Evaluation of Protein C and Protein S in Young Patients with Thrombosis

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Abstract

Objective: The current study aims to evaluate protein C and protein S levels in young patients with thrombosis and compare our results with others in surrounding countries.

Methods: Themeasurement of protein C, total protein S, and free protein S were done for one hundred young patients (younger than thirty years) who had thromboembolic disease either deep venous thrombosis (DVT), pulmonary embolism (PE), acute myocardial infarction (AMI), or stroke who were referred to Ibn-Sina and Al-Salam Teaching Hospitals in Mosul between December 2009 and December 2011. The diagnosis was confirmed by ultrasound with Doppler, magnetic resonance imagining (MRI), electrocardiography (ECG), cardiac enzymes, and angiography according to the case. Family history was taken to establish a familial occurrence of thrombosis. The measurement was done by enzyme linked immunoassay using kits from HELENA.

Results: Protein C deficiency was detected in 4 cases (4%), female to male ratio was 3:1, and their ages ranged from 16 to 28 year with a mean of 21 years. About 50% of the protein C deficient patients were presented in the form of deep venous thrombosis, 25% as stroke and 25% as acute myocardial infarction. Free protein S deficiency was detected in 6 cases (6%), with female to male ratio of 1:1. Their ages were in the range of 14-30 years with a mean of 22 years. About 33.3% of the protein S deficient subjects had repeated deep venous thrombosis, 33.3% had pulmonary embolisms, 16.7%had strokes, and 16.7%had deep venous thrombosis and pulmonary embolisms.

Conclusions: It appears from this study that protein C and protein S deficiency play a role in young patients with thromboembolic disease. Screening tests for PC and PS should be done in young subjects less than thirty years with thromboembolic disease in our locality because the diagnosis of these deficiencies has a clinical implication for the prevention of recurrent thromboembolic illness. The incidence was comparable to the surrounding areas.

Keywords: Thrombosis, Protein C, Protein S.

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Introduction

Inherited thrombophilia is a genetic tendency to thromboembolism, the most common inherited abnormalities of natural anticoagulants associated with venous thrombosis at young age are deficiency of protein S (PS), protein C (PC) and anti-thrombin III.[1-5] Hereditary deficiency of these inhibitors leads to a change in the balance

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between pro-clotting and anti-clotting making these patients more susceptible to thromboembolism. Both PC and PS are vitamin K dependent proteins and are inherited as autosomal dominant. The most common of these deficiencies is PS which circulates in free form and in an active complex withC4 binding protein. It has a functional activity as a co-factor for activated PC in proteolytic degradation of activated coagulation of FV and F VIII.[6,7] Thus, the assessment of such cases for PS deficiency includes the measurement of the level or activity of free PS.[8] Although PS deficiency is uncommon in the general population, it is detected approximately in 2% of unselected patients and 1-13% of patients with venous thromboembolism (VTE).[9,10]

The role of activated PC is the proteolytic inactivation of FVa activated PC (APC) cleaves and inactivates F VIIIa, but the rate of APC inactivation of FVa and FVIIIa are enhanced by PS.[11,12] Patients with inherited (PC) deficiency may have provokedor unprovoked thrombosis. Protein S deficiency has similar clinical symptoms with PC but thrombosis mostly occur before the age of 35 years and unprovoked VTE is less frequent.[13,14] The deficiency of these natural anticoagulants is reported to occur in less than 1% of the general population.[15,16,17] Depending on the nature of the inherited defect, the spectrum of clinical complication varies from mild to severe venous thrombosis.[18]

Materials and Methods

Aim of the Study

To evaluate the frequency of PC and PS deficiency in young patients with thrombosis in Mosul, Iraq and to compare our results with the neighboring countries.

Subjects (Control)

The normal ranges (mean ±SD) of PC and PS, total and free, were determined in 50 healthy blood donors who had no family history of thromboembolism, were not pregnant, and had not been on oral contraception for three months prior to the test.

Patients

Patients were included in the study if they were less than thirty years of age with confirmed venousor arterial thromboembolic disease (DVT, PE, AMI, or stroke) confirmed by ultrasound with Doppler, MRI, ECG, cardiac enzymes, and an angiography according to the case. Subjects had to have a positive family history with a thrombotic episode occurring in more than 2 members of the family.

Methods

All blood samples were collected between December 2009 and December 2011, before the administration of heparin and warfarin therapy. Venous blood was collected in a polypropylene tube with 3.8% trisodium citrate and was immediately centrifuged at 12500xrpm in an Eppendorf micro centrifuge 3200 for 3 min at 22^{o}C. The plasma was immediately a liquoted and frozen. An enzyme linked immunoassay method (Helena Laboratories, Beaumont, Texas USA)was used for the determination of PC, total PS, and free PS.

The mean, 2±SD was determined for PC and PS in both controls and patients. Protein S deficiency was diagnosed if the total PS antigen level was< 50% and the free PS level <60%. Protein C deficiency was defined by a reduced level of PC antigen < 70%.

The deficiency was considered inherited if it was confirmed by the measurement of a second

sample that was collected three months later while the patient was off anticoagulant for at least one week.

Results

A total of 100 patients (65 females and 35 males) with confirmed thromboembolic disease were included. As shown in table 1, a total of 10 patients were found to have an inherited deficiency; of these, four patient shad PC deficiency and six patients had PS deficiency.

In protein C deficiency, deep venous thrombosis developed in 2 patients, stroke in 1 patient, and acute myocardial infarction in 1 patient.

In Protein S deficiency, deep venous thrombosis appeared in 2 patients, pulmonary embolism in 2 patients, stroke in 1patient, and recurrent deep venous thrombosis and pulmonary embolism in 1 patient.

The mean level of PC in deficient cases was 34% and the free PS in deficient cases was 40.2%. These were significantly lower as compared to the control group with P values of0.001 and 0.05, respectively. All patients with PS deficiency had a type I deficiency (decreased total and free PS antigen).The plasma levels of PC and PS of the control in comparison to the normal reference range is shown in table (2).

Table (1): Clinical findings of PC and PS deficient cases

Type of deficiency	***Gender***	***No. of cases***	***Age Year***	***%***	***Level of PC and free PS%***	***Clinical presentation***
PC	*Female**	*2*	*22* *26*	*50*	*32* *36*	*Deep venous thrombosis*
	*Female***	*1*	*16*	*25*	*28*	*Stroke*
	*Male****	*1*	*28*	*25*	*40*	*Acute myocardial infarction*
PS	*Female***	*2*	*27* *21*	*33.3*	*30* *38*	*Recurrent deep venous thrombosis*
	*Male****	*2*	*25* *16*	*33.3*	*45* *22*	*Pulmonary embolism*
	*Female**	*1*	*14*	*16.7*	*18*	*Stroke*
	*Male****	*1*	*30*	*16.7*	*40*	*Deep venous thrombosis andpulmonary embolism*

* *positive family history of DVT in 2 members of their family*

** *positive family history of stroke*

*** *Positive family history of DVT and pulmonary embolism.*

The remaining ninety patients with thromboembolic disease and normal PC and PS level, presented with DVT in 62 (68%) cases, with PE in 18 (20%) cases and AMI in 5 (12%) cases.

Table (2): The plasma levels of PC, PS of the control in comparison with normal reference range

	Mean ±2 SD of patients with no PC or PS deficiency	***Normal reference range%***	***Range of control %***
PC	*107± 16.3*	*70-130%*	*70-124%*
TPS	*78 ± 15.6*	*50-140%*	*50-133%*
FPS	*67 ± 10.5*	*60-140%*	*60-132%*

Discussion

The lifetime risk of thromboembolic disease is 2 fold higher in individuals with deficiency of PC, PS, or both compared with non-deficient individuals. The risk of thromboembolic onset in deficient subjects, on average, is eleven years younger compared with non-deficient subjects.[19,20,21] Two large studies of consecutive patients with deep venous thrombosis have found a prevalence of PS deficiency of (**1%-2**%).[22,23]

Awidi et al. conducted a four-year prospective study on patients admitted with thromboembolic disease in Jordan and found PC and PS deficiency in 7.8% and 6.9%, respectively.[24]

Eid studied 602 patients in Jordan with thrombotic events and found the prevalence of hereditary PC and PS deficiency 3.8% and 2.3%,respectively.[25]

Al-Jaouni conducted a retrospective analysis in 179 consecutive Saudi patients with recurrent venous thrombosis at a young age with or without positive family history and found PS deficiency in 14.5% and PC deficiency in 8.4%.[26]

In this study (the first study which has been done in Iraq), the incidence of PC deficiency was comparable to the incidence in Jordan found by Eid,[25]but lower than the incidence in Saudi Arabia. The incidence of PS deficiency was comparable to the incidence in Jordan by Awidi et al.[24]

In another study on 680 consecutive patients with his toryof venous thrombosis, the prevalence of protein C deficiency was 2.5%, protein S deficiency was 1.3%, and combined deficiency was 0.4%.[27]

In the study by Miljic et al, the frequency of protein C deficiency was 4.1% and protein S deficiency was 1.6%.[28]

In another study conducted in Israel, they found the frequency of protein C and protein S deficiency were 5.6% and 2.8%, respectively.[29]

In a study conducted in Taiwan,85 consecutive and unrelated patients with unexplained thrombophilia were studied, and relatively higher prevalence of protein C and protein S deficiency was found.[30]

Khalid et al. stated that the incidence of protein C and protein S deficiency was2.3% and 1.4%, respectively.[31]

The prevalence of protein C and protein S deficiencies in the Trakya region of Turkey was found to be higher than in other reported studies.[32]

We noticed recurrent episodes of thrombosis in our cases. It is important to note that the recurrence rate in our cases was lower than in those reported in other studies because of the prophylactic therapy. However, spontaneous recurrence of thrombosis can be expected in almost (50%) of patients in whom their first thrombotic episode occurred after a triggering event.[33]

Conclusion

Protein C and Protein S deficiency play a role in young patients with thrombosis. Screening tests for PC and PS should be done in young subjects less than thirty years with thromboembolic disease in our locality because the diagnosis of these deficiencies has clinical implications for the prevention of recurrent thromboembolic illness.

The incidence of PC and PS deficiency were comparable to those of neighboring countries.

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تقييم مستوى بروتين C وبروتين S في المرضى الشباب الذين يعانون من التخثر

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الملخص

الهدف: تهدف هذه الدراسة إلى تقييم مستوى بروتين C وبروتين S في المرضى الشباب الذين يعانون من التخثر ومقارنة نتائج هذه الدراسة في العراق مع دراسات أخرى.

الطريقة: شمل البحث قياس مستوى بروتين C، بروتين S الكلي والحر في مائة من المرضى الشباب دون عمر الثلاثين الذين قد ثبت تشخيصهم بأنهم يعانون من تخثر وريدي أو شرياني وراقدين في مستشفى ابن سينا التعليمي ومستشفى السلام التعليمي في الموصل خلال الفترة بين كانون الأول 2009 وحتى كانون الأول 2011، وتم القياس بواسطة المقايسة المناعية المرتبطة بالانزيم (ELISA).

ويشمل البحث أيضاً تحديد مستوى بروتين C، بروتين S الكلي والحر في خمسين من الأشخاص من الأصحاء كمجموعة ضابطة مقاربة بالنسبة للعمر للفئة العمرية للمرضى مع الأخذ في الاعتبار بالنسبة للإناث (عدم وجود حمل أو استعمال أي من حبوب منع الحمل خلال الأشهر الثلاثة الماضية قبل إجراء الفحص).

النتائج: تم الكشف عن نقص في بروتين سي في 4 حالات بنسبة (4%) ونسبة الإناث إلى الذكور (3: 1) تراوحت أعمارهم بين 16-28 سنة (وسطي 23 سنة) وكان (50%) من حالات التخثر هي بشكل تخثر وريدي عميق.

تم أيضاً الكشف عن نقص في بروتين S الكلي والحر في 6 حالات بنسبة (6%) ونسبة الإناث إلى الذكور (1:1) وتراوحت أعمارهم بين 14-30 سنة (وسطي 22 سنة) وكان (33.3%) من الحالات تعاني من تكرار التخثر الوريدي العميق و(33.3%) لديها تخثر رئوي ثم (16.7%) لديها طارئة دماغية وعائية والنسبة نفسها (16.7%) لديها تخثر وريدي عميق مع تخثر رئوي.

الاستنتاجات: هذه الدراسة تبين اهمية التحليل المنهجي لعينات رأب الثدي النسيجية للمساعدة في تحديد وتوجيه العلاج للمريضات اللواتي لديهن خطر متزايد للإصابة بسرطان الثدي بعد رأب الثدي التصغيري، ومن ثم يجب تأكيد أخذ عينات شامل والتعامل مع عينات رأب الثدي.

الكلمات الدالة: التخثر، بروتين (C)، بروتين (S).