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Dysfunctional fibrinolysis and cerebral venous thrombosis

Aniket Prabhudesai[a], Shrimati Shetty[a], Kanjaksha Ghosh[b], Bipin Kulkarni[a,*]

[a] National Institute of Immunohaematology (ICMR), Department of Thrombosis and Haemostasis, KEM Hospital, Parel, Mumbai, India
[b] Surat Raktadan Kendra & Research Centre, Regional Blood Transfusion Centre, Surat, Gujarat, India

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ABSTRACT

Cerebral venous thrombosis (CVT) is an uncommon neurological disease with high morbidity and mortality. Even after extensive thrombophilia screening, majority of the thrombosis cases remain with unknown etiology. Hypofibrinolysis due to acquired or congenital deficiencies or abnormalities in factors in the fibrinolytic cascade is a known cause of thrombosis at any site. In the present study 104 cases of radiologically confirmed CVT cases were investigated for the conventional thrombophilia along with factors in the fibrinolytic cascade to find a possible etiology for the clinical manifestation. Conventional thrombophilia markers which included PC, PS, AT and FVL mutation were detected in 16.3% of the patients. Approximately 19% cases had grossly elevated plasma PAI-1 levels. PAI-1 4G/4G genotype was found to be strongly associated with high PAI-1 levels. 2.9% cases had reduced tPA levels, 1.9% had plasminogen deficiency and 1.9% cases had increased alpha-2-antiplasmin levels. Along with conventional thrombophilia, dysfunctional fibrinolysis is found to be strongly associated with CVT. Understanding the role of risk factors is important for appropriate treatment of this serious disorder.

1. Introduction

Cerebral venous thrombosis (CVT) is a rare cerebrovascular disease which is multifactorial in nature with an annual incidence of 1.32 cases per 100,000 person-years [1]. CVT is difficult to diagnose due to varied clinical presentation and etiologically heterogeneous factors [2]. The onset of CVT may be acute, sub-acute or chronic. Mortality rate has been brought down due to immediate initiation of anticoagulation therapy in diagnosed CVT patients. Presence of multiple risk factors including hereditary thrombophilia markers i.e. protein C (PC), protein S (PS), antithrombin III (AT), factor V Leiden (FVL) mutation, prothrombin G20210A mutation and acquired or transient risk factors such as pregnancy, puerperium, antiphospholipid syndrome, trauma, surgery, cancer, exogenous hormones, oral contraceptive use, inflammation, infection and hyperhomocysteinemia is known to account for CVT [3–5]. However, these multiple risk factors account for a fraction of these cases and many cases still remain unexplained despite of a strong family history or recurrence.

Fibrinolysis plays an important role in dissolution of blood clots formed in response to tissue injury. Fibrinolytic system consists of activators and inhibitors of plasminogen and their molecular interplay regulates fibrinolysis. Dysfunctional fibrinolysis or hypofibrinolysis is a state wherein there is decreased clearance of blood clots thereby leading to a state of thrombosis. A clear association has been established between hypofibrinolysis and risk of venous thrombosis in the Leiden Thrombophilia Study [6].

2. Materials and methods

One hundred and four patients with a proven diagnosis of CVT based on magnetic resonance imaging (MRI) combined with magnetic resonance venography (MRV), and/or computed tomography (CT) venography from a tertiary care centre in Mumbai were included in the present study. This is a prospective study which included (a) patients in acute/subacute/chronic phase of CVT who were not started on anticoagulants and (b) patients who had stopped anticoagulation after completing at least three months of anticoagulation therapy. Blood samples obtained from patients who had received anticoagulation ranged from 4 months to 13 years relative to CVT (average: 1 year 11 months). The study was approved by the Institutional Ethics Committee Review Board in accordance with declaration of Helsinki. A written informed consent was obtained from all the participants. Patients were initially screened for thrombophilia markers PC, PS, AT and FVL mutation using standard procedures. Normal range for PC, PS,

Abbreviations: CVT, cerebral venous thrombosis; PAI-1, plasminogen activator inhibitor − 1; PC, protein C; PS, protein S; AT, antithrombin III; FVL, Factor V Leiden; tPA, tissue plasminogen activator; PLG, plasminogen; A2-AP, alpha-2-antiplasmin; APLA, antiphospholipid antibodies; LA, lupus anticoagulant

* Corresponding author at: Department of Haemostasis and Thrombosis, National Institute of Immunohaematology (ICMR), 13th Floor, New MS Building, KEM Hospital Campus, Parel, Mumbai 400 012, India.
E-mail address: bipinp.kulkarni@gov.in (B. Kulkarni).

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and AT was 70–140% and all patients were diagnosed as PC, PS, or AT deficiency if the values were below 60% [Diagnostica stago, Asnires, France]. Fibrinolysis markers were studied using commercial ELISA kits. [Plasminogen (PLG), Boster biological, CA, USA; tissue plasminogen activator (tPA), Assaypro, MO, USA; plasminogen activator inhibitor-1 (PAI-1), Molecular Innovations, MI, USA; alpha-2-antiplasmin (A2-AP), Bioassay technologies, Shanghai, China]. Normal ranges were set for fibrinolytic parameters on studying 50 healthy controls. Patients were diagnosed with high PAI-1 if values were above 110 ng/ml, with reduced tPA if values were below 0.75 ng/ml, with plasminogen deficiency if values were below 300 μg/ml and with high A2-AP if values were above 200 ng/ml. Homocysteine levels were measured by chemiluminescence immunoassay [normal range: 3.7–13.9 μmol/l, above which was considered hyperhomocysteinemia]. Lupus anticoagulant screening was done using LA1 and LA2 reagents (Siemens Healthcare GmbH, Erlangen, Germany). FVL mutation [rs6025] was studied using PCR-RFLP method (*MnlI* digestion) and PAI-1 4G/5G promoter polymorphism [rs1799768] was studied using allele specific PCR.

3. Results

Mean patient age in the study was 32.7 ± 11.8 years. Study included sixty four (61.5%) male patients and forty (38.5%) female patients with a mean age of 34.3 ± 11.7 and 30.9 ± 11.9 years respectively. Thrombosis was predominantly seen in superior sagittal sinus (60.4%), followed by transverse sinus (57.3%), cortical veins (24%), extending to internal jugular veins (12.5%), straight sinus (10.4%), vein of Galen and internal cerebral veins (8.3%) and cavernous sinus (2.1%). The major clinical manifestations were headache (56.4%), convulsions (38.5%), blurring of vision (15.4%), vomiting (12.8%), altered consciousness (10.2%), hemiparesis (7.7%), vertigo and migraine (5.1%).

Hereditary thrombophilia was present in 17 (16.3%) cases. Seven (6.7%) cases were PC deficient; four (3.8%) were PS deficient (all females). AT deficiency was seen in one (1%) case. None of the patients had a combined deficiency of the natural anticoagulants in the present study. Five (4.8%) cases had heterozygous FVL mutation. APLA (antiphospholipid antibodies) and LA (lupus anticoagulant) were present in 6 cases (5.8%).

Twenty (19.2%) patients had hyperhomocysteinemia. Fourteen (13.5%) patients had high CRP levels (> 10 mg/l). Seven (6.7%) patients had hypertension, 17 (16.3%) had trauma (recent history of fall/head injury/accident/surgery); 2 (5%) patients had history of pregnancy loss and pre-eclampsia while 2 (5%) were on oral contraceptive pills. Three (2.9%) patients had tuberculosis and two (1.9%) had suffered from typhoid fever. Two patients (1.9%) had polycythemia vera.

Of all the fibrinolytic markers studied, 20 (19.2%) patients had grossly elevated levels of PAI-1 levels. High PAI-1 is found to be a risk factor for CVT ($P = 0.0182$, OR = 11.6) in the present study. High plasma PAI-1 level has been attributed to the 4G allele of 4G/5G promoter polymorphism [7]. In our study, 19 (18.2%) patients had a homozygous 4G/4G PAI-1 promoter polymorphism. Patients with a 4G/4G genotype had significantly high mean of 117.6 ± 83.6 ng/ml PAI-1 level when compared to those with 4G/5G genotype (mean: 62.5 ± 42.1 ng/ml) and 5G/5G genotype (mean: 53.3 ± 40.7 ng/ml) ($P = 0.0103$, Kruskal Wallis test, Fig. 1). Three (2.9%) patients had reduced tPA levels. Two (1.9%) cases had plasminogen deficiency. Two (1.9%) cases had increased A2-AP. Demographic and laboratory data of CVT patients of the present study along with the scatter plots is shown in Table 1 and Fig. 2.

Presence of multiple risk factors was seen in five cases (4.8%) [Table 2]. Two patients with a history of familial thrombosis were enrolled in the study. Of these, first patient had PS deficiency along with homozygous 4G/4G PAI-1 polymorphism and high PAI-1 levels.

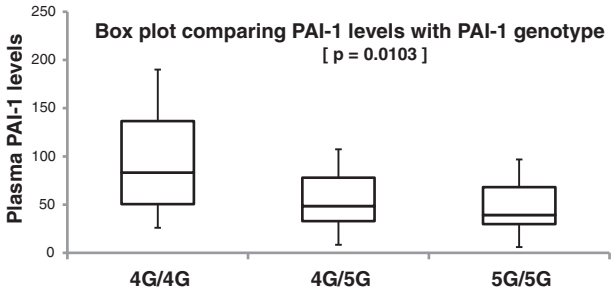

Fig. 1. Comparison of PAI-1 level with genotypes of PAI-4G/5G promoter polymorphism. There is a significant difference of PAI-1 (plasminogen activator inhibitor-1) levels observed in 4G/4G when compared to 4G/5G and 5G/5G genotypes ($P = 0.0103$, Kruskal Wallis test).

Table 1
Demographic and laboratory data of CVT cases in the present study.

CVT cases	104
Age (years)	32.7 ± 11.8
Sex	
Male	64 (61.5%)
Female	40 (38.5%)
Hereditary thrombophilia	
PC deficiency (< 60%)	7 (6.7%)
PS deficiency (< 60%)	4 (3.8%)
AT deficiency (< 60%)	1 (1%)
FVL mutation	5 (4.8%)
Hypofibrinolysis	
High PAI-1 (> 110 ng/ml)	20 (19.2%)
PAI-1 4G/4G polymorphism	19 (18.2%)
Reduced tPA (< 0.75 ng/ml)	3 (2.9%)
PLG deficiency (< 300 μg/ml)	2 (1.9%)
High A2-AP (> 200 ng/ml)	2 (1.9%)
Acquired or transient risk factors	
Hyperhomocysteinemia	20 (19.2%)
Trauma	17 (16.3%)
High CRP (> 10 mg/l)	14 (13.5%)
Hypertension	7 (6.7%)
APLA/LA	6 (5.8%)
Tuberculosis	3 (2.9%)
Typhoid	2 (1.9%)
Oral contraceptive use	2 (5%)[a]
Pre-eclampsia	2 (5%)[a]
History of pregnancy loss	2 (5%)[a]
Polycythemia vera	2 (1.9%)

Abbreviations: PC: protein C; PS - protein S; AT: antithrombin III, FVL: Factor V Leiden, PLG - plasminogen, PAI-1: plasminogen activator inhibitor-1, tPA - tissue plasminogen activator, A2-AP: alpha-2-antiplasmin, APLA: antiphospholipid antibodies, LA: lupus anticoagulant, CRP: C-reactive protein.

[a] Risk factors in females alone (out of forty).

Second patient had PC deficiency and hyperhomocysteinemia. His elder brother (also diagnosed as PC deficiency) had an episode of deep vein thrombosis.

4. Discussion

In the present study it was seen that 16.3% CVT cases had a hereditary thrombophilia marker. PC deficiency was the most prevalent thrombophilia marker seen in 6.7% CVT cases. PS and AT deficiency was noted in 3.8% and 1% cases respectively. Prevalence of PC, PS and AT deficiencies has been reported to range between 0%–20% in CVT cases in several earlier studies. The prevalence of FVL heterozygous mutation was 4.8% in the present study as against 3% - 17.3% prevalence in CVT cases reported from various countries [Table 3]. Overall prevalence of FVL mutation in Indian population is very low. Dentali F et al. have shown a strong association between CVT and FVL, Prothrombin G20210A mutation and hyperhomocysteinemia [16]. Prothrombin G20210A mutation is a risk factor associated with CVT