteins deficiencies associated with increased thrombophilia and severe early-onset pre-eclampsia.

In the present study the mean plasma level of protein C and protein S in preeclamptic patients were significantly lower than their counterpart in the normotensive group.

In the current study the mean level of protein S in both preeclamptic and the control groups were within normal range, despite the significant difference between their mean level and despite the fact that protein S levels decrease gradually in healthy women during pregnancy, which could be caused by increased protein C resistance and increased levels of coagulation factors [11].

Similarly, a related study done in a Turkish population, Demir et al. (2010) [12] found that mean levels of protein S was significantly reduced in women with pre-eclampsia as compared with normotensive pregnant and non-pregnant females. Other studies had been found no significant difference in mean levels of protein S between the study groups, probably because of differences in the study participants, in the period of sample collection, and in the assay methods. For example, Yalinkaya et al. (2006) [13] had analyzed samples collected during postpartum period and had used a coagulation-based assay. The expected changes in the levels of these proteins, by the effects of pregnancy and PE, might have completely or partially resolved by this stage. While the present study conducted among third trimester pregnant women using immunologic (antigen) method, which are subject to less interference as compared with the coagulation-based (activity) assay.

Among the total 8 protein C deficient preeclamptic patients, 3 of them had previous history of PE, and 2 out of total 6 protein S deficient patients had such history. Both of these results were significantly higher than their preeclamptic patients counterparts with normal protein C and protein S levels, this finding can be explained by the fact that previous history of PE is one of the documented risk factor of PE and may reveal a role of protein C and protein S in its pathogenesis that require further study. Regarding gravidity and parity there were no significant differences between preeclamptic patients and the control group which were similar to studies in Nigeria, India and Pakistan [4,6,7]. While within the preeclamptic group, those with protein C and S deficiencies showed higher gravidity and parity in contrast to the well-known fact of decreased risk of PE among multiparas patients.

Concerning the severity of PE, half of both protein C and protein S deficient preeclamptic patients had a severe form of the disease and the other half suffered from mild disease, while (95.45%)of

preeclamptic patients who had no protein C deficiency and (91.67%) of preeclamptic patients who had no protein S deficiency were suffered from mild PE which were significantly higher than that in the deficient group. This might suggest some correlation between these proteins deficiencies and the severity of the disease.

Collectively, these data suggest that both protein C and protein S are reduced in women with PE and inversely associated with the severity of the disease may be through affecting some inflammatory factors. However, further studies are required to more illustrate the role of these proteins in PE.

CONCLUSION:

Collectively these data highly indicated that both protein C and protein S are significantly reduced in pregnant women with PE and are associated with the severity of the disease.

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