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Relation between the Level of Soluble Endothelial Protein C Receptor and the Risk of Deep Venous Thrombosis in Sudanese

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Abstract

Background: Deep vein thrombosis (DVT) may lead to serious complication; the pulmonary embolism; one of the most serious conditions that cause morbidity and mortality worldwide. sEPCR circulates in the plasma and has high affinity to bind protein C and activated protein C (APC). This binding interferes with the anticoagulant function of APC and results in increased risk for DVT. The aim of this study is to explore the role of sEPCR as a risk factor for DVT in Sudanese individuals. Methods: A total of 100 Sudanese DVT patients and 100 apparently healthy individuals were recruited for this study. EDTA-anticoagulated venous blood samples were collected from all participants. Plasma sEPCR levels were measured by enzyme linked immunosorbent Assay (ELISA). All results were analyzed using SPSS. Results: The plasma level of sEPCR was higher in DVT group than in healthy individuals, while male patients showed higher level when compared to females. Age correlates positively with sEPCR, whilst BMI and ethnicity showed no effect on the level of sEPCR. Individuals in the top quartiles of showed to increased risk of DVT when compared to those in the lower quartile. Conclusion: It can be concluded that elevated sEPCR level increases the risk to develop DVT in Sudanese. The level of the soluble receptor is influenced by the gender of individual more than by his/her ethnicity or body mass index. Results are also indicative of a much higher risk in those with sEPCR level more than 120 ng/ml when compared to those with lower levels.

Keywords

Deep Vein Thrombosis, sEPCR, Pulmonary Embolism, EPCR, Sudanese

Subject Areas: **Hematology**

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1. Introduction

Deep vein thrombosis (DVT) is the formation of blood clots (thrombi) in deep veins [1]. It commonly affects the deep veins of the leg (such as calf veins, femoral vein, or popliteal vein) or the deep vein of the pelvis [2]. DVT derives its importance from its association with risk of fatal pulmonary embolism (PE), chronic post phlebitic syndrome and pulmonary hypertension. Many genetic and acquired factors are linked with the risk of DVT [3]. The strongest genetic risk factors are antithrombin deficiency, protein C deficiency, and protein S deficiency, while moderate risk factors include factor V Leiden, Prothrombin G20210A, fibrinogen 10034T, non-O blood type, and variants of factor XIII and XI [2]. Acquired risk factors for DVT include old age, trauma, surgery, contraceptive pills [4], cancer [5], obesity, pregnancy, and nephrotic syndrome [2]. PC is an important naturally occurring anticoagulant activated by thrombin-thrombomodulin complex and its activation is augmented by endothelial protein C receptor (EPCR) [6]. Kurosawa *et al.* [7], identified a soluble form of EPCR in human plasma, which has a molecular weight of 43,000 D, and circulates at a concentration of about 100 ng/ml; they found that sEPCR has ability to bind PC and activated protein C (APC) with an affinity similar to that of membrane bound EPCR. The physiological function of sEPCR is still undefined and may be multifaceted; it appears to inhibit the anticoagulant and cell signaling activities of APC but it may promote the anti-inflammatory effects of APC by enhancing its binding to activated neutrophils and preventing neutrophil adhesion to endothelium [8], therefore, binding of sEPCR to APC can increases the risk of thrombus formation. The relation between sEPCR and the risk of DVT was suggested by many studies [9]-[11]. Elodie Ducros *et al.* [12] reported an association between sEPCR level and thrombosis in patients with haematologic malignancies, another association between sEPCR level and thromboses development was reported by Abd-Elghaffar, A.A *et al.* [13] in patients under haemodialysis. The aim of this study is to determine the association of sEPCR level with the risk of DVT among Sudanese.

2. Materials and Methods

In a prospective case control study a sample of 200 Sudanese were recruited, out of which 100 DVT patients (43% male and 57% female) with age ranged from 18 to 59 years, attended different Khartoum state hospitals, were assigned to the cases group. All DVT cases were confirmed with Doppler ultrasonography (Philips Healthcare, Netherlands). Patients with chronic diseases, malignancies or under thrombolytic medications were excluded, while the control group comprised of 100 apparently healthy individuals (58% male and 42% female) with age ranged from 17 to 55 years. All controls were recruited from the co-patients of the DVT patient to ensure the maximum homogeneity between the cases and controls. The study was approved by the committee of research department, Ministry of Heath, Khartoum state, Sudan. A written informed consent was obtained from every participant.

2.1. Blood Sample Collection

A total of 4 ml venous blood was drawn from all participants into EDTA vacuum blood containers, plasma was separated and stored at −80˚C until further processing.

2.2. Determination of sEPCR

Plasma sEPCR levels were measured by enzyme linked immunosorbent Assay (ELISA) sEPCR ELISA kits from WKEA Med Supplies corporation, China. The assay included the use of microtitre plate wells that coated with a purified human sEPCR antibodies, which make a solid-phase antibody, then sEPCR was added to the wells, enzyme-linked anti-sEPCR antibody was then added, followed by thorough washing, then substrate was added to give blue color at horseradish peroxidase enzyme-catalyzed reaction, the reaction was terminated by the addition of Sulphuric acid solution and the color change was measured spectrophoto metrically at a wave length of 450 nm. The concentration of sEPCR in the samples was then determined by comparing the optical density of the samples to the standard curve.

2.3. Data Analysis

The demographic data and ELISA results were analyzed using statistical package for social sciences (SPSS) software version 16. Level of significance for the statistical tests was set at 0.05.

3. Results

The demographic data of the cases group comprised of three ethnic groups, Nilo-Saharan 26%, Afro-Asiatic 60%, and Congo-Niger 14%, and their distribution according to the BMI was; underweight 10%, normal weight 72%, overweight 12% and obese 6%. While the ethnic distribution of the control group was; 22% of them were Nilo-Saharan, 56% were Afro-Asiatic, and 22% were Congo-Niger and the BMI distribution; 4% were underweight, 70% normal weight, 20% over weight, and 6% obese. The comparison of sEPCR level between the case and control groups showed a statistically significant difference ($P < 0.05$) as in **Table 1**. In the cases group alone statistically significant difference was also observed in the sEPCR level between males and females ($P < 0.05$) as shown in **Table 2**. According to ethnicity and BMI of the cases group, the level of sEPCR showed no statistical significant differences ($P > 0.05$) as shown in **Table 3** and **Table 4**, respectively.

A positive correlation was also seen between level of sEPCR and age of individual with P. value < 0.05 as shown in **Table 5**.

To assess the association of different levels of sEPCR with the risk of DVT, we divided the sEPCR levels into quartiles, as measured in the healthy control group (<80 ng/ml, 80 - 100 ng/ml, 100 - 119.75 ng/ml, and >119.75 ng/ml). The top quartile group (>119.75 ng/ml) showed an increased risk for DVT as compared to the first quartile (<80 ng/ml) (OR = 3.409; 95% CI, 1.542 - 7.538) ($P < 0.05$), while those with plasma level in the second quartile (80 - 100 ng/ml) and the third quartile (100 - 119.75 ng/ml) showed no statistically significant risk as shown in **Table 6**.

Table 1. Level of sEPCR in the cases and control.

	Study groups	N	Mean	Std. Deviation	*P*
sEPCR ng/ml	Control	100	100.75	20.642	0.000
	Case	100	118.40	30.516	

Table 2. Level of sEPCR in the cases according to gender.

	Gender	N	Mean	Std. Deviation	*P* value
sEPCR ng/ml	Male	45	129.84	33.582	0.001
	Female	55	109.04	24.287	

Table 3. Comparison of sEPCR level between ethnic groups.

sEPCR ng/ml	Sum of Squares	df	Mean Square	F	*P* value
Between Groups	2973.399	2	1486.699	1.616	0.204
Within Groups	89,216.601	97	919.759		
Total	92,190.000	99			

Table 4. Comparison of sEPCR level based on BMI.

sEPCR ng/ml	Sum of Squares	df	Mean Square	F	*P* value
Between Groups	1748.924	3	582.975	0.619	0.604
Within Groups	90,441.076	96	942.095		
Total	92,190.000	99			

Table 5. Correlation between the level of sEPCR and age.

		Age/year
sEPCR ng/ml	Pearson Correlation	0.279
	P value	0.005
	N	100

Table 6. Odd ratio of sEPCR level in quartiles as a risk factor for DVT.

		Sig.	Exp(B)	95.0% CI. for EXP (B)	
				Lower	Upper
Step 1	sEPCR level	0.001			
	80 - 100 ng/ml	0.644	0.802	0.315	2.043
	100 - 119.75 ng/ml	0.902	0.949	0.412	2.185
	>119.75 ng/ml	0.002	3.409	1.542	7.538
	Constant	0.220	0.680		

4. Discussion

In the present study DVT patients showed higher sEPCR level than in healthy individuals, this finding consistent with that stated by many studies [10] [11] [14]-[18]. In DVT patients there was a typically high production of thrombin, which in turn induces metalloprotease shedding of EPCR from endothelial cell membranes [19] [20] and this results in increased sEPCR level. Alternatively, previous reports showed a positive association between sEPCR and PC level [10] [21], though the mechanism is still unknown, hence, increased production of PC in thrombotic patient may contribute to their high level of sEPCR. On the other hand, over-production of proinflammatory cytokines such as TNF-α, interleukins-1β, in thrombotic patients could result in an increase in sEPCR level in their plasma [22].

Current results also showed that the level of sEPCR in DVT patients was slightly higher in males than in females, a finding goes in concordance with many studies [7] [10] [23]-[25] that reported a high level of sEPCR in male than females. This gender difference in the sEPCR may suggest a hormonal influence or it may be indicative to increase thrombin production and a hypercoagulable state in male individuals, which in turn results in elevation of sEPCR level.

No difference was observed in sEPCR level among different ethnic groups as well as BMI groups in agreement with two studies [10] [26] that found no association between BMI and plasma level of sEPCR.

When taking into account parameters affecting sEPCR concentration, present study indicate a positive correlation between age and sEPCR level among the DVT patients, this finding was consistent with that reported by Yamagishi, *et al*. [27]. Uitte De Willige. S *et al*. [10] who found high level of sEPCR in individuals older than 45 years than individuals younger than 45 years. A study by F. S. Orhon *et al.* [25] determined plasma sEPCR levels in a group of Turkish healthy population had shown a negative correlation between the level of sEPCR and individual age in the children group but they failed to find similar correlation in adult group. Accumulation of many genetic and environmental factors such as mutations, infections, and decrease activity, that may affect the hemostatic balance occur with age, causing increased production of thrombin, which in turn induces metalloproteinase shedding of EPCR and elevate level of sEPCR.

In order to assess the variation in sEPCR level as a risk factor for DVT, we divided the sEPCR levels into quartiles, as measured in the healthy control group. Current results showed that subjects with plasma level in the top quartile (>119.75 ng/ml) at risk to develop DVT when compared to those in the first quartile (<81 ng/ml), while no risk was showed by those in the second and third quartile. S. Uitte de Willige *et al.* [10] found that when compared with the first quartile of plasma sEPCR levels (<81 ng/ml) the top quartile (>137 ng/ml) was associated with an increased risk of venous thrombosis, and the risk of the 2nd and 3rd quartiles was also increased relative to that of the first quartile. Yamagishi *et al.* [27] found no association between sEPCR and risk of VTE, and the Odds ratio for the highest quartile of sEPCR (170.4 - 1381 ng/ml) versus the lowest quartile (65.4 - 121.7 ng/ml) was 1.84 for non white and 0.96 for white VTE patients. In the present results, although the effect of the second and the third quartile was not significant there was an increment in the OR as the level of the sEPCR increases indicating a true relation between the level of the receptor and the risk of DVT.

5. Conclusion

In conclusion the results of the current study are suggestive of an increased risk of DVT with elevated levels of sEPCR, and that receptor level is influenced by the gender of individual more than his/her ethnicity of body

mass index. It is also safe to say that the Sudanese with plasma sEPCR level more than 120 ng/ml are more than 3 folds at risk to develop DVT than those with lower levels.

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