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ScienceDirect
Clinical Biochemistry 40 (2007) 41–45
CLINICAL BIOCHEMISTRY

Hyperhomocysteinemia is associated with deep venous thrombosis of the lower extremities in Tunisian patients

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Received 2 May 2006; received in revised form 17 July 2006; accepted 13 August 2006
Available online 30 August 2006

Abstract

Objectives: To test the association between hyperhomocysteinemia (HHC) and deep venous thrombosis (DVT) of lower extremities in Tunisians.

Design and methods: This case–control study included 90 patients with DVT of the lower extremities and 160 healthy controls. Plasma homocysteine, vitamin B_{12} and folate were determined using immunoenzymatic methods. Logistic regression models were performed to test whether the association between HHC and DVT is independent and to precise determinants of HHC in DVT patients.

Results: Plasma total homocysteine concentrations were significantly higher in patients with DVT (17.4±11.5 μmol/L) and in patients with idiopathic DVT (15.2±6.4 μmol/L) as compared to controls (11.5±3.3 μmol/L). HHC was significantly associated ($p<0.001$) with all DVT (OR, 8.82; 95% CI, 3.96–19.6) as well as idiopathic DVT (OR, 7.40; 95% CI, 3.01–10.8). These associations persisted after adjustment for several thrombosis risk factors. In patients with DVT, HHC was related to folate and vitamin B_{12} concentrations, but neither to the type of occurrence nor to the recurrence of DVT.

Conclusion: HHC is independently associated with first DVT of lower extremities in Tunisians. Homocysteine should be assessed in patients with DVT and the effect of vitamin B supplementation should be tested among them.

Keywords: Folic acid; Deep venous thrombosis; Homocysteine; Hyperhomocysteinemia; Venous thromboembolism; Recurrent deep venous thrombosis; Vitamin B_{12}

Introduction

Venous thromboembolism is believed to be a multifactorial disease. Several genetic and acquired conditions such as coagulation factors defects, trauma, surgery, childbirth, obesity and malignancy may predispose to venous thrombosis [1]. Homocystinuria, the rare disease caused by a genetic defect of homocysteine metabolism that leads to marked elevation of plasma total homocysteine (tHcy), is characterized by frequent and premature arterial and venous thromboembolism [2]. Mild hyperhomocysteinemia (HHC), meaning mildly increased plasma tHcy (tHcy 15–50 μmol/L), is common in general population [3,4]. This condition is caused by either genetic factors (mutations of homocysteine metabolism enzymes) or acquired conditions, such as deficiencies in B vitamins, renal insufficiency and some medications. Several clinical studies support that HHC is associated with increased risk of cardiovascular disease, atherothrombotic stroke and peripheral vascular disease [5–7]. However, the association between HHC and venous thrombosis remains controversial [8–16]. This study aimed to verify whether HHC is associated with deep venous thrombosis (DVT) of the lower extremities and to identify factors associated with HHC in Tunisian patients with DVT.

Methods

Subjects

Consecutive patients presenting with DVT of the lower extremities at the Internal Medicine Department of Rabta

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doi:10.1016/j.clinbiochem.2006.08.007

hospital (Tunis) between June 2002 and June 2004 were prospectively included. Patients with the following coagulation abnormalities (protein C, protein S or anti-thrombin deficiency, resistance to activated protein C or anti-phospholipid antibodies) were excluded as well as patients with kidney, liver, thyroid, systemic or malignant diseases. A total of 90 patients (47 men and 43 women), aged 18 to 92 years, with DVT of the lower extremities were included. The control group comprised 160 apparently healthy subjects (90 men and 70 women), aged 18 to 82 years who were not diagnosed with venous thromboembolism or cardiovascular disease. Patients and controls gave their written consent to participate to the study, and approval of the hospital's ethics review board was obtained. Venous thrombosis was confirmed by ultrasonography and/or phlebography. Thrombosis was proximal in 67 patients (74.4%) and was recurrent in 20 patients (22.2%). DVT were defined as "idiopathic" when they were unrelated to obesity or recent trauma, surgery, immobilization or childbirth ($n=44$). No participant had knowingly received B vitamin supplements within the last year. Obesity was considered for subjects with body mass index (BMI) >30 kg/m^2 [17]. Dyslipemia was considered for subjects with total cholesterol >2.5 g/L and/or triglycerides >2 g/L.

Methods

Blood was collected at least 4 weeks after the thrombotic event from fasting individuals into EDTA-containing tubes. Tubes were centrifuged within the following hour at −4°C and the plasma was stored at −80°C until analysis (within 3 months). Plasma tHcy was assessed by a fluorescent polarizing immunoassay method. Plasma vitamin B_{12} and folate were determined by micro particular enzyme immunoassay and ion capture, respectively. Determinations were performed using commercial kits on an Abbott-Axsym system (Abbott Laboratories, IL, USA). The long-term ($n=20$) imprecision (CVs) were <6% for tHcy, and <10% for folate and vitamin B_{12}. Serum total cholesterol, triglycerides and creatinine were determined using commercial kits (Roche Diagnostics, Mannheim, Germany) on a Hitachi 912 auto analyzer. Clearance of creatinine was calculated by Cockroft formula [18]. HHC was defined as tHcy above the 95th percentile of the controls' values (17.8 μmol/L).

Statistical analysis

Statistical analysis was carried out using SPSS for windows 10.0 software (SPSS Inc., Chicago, IL, USA). Values were reported as means and standard deviation, or percents. The difference between groups was compared by independent-samples *t* test or Mann–Whitney test for continuous variables, and by chi-square tests for categorical variables. We calculated unadjusted and multi-adjusted odds ratio as estimate of relative risk of DVT for increased tHcy levels. Backward binary logistic regression models were applied to assess how the association between DVT and HHC depended on confounding factors and to identify factors independently associated with HHC in DVT patients.

Results

Main clinical characteristics of study subjects are summarized in Table 1. No differences were observed in sex, age, prevalence of smoking and obesity between patients and controls. Dyslipemia and diabetes were more frequent in patients group.

Plasma tHcy concentrations mean and prevalence of HHC were significantly higher in patients with all DVT as well as in patients with idiopathic DVT (Table 2, Fig. 1). No difference was observed in plasma vitamin B_{12} and folate concentrations and clearance of creatinine between patients and controls (Table 2). However, the prevalence of vitamin B_{12} deficiency (vitamin B_{12} <200 ng/L) was significantly higher in DVT patients (35.8% vs 15.1%; $p<0.001$). HHC was significantly associated with all DVT [odds ratio (OR), 8.82; 95% confidence interval (CI), 3.96–19.6; $p<0.001$] as well as with idiopathic DVT [OR, 7.4; 95% CI, 3.1–18.2; $p<0.001$]. In a logistic regression model including HHC, age, sex, smoking, obesity, dyslipemia and immobilization as dependent variables, DVT was associated with HHC (multi-adjusted OR, 9.77; 95% CI, 3.64–26.53; $p<0.001$), dyslipemia ($p=0.001$) and age ($p=0.01$).

In the DVT group, mean tHcy concentration was significantly higher in males than females and in patients with proximal DVT than patients with distal DVT, but was lower in patients with recurrent DVT than those with first DVT. However, no significant difference was observed between idiopathic and secondary DVT (Table 3).

To precise main determinants of HHC in DVT patients, we applied a backward logistic regression model adjusting on several potential confounding factors (age, sex, smoking, clearance of creatinine, plasma vitamin B_{12} and folate, obesity and dyslipemia) on type of occurrence (unprovoked or secondary), location (proximal or distal) and recurrence of DVT. In multivariate analysis, HHC was related to plasma folate and vitamin B_{12} concentrations, but not to type of occurrence, location or recurrence of DVT.

Discussion

This study showed significantly higher prevalence of HHC in patients with DVT of the lower extremities. This association

Table 1
Main clinical characteristics of study subjects

	DVT patients ($n=90$)	Controls ($n=160$)	p
Men/Women	47/43	90/70	0.53
Age*, years	50.8 (13.1)	51.4 (16.0)	0.68
Smoking, %	33.3	35.6	0.73
Obesity[a], %	13.3	14.4	0.82
Dyslipemia, %	26.7	2.5	<0.001
Diabetes, %	15.6	–	–

Data are expressed as percents or mean (standard deviation)*; DVT, deep venous thrombosis [a], body mass index >30 kg/m^2.