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Plasma *S*-adenosylhomocysteine: A potential risk marker for cerebral venous thrombosis

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ABSTRACT

Background: Despite a plethora of studies suggesting that hyperhomocysteinemia is associated with an increased risk for arterial and venous thrombosis, there is paucity of data on the role of the *S*-adenosylhomocysteine (SAH), the metabolic precursor of homocysteine (Hcy) as a risk predictor for cerebral venous thrombosis (CVT).
Method: We estimated fasting plasma concentrations of total homocysteine (tHcy), SAH and *S*-adenosylmethionine (SAM), in 185 CVT patients and 248 healthy controls, by reverse-phase high performance liquid chromatography coupled with coulometric electrochemical detection.
Results: Fasting tHcy, SAH and SAM were significantly higher in patients compared with controls. Increased tHcy and SAH concentrations were associated with 4.54-fold (95% CI, 2.74–7.53) and 35.77-fold (95% CI, 19.45–65.79) increase in risk for CVT, respectively. Receiver operating characteristic (ROC) curve analysis showed that the area under curve, sensitivity and specificity was higher for SAH compared to tHcy. Further, discriminant analysis to distinguish between tHcy and SAH showed that SAH had a significantly higher percentage classification, with lower Wilk's lambda and higher χ^2, compared to tHcy.
Conclusion: Increased plasma SAH may be a more sensitive risk marker for CVT than plasma tHcy.

1. Introduction

Plasma total homocysteine (tHcy) has been identified as an independent risk factor for vascular disorders in a variety of studies [1–5]. However, it is still unclear whether tHcy is causally involved in the etio-pathogenesis of vascular disease or whether hyperhomocysteinemia is just an indirect marker of a more complex mechanism [6]. The only intracellular source of homocysteine (Hcy) is the hydrolysis of *S*-adenosylhomocysteine (SAH) by the enzyme SAH hydrolase. This reaction is reversible and therefore increased Hcy results in increased SAH [7]. SAH is a potent inhibitor of *S*-adenosylmethionine (SAM)-dependent methyltransferases. In addition to DNA methylation, SAM-dependent methyltransferases are essential for numerous other cellular methylation reactions including synthesis of creatine, membrane phosphatidylcholine, neurotransmitters, detoxification reactions, and RNA and protein methylation [8]. Experimental studies have shown that increased tissue SAH concentrations have profound physiologic consequences [9–11] and that SAH mediates the harmful effects of Hcy in the vascular system [12–14]. The plasma concentration of SAM is 500 times less than that of homocysteine [7]. The procedure for estimation of plasma SAH is rather challenging and it is likely that this may be a reason for the relatively few studies conducted to assess the role of plasma SAH as a risk marker of vascular disease [15].

Cerebral venous thrombosis (CVT) which includes thrombosis of the cerebral veins and dural sinuses, is a potentially life threatening disorder which is rare in developed countries but relatively common in India [16]. CVT represents almost 0.5 to 3% of all strokes, and affects predominantly younger people. The etiology of CVT is multi-factorial, involving inherited and acquired factors [17]. Despite the wide range of known causes, the etiology remains undetermined in approximately 15% to 35% of cases [18]. Previous studies suggest that hyperhomocysteinemia is associated with a 4-fold increase in risk of CVT [19] and could possibly contribute to the relatively high frequency of CVT in India [4].

2. Materials and methods

2.1. Ethics statement

The study was approved by the review board of the National Institute of Mental Health and Neuro Sciences (NIMHANS), Bangalore, India, and the work was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for

Abbreviations: CVT, cerebral venous thrombosis; tHcy, total homocysteine; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine.

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experiments involving humans. Written informed consent was obtained from all participants/spouses for participation in this study.

2.2. Study participants

Patients diagnosed with CVT and treated in the Stroke Unit of NIMHANS were recruited. The diagnosis of CVT was confirmed by neuroimaging studies: magnetic resonance imaging (MRI)/computed tomography (CT) scanning, and magnetic resonance venography (MRV)/digital subtraction angiography (DSA). The type of study depended on the judgment of the treating neurophysician. Patients with CVT secondary to neuroinfection, head trauma, invasive neurosurgical procedures, malignancy, or major systemic diseases known to predispose to venous thrombosis, or with clinical and laboratory evidence of thyroid, hepatic or renal dysfunction, were excluded. Control subjects were recruited from friends of patients attending our Institute and from other hospital staff during the same period as the cases. Previous thrombosis was excluded in them by clinical history.

2.3. Assessments

The baseline clinical and demographic data and information concerning major risk factors for venous thrombosis was recorded. Hypertension was defined as diastolic blood pressure (DBP) ≥ 90 mm Hg and/or systolic blood pressure (SBP) ≥ 140 mm Hg and/or the use of antihypertensive medication. Diabetes mellitus was defined as venous plasma glucose concentration of ≥126 mg/dl after an overnight fast and/or ≥200 mg/dl 2 h after a meal or the use of insulin or oral hypoglycemic agents.

Routine blood chemistry and hematology investigations were carried out for the study participants. Other tests conducted on the patients to indicate hypercoagulable states included protein S, protein C, antithrombin III, lupus anticoagulant, and factor V Leiden mutation testing. Antinuclear antibody studies were performed to screen for systemic lupus erythematosus. For estimation of plasma tHcy, SAH and SAM, fasting venous blood samples were collected into EDTA vacutainer tubes, immediately placed on ice and after transport to the laboratory, were centrifuged at 4000 rpm for 10 min. Plasma was separated and stored at −80 °C until analysis.

2.4. Estimation of plasma tHcy, SAH and SAM

Plasma tHcy, SAH and SAM were estimated by reverse-phase high-performance liquid chromatography and coulometric electrochemical detection using a Shimadzu HPLC system (LC-10ADVP) equipped with an auto sampler (SIL-HT_A) and a ESA Coulochem III detector (ESA Inc.) [20,21]. Chromatographic separation was performed at ambient temperature at a flow rate of 1.0 ml/min and a pressure of 120–140 kg/cm^2 (1800–2100 psi), using a reverse phase C18 Supelco column (5 μ, 4.6 × 250 mm, Shimadzu). To assure precision and accuracy between sample runs, standards were injected and duplicate reference plasma standards were included. Detection of HPLC separation was achieved by the ESA Coulochem III EC detector equipped with a dual analytical cell (model 5010) and a guard cell (model-5020). The peak area analysis of analytes was provided by Class Vp software (Shimadzu Corporation, Japan) based on calibration curves generated.

2.5. Statistical methods

Statistical analysis of data was performed using the SPSS (Version 11.0) for Windows. Data of CVT patients and controls were compared using Student's *t*-test and χ^2 test. Hyperhomocysteinemia and increased SAH were defined as concentrations of plasma tHcy and SAH above the 90th percentile of tHcy and SAH value distribution in healthy controls. The estimated risk for CVT associated with analytes were expressed as odd's ratio (OR) and its 95% confidence interval (95% CI). Crude OR was calculated by simple cross-tabulation. Adjusted OR were obtained to determine the influence of other independent variables through multiple logistic regression analysis. Receiving operating characteristics (ROC) analysis has been used to find the accuracy of Hcy and SAH as risk predictors for CVT. Discriminant function analysis has been used to analyze the study parameters between control and CVT.

3. Results

3.1. Characteristics of the study participants

The study group included 185 CVT patients (60 males, 125 females) and 248 healthy controls (101 males, 147 females). There were no statistically significance difference in age and sex between patients and controls. The demographic data and risk factors are shown in Table 1. Patients and controls were from the same socio-economic background. Factors such as smoking, alcohol consumption, hypertension, diabetes and history of oral contraceptive use were more prevalent in the patient group. Headache, vomiting, limb weakness, altered sensorium, and seizures were the most commonly observed symptoms in CVT patients. All participants in the control group were clinically normal and symptom-free.

3.2. Plasma tHcy, SAH and SAM

Fasting mean concentrations of plasma tHcy, SAH, SAM were significantly higher in CVT patients compared to the control group ($p < 0.001$ with effect size (d) 0.60, 1.28, 1.14 respectively), whereas the SAM:SAH ratio was significantly lower in patients ($p < 0.001$, effect size (d) 1.14) (Table 2).

3.3. Odds ratio for tHcy and SAH

The 90th percentile of tHcy which was 15.4 μmol/l (OR 5.31 [95% CI, 3.21–8.79]), was used as the cutoff to define hyperhomocysteinemia in our study. This also coincided with previously established cut-off values for hyperhomocysteinemia (Kang et al., 1992). Among patients, a significant proportion, (41.08%), had hyperhomocysteinemia compared to controls (13.31%), $p < 0.001$. The crude OR for CVT in subjects with increased tHcy was 4.54 (95% CI, 2.84–7.26). After adjustment with the conventional risk factors for thrombosis such as age > 40 years, gender, smoking, alcohol intake, diabetes, hypertension and oral contraceptive usage, the OR was not significantly altered (4.54 [95% CI, 2.74–7.53]) (Table 3).

For SAH, the 90th percentile was 18.5 nmol/l (OR 32.54 [95% CI, 18.41–57.51]). Therefore, we used 18.5 nmol/l as the cut-off value for identifying increased plasma SAH. In the patient group, 73.51% had increased SAH values, whereas only 7.66% had increased values in controls with $p < 0.001$. The crude OR for CVT in subjects with increased SAH was 33.45 (95% CI, 18.90–59.19) (Table 3). After adjustment with the conventional risk factors there was slight positive attenuation.

Table 1
Demographic, conventional life style and vascular risk factors in the study group.

Parameters	Cases (n = 185)	Controls (n = 248)	p value
Age, years	27.45 ± 8.27	28.91 ± 10.19	NS
Sex, male, n (%)	60 (32.4)	101 (40.7)	NS
Smoking, n (%)	24 (12.9)	14 (5.6)	0.010*
Alcohol consumption, n (%)	26 (14.1)	16 (6.5)	0.013*
Hypertension, n (%)	9 (4.9)	2 (0.8)	0.011*
Diabetes mellitus, n (%)	17 (9.2)	2 (0.8)	<0.001*
H/o oral contraceptive intake, n (%)	11 (5.9)	3 (1.2)	0.011*
Platelets (×10^3/mm^3)	261 ± 43	257 ± 29	NS
Glucose (mg/dl)	90.12 ± 22.05	87.02 ± 14.85	NS
Cholesterol (mg/dl)	160.20 ± 34.85	155.96 ± 33.04	NS
Triglycerides (mg/dl)	141.21 ± 47.94	134.05 ± 63.37	NS

* p value < 0.05 is statistically significant.