Hyperhomocyst(e)inemia and the Increased Risk of Venous Thromboembolism

More Evidence From a Case-Control Study

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Background: Elevation of plasma homocyst(e)ine level is an independent risk factor for arterial and venous thrombosis. We studied the degree to which hyperhomocyst(e)inemia contributes to the development of venous thromboembolism, using a retrospective case-control study design.

Methods: Cases were individuals with objectively confirmed venous thromboembolism and no history of atherosclerosis seen at the Toronto Hospital Thrombosis Clinic, Toronto, Ontario, between January 1, 1996, and July 31, 1998. Three controls were matched for every case according to sex and age within 5 years and were derived from a large community cohort. All subjects underwent assessment for fasting plasma homocyst(e)ine levels. Hyperhomocyst(e)inemia was defined as a fasting total homocyst(e)ine concentration above the 95th percentile control value.

Results: Seventy cases and 210 matched controls were included. Men and women were equally represented, and most were younger than 60 years. Among cases with venous thromboembolism, the mean (± SD) plasma homocyst(e)ine level was significantly higher than in controls (13.0 ± 6.9 µmol/L vs 9.0 ± 4.8 µmol/L, respectively; P < .001). Sixteen (23%) of 70 cases had hyperhomocyst(e)inemia compared with 10 (5%) of 210 controls (odds ratio, 5.9; 95% confidence interval [CI], 2.5-13.8). Among subjects aged 60 years or younger, the odds ratio was 4.9 (95% CI, 1.4-16.4), while for those aged 60 years or older, it was 7.3 (95% CI, 2.2-24.0). Even with the exclusion of cases showing abnormal renal function or low serum vitamin B12 or folate levels, the odds ratio remained significantly elevated at 3.3 (95% CI, 1.1-10.0).

Conclusions: We found that fasting hyperhomocyst(e)inemia is a significant risk factor for venous thromboembolic disease in patients at a thrombosis clinic. Given the magnitude of effect and consistency across these studies, it is likely that homocyst(e)ine plays a causative role in the development of venous thrombosis, and it should be considered in the workup for venous thromboembolism.

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The role of mild to moderate hyperhomocyst(e)inemia (hyper-Hcy) in the development of vascular disease has been documented and reviewed extensively. Unlike some thrombophilia defects, hyper-Hcy is associated with both venous and arterial thrombosis. In a recent meta-analysis, including 9 published studies, Ray found that hyper-Hcy was a significant risk factor for venous thromboembolism (VTE), with a pooled odds ratio (OR) of 2.95 (95% confidence interval [CI], 2.08-4.17). In the subgroup analysis, the OR appeared to be higher among patients with VTE before 60 years of age (OR, 4.13; 95% CI, 1.25-13.72).

In the current study, we examined whether hyper-Hcy is a risk factor for VTE in a thrombosis clinic population, with an effort to respond to the apparent limitations of previous studies. Specifically, we also omitted subjects with abnormal serum creatinine, folate, and vitamin B12 levels, which are known to influence plasma total homocyst(e)ine (Hcy) levels.

RESULTS

Of the 70 cases and 210 controls, men and women were equally represented, and the majority of subjects were less than 60 years of age (Table 1). Most cases had experienced either deep vein thrombosis or pulmonary embolism, of whom 13 (19%) of all cases had recurrent disease. Twenty (29%) of the cases had either activated protein C resistance or antiphospholipid antibodies as an identified thrombophilic defect, but none had both. Almost half of all cases had another predisposing factor for VTE (Table 1). The mean (± SD) plasma total Hcy level was not statistically different between male and female cases (13.2 ± 8.0 µmol/L vs 12.8 ± 6.1 µmol/L;
SUBJECTS AND METHODS

We conducted a case-control study of subjects prospectively recruited from the Toronto Hospital Thrombosis Clinic, Toronto, Ontario, between January 1, 1996, and July 31, 1998. Cases were individuals seen as outpatients with objectively confirmed VTE and no history of atherosclerosis. Venous thromboembolism was confirmed by the following techniques: for deep vein thrombosis, duplex ultrasonography and rarely venography; for pulmonary embolism, ventilation-perfusion scan; for VTE in the inferior vena cava, computed tomography with venous phase contrast or Doppler ultrasound; for VTE in the upper arm, Doppler ultrasound; and for intracranial VTE, magnetic resonance imaging. Three controls were matched for every case, according to both sex and age within 5 years, and were from a convenience sample derived from a large community cohort of ambulatory adults whose primary care physicians had ordered measurement of plasma total Hcy.11 Specific health details, including history of VTE or vitamin supplementation, or reasons for testing of controls, were not available. None of the cases were prescribed vitamin supplements, although some may have been taking over-the-counter vitamin supplements on their own.

Subjects were instructed to fast for at least 8 hours before collection of whole blood anticoagulated with EDTA; the blood was placed on ice and the plasma was separated within 2 hours of venipuncture. Plasma was frozen at −70°C until analysis. Plasma total Hcy was measured by high-performance liquid chromatography, with electrochemical detection and pulsed integrated amperometry.14,15 Serum creatinine was measured by the Jaffe method on an autoanalyzer (AU800; Olympus Diagnostic Systems, Melville, NY), while serum folate and vitamin B12 were measured by radioimmunoassay (Quantaphase II; Bio-Rad Laboratories Inc, Toronto). Activated protein C resistance16 and antiphospholipid antibodies were measured by previously described techniques17 (Sanofi ACA kit 31057; Sanofi Diagnostics, Redmond, Wash).

Mean values of plasma total Hcy were compared between cases and controls by the t test with a 2-sided P value of .05. Hyperhomocyst(e)inemia was defined as a value above the 95th percentile of controls. The crude ORs, along with 95% CIs, were calculated for cases and control groups. The calculated OR for the presence of hyper-Hcy among subjects aged 60 years or younger was measured as a secondary outcome. In a third analysis, cases with a serum creatinine level greater than 150 µmol/L (1.7 mg/dL), serum folate level less than 4.1 nmol/L, or serum vitamin B12 level less than 100 pmol/L were excluded, and the ORs were re-calculated.

Data were analyzed by the SPSS 7.5 software package (SPSS Inc, Chicago, Ill) and Instat (GraphPad version 3.0; GraphPad Software Inc, San Diego, Calif). On the basis of previously published studies, we fixed the prevalence of hyper-Hcy in the unaffected control group at 5%, and we estimated that a tripling of this value among cases with VTE, equivalent to an OR of 3.0, would be clinically important. With a 2-sided α of .05 and β of .30, and an availability of at least twice as many controls as cases, we estimated that 70 cases and 140 controls would be required for our study. Since 3 controls were available for each case, we included 70 cases and 210 controls.

The OR (and 95% CI) obtained with data pooled from this study and those previously used in Ray’s meta-analysis6 was calculated, using the random effects model of DerSimonian and Laird.8 Heterogeneity across the studies was assessed by visual inspection and statistical analysis,9 with a threshold P value of .10.

P = .78) or between male and female controls (9.1 ± 5.5 µmol/L vs 8.9 ± 4.3 µmol/L; P = .72). Among the 70 cases, the mean plasma total Hcy level (13.0 ± 6.9 µmol/L) was significantly higher than for controls (9.0 ± 4.8 µmol/L; P < .001) (Table 1). The 95th percentile for control Hcy level was 14.8 µmol/L (Figure). Of the 70 cases, 16 (23%) had total Hcy levels above this 95th percentile value, compared with 10 controls (5%) (OR, 5.9; 95% CI, 2.5-13.8) (Figure, Table 2).

With exclusion of subjects aged 60 years or older, the OR was 4.9 (95% CI, 1.4-16.4), while for those aged 60 years or older, it was higher (OR, 7.3; 95% CI, 2.2-24.0) (Table 2). On the exclusion of cases whose baseline serum vitamin B12 level was less than 100 pmol/L (262 [3%]); serum folate level, less than 4.1 nmol/L (1.64 [2%]); and serum creatinine level, greater than 150 µmol/L (1.7 mg/dL) (6/65 [9%]); the OR for VTE in the presence of hyper-Hcy remained significant (OR, 3.3; 95% CI, 1.1-10.0) (Table 2). Combining our data with those from 9 published studies,6 the pooled OR is 3.3 (95% CI, 2.3-4.6), with an absence of statistical heterogeneity (P = .42).

Our findings add to the observational evidence examining the relationship between hyper-Hcy and VTE.6 Given the consistency and the magnitude of the pooled OR across these 10 studies, it seems likely that elevated plasma total Hcy level is a clinically significant factor in the development of VTE. Hyperhomocyst(e)inemia may cause vessel wall endothelial dysfunction,18 vascular smooth muscle proliferation,19 and hemostatic changes consistent with the prothrombotic state.20 Such changes can be mimicked in vitro by exposure of cells or tissues to homocyst(e)ine.21,22 These lend support for a causal link that would explain the epidemiological association of hyper-Hcy with VTE we describe herein.23

Our study derived an OR of 5.9 for VTE in the presence of hyper-Hcy, redefining the pooled OR for all 10 studies to 3.3; but are these figures clinically important? Some argue that an OR above 2.0 influences clinical practice.24 However, clinical significance also depends on how common and lethal both exposure and disease are, as well as the magnitude of resources required for their detection, treatment, and prevention.25 Venous thromboembolism is a serious condition, and plasma total Hcy measurement is increasingly offered by routine diagnostic centers. Studies are now under way to evaluate the therapeutic efficacy of folate and vitamins B6 and B12 in the secondary prevention of VTE.26 Considering these factors, it can be argued that a fasting plasma total Hcy measurement should be part of any thrombophilia workup.
A striking aspect of hyper-Hcy is its association with earlier-onset, often severe vascular disease, particularly with a positive family history. However, our study fails to support this finding, as the OR was less pronounced among patients younger than 60 years compared with older adults. Thus, hyper-Hcy may be an important VTE risk factor regardless of age. Several publications confirm a high frequency of clinically silent vitamin B₁₂ deficiency in geriatric populations, even those with apparently “normal” vitamin B₁₂ levels. Willems and colleagues also noted a higher frequency of hyper-Hcy among a group of elderly adults with VTE, compared with disease-free controls of approximately the same age (OR, 2.4; 95% CI, 0.8-6.9).

Our study has several limitations and certain strengths. Although we attempted to match cases with controls for age and sex, we were unable to control for other factors known to affect plasma total Hcy level, such as renal insufficiency or vitamin supplementation. Studies have shown that homocyst(e)ine level is strongly correlated with serum creatinine level and inversely related to glomerular filtration. Several cases had cancer, were undergoing hemodialysis, or had received organ transplants, any of which could raise plasma total Hcy levels and increase the risk for vascular disease. Also of concern is the lack of information on the reasons leading to plasma total Hcy measurement in controls. However, if some controls had also experienced a previous VTE or atherosclerotic event, or had renal insufficiency, we would have expected to find a greater frequency of hyper-Hcy and, accordingly, a deflation in the OR.

Since the cause of VTE remains multifactorial, future research should evaluate the risk for VTE in the presence of hyper-Hcy and other common risk factors, such as activated protein C resistance, antiphospholipid antibodies, and defects in protein C, protein S, or anti-

Table 1. Characteristics of Study Cases and Controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases (n = 70)</th>
<th>Controls (n = 210)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y Mean ± SD</td>
<td>55.1 ± 17.6</td>
<td>54.9 ± 16.8</td>
</tr>
<tr>
<td>Range</td>
<td>22-92</td>
<td>22-97</td>
</tr>
<tr>
<td>Sex, No. F/M</td>
<td>39:31</td>
<td>117:93</td>
</tr>
<tr>
<td>Recurrent VTE, No. (%)</td>
<td>13 (18.6)</td>
<td>NA</td>
</tr>
<tr>
<td>Another thrombophilic defect, No. (%)</td>
<td>20 (29)</td>
<td>NA</td>
</tr>
<tr>
<td>Activated protein C resistance</td>
<td>11 (16)</td>
<td>NA</td>
</tr>
<tr>
<td>Antiphospholipid antibodies</td>
<td>9 (13)</td>
<td>NA</td>
</tr>
<tr>
<td>Predisposing factors for VTE, No. (%)</td>
<td>33 (47.1)</td>
<td>NA</td>
</tr>
<tr>
<td>Cancer</td>
<td>25 (35.7)</td>
<td>NA</td>
</tr>
<tr>
<td>Renal disease or dialysis</td>
<td>3 (4.3)</td>
<td>NA</td>
</tr>
<tr>
<td>Leg fracture</td>
<td>2 (2.9)</td>
<td>NA</td>
</tr>
<tr>
<td>Organ transplant</td>
<td>2 (2.9)</td>
<td>NA</td>
</tr>
<tr>
<td>Other</td>
<td>4 (5.7)</td>
<td>NA</td>
</tr>
<tr>
<td>Fasting plasma total Hcy, µmol/L (mean ± SD)</td>
<td>13.0 ± 6.9</td>
<td>9.0 ± 4.8</td>
</tr>
</tbody>
</table>

*VTE indicates venous thromboembolism; NA, not applicable; Hcy, homocyst(e)ine.

Distribution of total plasma homocyst(e)ine (tHcy) concentrations in cases (black bars) and controls (gray bars). The 95th percentile for the control group (14.8 µmol/L) is shown as a dashed line.
Table 2. Risk of VTE in the Presence of Hyper-Hcy

<table>
<thead>
<tr>
<th>No. (%) With Hyper-Hcy</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td>All subjects</td>
<td></td>
</tr>
<tr>
<td>Age ≤ 60 y</td>
<td></td>
</tr>
<tr>
<td>16/70 (22.9)</td>
<td>10/210 (4.8)</td>
</tr>
<tr>
<td>Age &gt; 60 y</td>
<td></td>
</tr>
<tr>
<td>9/30 (30.0)</td>
<td>5/90 (5.6)</td>
</tr>
<tr>
<td>Exclusion of cases with renal insufficiency, or serum folate, or vitamin B12 deficiency †</td>
<td>8/51 (15.7)</td>
</tr>
</tbody>
</table>

†Exclusion of cases with creatinine level greater than 150 µmol/L (1.7 mg/dL), serum folate level less than 4.1 nmol/L, or vitamin B12 level less than 100 pmol/L and their respective controls, as well as those who did not undergo analysis of all 3 measures.

*VTE indicates venous thromboembolism; hyper-Hcy, hyperhomocysteineemia (defined as homocyst(e)ine level greater than the 95th percentile of the mean value for the controls); and CI, confidence interval.

7. den Heijer M, Langman LJ, et al. Total plasma homocysteine concentrations are strongly correlated with whole blood cysteine and aminothiol concentrations and angio graphic coronary artery disease in heart transplant patients [abstract]. Transplantation. 1998;64(suppl):10S.