Original paper

Protein C Deficiency in Patients with Retinal Vein Thrombosis

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Abstract

Introduction: Retinal vein occlusions are the most common retinal vascular disorder after diabetic retinopathy with several ocular and systemic disorders associated with retinal veins thrombosis. Protein C deficiency as part of inherited thrombophilia due to deficiency of natural anticoagulant may associated with retinal vein thrombosis.
The aims of this study were to measure the Protein C concentrations in patients with retinal vein thrombosis and to evaluate its role in the etiology of those patients.
Patients, materials and methods: During a period of 6 months a total number of 42 patients who were diagnosed with retinal vein thrombosis while attending the out patient clinic in Karbala, Iraq. From each patient appropriate amount of venous blood was withdrawn and estimated for plasma protein C level, Complete blood count, blood film, ESR, Kaolin clotting time, anticardiolipin antibodies, antinuclear antibody, TSH, blood urea, serum creatinin, random blood sugar and serum cholesterol. For control group individual only protein C estimation was done.
Results: Forty-two patients with retinal vein thrombosis were investigated. There were 22 females and 20 males and a mean age of 48 ± 2 years with no significant statistical differences in mean age and sex between the patients and the control group. Decreased levels of PC were found in six (14%) of the patients, and a statistically significant difference was seen for PC concentration between the patients and the control groups.
Conclusions: Deficiency of natural anticoagulant proteins, especially protein C, may play a role in the etiology of retinal vein thrombosis and measurement of these parameters with proper prophylaxis especially in young patients may be useful in prevention of venous thrombosis.
Key words: Protein C deficiency, retinal vein thrombosis.

Introduction

Retinal vein occlusions are the most common retinal vascular disorder after diabetic retinopathy and an important cause of visual loss [(1,2)]. Several ocular and systemic conditions have been reported in association with retinal vein thrombosis. These systemic risk factors are hypertension, diabetes mellitus, smoking, obesity, hyperlipid-aemia, autoimmune disorders, chronic renal failure, hypothyrodism medication with oral contraceptive pills and diuretics [(3,4)]. Hematological disorders include; multiple myeloma, cryoglobulinemia, leukemias, lymphomas, Waldenstrom macroglobulinemia, polycythemia vera, and sickle cell disease [(5)] in addition to and abnormalities of hemostatic factors and blood viscosity including hereditary and acquired deficiencies of protein C (PC), protein S (PS) and antithrombin III (AT III), mutations that increase tendency to thrombosis such as factor V Leiden and prothrombin 20210 A are important risk

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factors of thrombosis and were reported in association with retinal vein thrombosis, especially in young patients [6,7].

Protein C is a vitamin K-dependent coagulation protein that serves a critical role in the regulation of thrombin. Protein C is synthesized in hepatocytes and circulates in plasma in a very low concentration. Plasma protein C is activated after complex formation with thrombin on the endothelial cell receptor thrombomodulin; this activation is facilitated by protein C binding to the endothelial protein C receptor (EPCR). Activated protein C (APC), augmented by protein cofactors (protein S and factor V) and lipid cofactors, cleaves critical sites in the activated procoagulant factors V and VIII, thus inactivating these enzymes [8].

Patients with protein C deficiency have a decreased capacity to down-regulate the propagation of thrombin generation by factor Va and VIIIa once they have been activated by the small amounts of thrombin generated in the initiation phase of coagulation activation. There are two main types of protein C mutations that lead to protein C deficiency: Type I of quantitative defects of protein C (low production or short protein half life) and Type II of qualitative defects, in which interaction with other molecules is abnormal. Defects in interaction with thrombomodulin, phospholipids, factors V/VIII and others have been described. Both types of no thrombotic risk differences [10].

Inherited heterozygous PC deficiency is found in 2–4% of first-episode thromboses in unselected patients and 5–7% of all recurrent thromboembolic episodes in young adults [9, 11].

Protein C deficiency may be acquired and caused by increased consumption like overt DIC or by decreased synthesis of the active carboxylated protein (e.g. administration of vitamin K antagonists, severe hepatic synthetic dysfunction, complications of prematurity). Rarely, antiphospholipid antibodies (APA) may also cause acquired protein C deficiency via antibody-mediated clearance. Protein C level unchanged in pregnancy [12].

The clinical phenotype of simple heterozygous protein C deficiency, characterized by mild deficiency in measured protein C activity, can range from asymptomatic to a potent thrombophilic state with recurrent thromboses. In addition to DVT and PE, the patients with heterozygous protein C deficiency may develop ischaemic arterial stroke, mesenteric thrombi and pregnancy-associated thrombosis. Patients with a significant positive family history, multiple thrombophilia traits, APA, or underlying inflammatory disorders are more likely to develop thrombotic manifestations, while more benign personal and family histories are often characterized by mild protein C deficiency as a single thrombophilic defect [13].

Authors employ a nomenclature of mild protein C deficiency to indicate activity levels greater than 20 IU/dL but below the age-appropriate lower limit of normal values (65-140 IU/dL), moderately-severe protein C deficiency as activity levels in the range of 1–20 IU/dL, and severe deficiency for activity levels less than 1 IU/dL [14].

The aims of this study was to measure the PC concentrations in patients with retinal vein thrombosis and to evaluate its role in the etiology of those patients.

Patients, materials and methods

Patients group: During a period of 6 months extended from April 2014 to September 2014, a total number of 42 patients who were diagnosed as patient with retinal vein thrombosis (central and branch retinal vein thrombosis) confirmed by suggestive clinical history and fundscopy examination done by ophthalmologist while attending the out patient clinic in Karbala, Iraq.

Inclusion criteria include all patients with newly diagnose retinal vein thrombosis with or without systemic disease, while exclusion done for patient with establish thrombosis on anticoagulant therapy.

A detail history and clinical information collected in data sheet including; patients name, age, sex, occupation, address, history of hypertension, hyperglycemia, hypercholesterolinemia, cardiovascular disease, renal failure, hypothyrodism, autoimmune diseases, local ophthalmological causes, drug history, personal and family history of thrombosis.

Control group: The control groups for this study were 47 healthy blood donor subjects without personal or family history of thromboembolic diseases or known systemic disorders, ages and sex matched.

Methods: From each patient group individual, appropriate amount of venous blood was withdrawn and divided into 3 aliquots for proper tubes including citrated tube for coagulation studies, EDTA tube for hematological tests and plain tube for immunological and biochemical tests.

- *Coagulation tests:* These were performed on platelet poor plasma (PPP) within two hours of sampling in the same day using commercially available kits for protein C [(15)] and KCT and KCT index estimation [(16)].
- *Hematological tests:* Complete blood count (Hematology autoanalyser, Swelab- Italy), blood film and ESR.
- *Immunological and biochemical investigation:* These were performed on patient serum for estimation of aCL antibodies (IgM and IgG) and antinuclear antibody using commercially available kits (Orgentec-Diagnostika/ Germany), TSH [(15)], blood urea, serum creatinin, fasting blood sugar and serum cholesterol.
- *Urine analysis.*

For control group individual only protein C estimation was done.

Biostatistical analysis: The results were expressed as (mean ± standard deviation). Pooled t-test was used for the comparison of significant difference between the patients and control groups in the measured parameters. Statistical significant was defined as a P value < 0.05.

Results

Forty-two patients with retinal vein thrombosis were investigated. There were a total of 22 females (52%) and 20 males (48%) with a mean age of 48 ± 2 years. There were 25 females (54 %) and 22 males (46%) with a mean age of 50 ± 4 years in the control group. There were no significant statistical differences in mean age and sex between the patients and the control group.

Eight of the patients had diabetes mellitus, four patient with hypercholesterolinemia and seven had hypertension. Two patients had history of venous thrombosis. Some patients had more than one of these diseases.

Twenty eight of the 42 retinal vein thrombosis cases were found to have none of the systemic diseases especially renal failure or autoimmune disorder or antiphospholipid syndrome and no patients with local ophthalmological causes of thrombosis. Table 1 showing patient characteristics, while table 2 and 3 document the reduced plasma levels of PC in the patients.

Decreased levels of PC concentration were found in six (14%) of the patients, and a statistically significant difference was seen for PC concentration between the patients and the control groups.

Reduce PC concentration level was found in six patients, five of them under age of 50s, and none of them had abnormalities in the systemic investigations and other laboratory findings, with M:F ratio was 1:1. Two of them had history of venous thrombosis.

Table 1. Patients' characteristics in retinal vein thrombosis.

Characteristics of patients	*No (%)*
Mean age	48 ± 2
Male	20/42 (48 %)
Female	22/42 (52 %)
Hypertension	7/42 (18%)
DM	8/42 (19 %)
Hypercholesterolinemia	4/42 (9 %)
Renal failure	---
Hypothyrodism	---
Antiphospholipid syndrome	---
Autoimmune disease	---
History of thrombosis	2/42 (5 %)
Normal systemic findings	28/42 (66%)
Local eye causes of thrombosis	---

Table 2. Reduced PC levels in the patients and control groups.

	Patients	*Controls*
Protein C deficiency	6/42 (14 %)	1/47 (2 %)

Table 3. Reduced PC levels in the six patients.

Range of protein C (%)	*Number of cases with deficiency of PC*
0-20 %	0
21-40 %	3
41-60 %	3
60-65 %	0

Discussion

Although several ocular and systemic risk factors were reported, exact etiology and pathogenesis of retinal vein thrombosis still remains unclear. Various haemostatic factors have been implicated in retinal vein thrombosis, for example the reduced PC, PS and AT III levels. The thrombin-thrombomodulin complex activates PC. Activated PC inhibits the blood coagulation cascade by selective degradation of the procoagulant factors, Va and VIIIa. PS acts as a cofactor in the PC catalyzed inactivation of factor Va [(17)].

Deficiency of PC, PS and AT III may result in a hypercoagulable state. Heterozygous PC deficiency represents an important risk factor for thrombosis. Thrombophilia in clinically dominant protein C-deficient families is probably caused by the combined action of heterozygous PC deficiency and an additional risk factor [(12)].

In this study, PC concentrations were measured in patients with retinal vein thrombosis, found to be low in six (14%) of the patients and only in one control person with statistical significantly associated with retinal vein thrombosis patients than control group, which come in similar with findings of **Oya Tekeli 1999.**

Reduce PC level was found in six patients, majority in the young age group less than the age of 50s, without abnormalities in the systemic investigations and other laboratory findings. Two of them had history of venous thrombosis. So many cases with thrombosis especially in young patients without any associated or explained systemic factors for thrombosis may related to defective coagulation pathways in inherited thrombophilia or in combination manner with other risk factors. In addition patients with thrombosis at unusual sites like retina vein thrombosis should searching for inherited thrombophilia like PC, PS, AT III, Factor V Leiden mutation and prothrombin mutation which increase the possibility of

recurrent thrombosis at usual or unusual sites.

Conclusions

Deficiency of natural anticoagulant proteins, especially protein C, may play a role in the etiology of retinal vein thrombosis and measurement of these parameters and proper prophylaxis especially in young patients may be useful in preventing venous thrombosis. It recommended to investigate every patients with retinal vein thrombosis for protein C plasma level in addition to other important inherited thrombophilic factors like protein S, antIthrombin III, Factor V Leiden mutation.

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