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Determination of Protein C Level among Sudanese Patients with Deep Venous Thrombosis

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Abstract

Background: Protein C deficiency is a congenital or an acquired condition that leads to increased risk for thrombosis. Congenital protein C deficiency is one of several inherited thrombophilias, which are a heterogeneous group of genetic disorders associated with an elevated risk of venous thromboembolism. One of the major clinical manifestation of venous thromboembolism (VTE) is deep vein thrombosis (DVT).

Objective: To determine protein C level among Sudanese patients with deep venous thrombosis

Materials and methods: This was an analytical case control study that was conducted in Aliaa Specialist Hospital (Omdurman, Sudan). A total of 90 patients were enrolled in this study: 45 DVT patients (test group) and 45 apparently healthy participants (control group). 2.5 ml of venous blood were collected from both groups in trisodium citrate containers. The protein C level was determined by using the enzyme linked-immunosorbent assay (ELISA) method. Data were analyzed by the SPSS program, version 21.

Results: Out of 45 deep venous thrombosis patients, 12 (26.67%) were males and 33 (73.33%) were females. The age incidence range was 25-65 years (mean age 42.60 ± 9.866). Protein C level was significantly decreased in DVT test group patients (70.02 ± 23.134) as compared with control group participants (83.04 ±11.975), p = 0.000.

Conclusion: Deep venous thrombosis was significantly associated with low protein C level among test group patients.

Keywords: Protein C, Deep venous thrombosis, Venous thromboembolism, ELISA.

Introduction

Normal homeostasis, using several inhibitor mechanisms, prevents fibrin clots in the blood vessels. In addition, fibrinolysis system has the main role in the lysis and solution of Small amount of fibrin that has been made in bloodstream and therefore be considered as a first line of defense against thrombosis. The main inhibitors of coagulation pathway are anti-thrombin (AT), protein C and protein S. These inhibitors are necessary to prevent thromboembolism. Hereditary deficiency of coagulation inhibitors lead to a change in balance between the anti-clotting and the formation of thrombin. Venous thromboembolism is the third most common vascular disorder in the world after the ischemic heart failure and stroke[1].

One of the major clinical manifestations of venous thromboembolism (VTE) is deep vein thrombosis (DVT). Protein C is a precursor to the serine protease found in plasma, and plays an important physiological role in the regulation of blood coagulation. Protein C is vitamin K dependent produced in the liver and normally circulates in the blood as a glycoprotein, and is activated by a complex of thrombin and thrombomodulin. Activated protein C forms a complex with its cofactor protein S and inactivates the clotting factors Va and VIIIa , thus preventing the formation of thrombin[2].

Protein C deficiency causes thrombophilia and increased risk of venous thrombosis, hence individuals lacking protein C often have a history of thrombotic disease. Heterozygous protein C deficiency is inherited in an autosomal dominant fashion. The gene for protein C is located on the long arm of chromosome 2. These mutations are divided into 2 types: type I and type II on the basis of whether they cause a quantitative (type I) or functional (type II) deficiency of protein C[3].

Materials and methods

This was a case control study conducted in Khartoum State at Aliaa Specialist Hospital. The study has been approved by the local ethics committee of AL Neelain University. The procedure of blood collection was explained to study population, and they were informed about the research objectives and procedures. The study was approved by the Ethical Board of Al-Neelain University, Khartoum (Sudan). Permission to collect the specimens was obtained from authorities of Aliaa Specialist Hospital (Omdurman, Sudan). The information collected had not been used for any purpose other than this study. Confidentiality of information obtained was maintained.

A total of 90 participants were enrolled in this study: 45 DVT patients (test group) and 45 apparently healthy participants (control group). 2.5 ml of venous blood was collected, from each participant in 3.8% trisodium citrate (9:1 vol/vol), kept on ice until centrifugation at 2500 g for 30 minutes at 4°C. Plasma samples were immediately frozen and stored at - 80°C for subsequent coagulation analysis.

Protein C level was measured by the sandwich technique of the enzyme immunoassay (ELISA) using commercial assay kits from Aesku Diagnostica (Germany) and following the manufacturer's recommendations. The micro ELISA plate was coated with capture antibody specific for human protein C and the enzymatic activity was obtained by using TMB as a substrate and 1mol/L HCI to block the reaction.

Data were analyzed using statistical package for social science software (SPSS) version 21.The results were expressed as percentage and means. Independent t-test was used to compare protein C level between case and control groups as well as across the gender distribution. Pearson correlation was done to study the association between protein C and age.

Results

A total of 90 participants: 45 DVT patients (test group) and 45 apparently healthy participants (control group) were enrolled in this study. The mean age of DVT patents was (42.60 ± 9.866).

Out of the 45 DVT patients, 12 (26.67%) were males and 33 (73.33%) were females. The independent t-test analysis showed that, protein C level was significantly decreased in deep venous thrombosis patients (70.02 ± 23.134) as compared to the control group (83.04 ±11.975), with p = 0.000.

Moreover the protein C level was insignificantly increased in female DVT patients (73.55 ±21.806) and the protein C level was insignificantly decreased in male DVT patients (60.33 ±21.806), with p = 0.09.

As shown in Fig. (1), the Pearson correlation showed no correlation observed between protein C level and age incidence (R=0.255, p = 0.09).

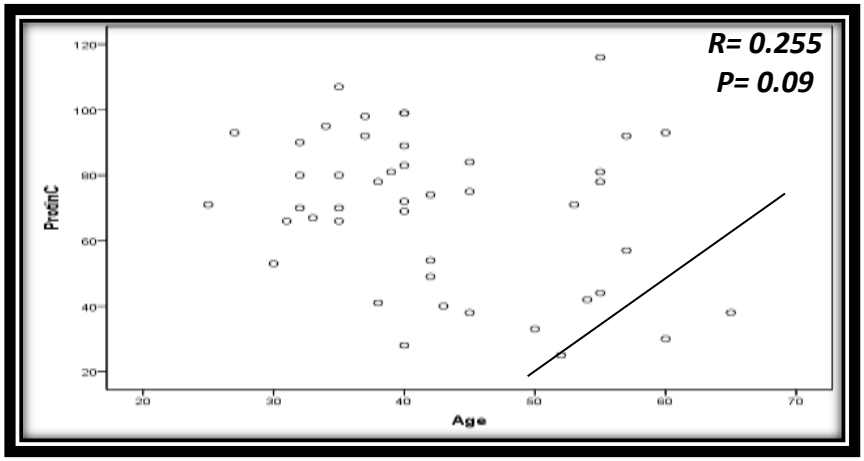

Fig. (1). Correlation of protein C and age incidence

Discussion

Venous thromboembolism is the third most common vascular disorder in the world after the ischemic heart failure and stroke. One of the major clinical manifestations of venous thromboembolism (VTE) is DVT. Risk factors of DVT include past history of DVT, pulmonary edema, operative intervention, immobilization, trauma, neurological deficit, malignancies, sepsis, central venous catheter and hypercoagulable state[4].

Protein C is a vitamin K-dependent anticoagulant serine protease zymogen in plasma which upon activation by the thrombin-thrombomodulin complex down-regulates the coagulation cascade by degrading cofactors Va and VIIIa by limited proteolysis. In addition to its anticoagulant function, activated protein C (APC) also binds to endothelial protein C receptor (EPCR) in lipid-rafts/caveolar compartments to activate protease- activated receptor 1 (PAR-1) thereby eliciting antiinflammatory and cytoprotective signaling responses in endothelial cells. These properties have led to FDA approval of recombinant APC as a therapeutic drug for severe sepsis. The mechanism by which APC selects its substrates in the anticoagulant and antiinflammatory pathways is not well understood. Furthermore, two negatively charged residues on the opposite side of the active-site of APC on a helical structure have been demonstrated to determine the specificity of the PAR-1 recognition in the cytoprotective pathway[5].

The present context found a lower protein C level among DVT patients (70.02±23.134) as compared with the control group (83.04 ± 11.975). This difference was significant (p = 0.000). Our finding is in agreement with Ridker and his colleagues (1995)[6]; and with Andrzej and his co-workers (2011)[7]. This result indicates that low level of protein C is a risk factor for developing DVT.

Conclusion: Deep venous thrombosis was significantly associated with low protein C level among test group patients.

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