High Prevalence of Hyperhomocyst(e)inemia in Patients With Juvenile Venous Thrombosis

Cristina R. Falcon, Marco Cattaneo, Daniela Panzeri, Ida Martinelli, Pier Mannuccio Mannucci

Abstract We determined the prevalence of hyperhomocyst(e)inemia before and 4 hours after a methionine load (3.8 g/m²) in 80 patients (25 men and 55 women) who had had at least one verified episode of venous thromboembolism before the age of 40 years and in 51 healthy control subjects. No patient had any of the hemostatic abnormalities known to be associated with increased risk of venous thrombosis, and all had normal renal and liver function and no evidence of neoplastic disease. Hyperhomocyst(e)inemia was defined as fasting plasma homocyst(e)ine levels or absolute postload increments of homocyst(e)ine above the normal range. According to these diagnostic criteria, 15 patients (18.8%) and 1 control subject (1.9%) had hyperhomocyst(e)inemia. In 1 of these patients only, hyperhomocyst(e)inemia could be explained by low serum concentrations of vitamin B₁₂ and folates.

Homocysteine is a sulfhydryl amino acid derived from metabolic conversion of methionine. Homocysteine is oxidized in plasma to the disulfides homocysteine-homocysteine (homocystine) and homocysteine-cysteine. Homocysteine and the two disulfides exist in both free and protein-bound forms and are usually referred to as homocyst(e)ine. Very high plasma levels of homocyst(e)ine are found in the rare patients with homozygous homocystinuria (prevalence, 1 in 80 000 to 200 000), which is usually due to a deficiency of the enzyme cystathionine β-synthase, which transforms homocysteine to cystathionine and is associated with early atherosclerosis and juvenile arterial and venous thrombosis. Vascular complications of homocystinuria are probably due to the toxic effects of homocyst(e)ine on the vascular wall and ensuing changes in hemostasis.

Several studies, reviewed in Reference 13, have demonstrated that even moderately high plasma levels of homocyst(e)ine, such as those that can be found in heterozygotes for enzyme deficiency (prevalence, 1 in 70 to 200), are associated with an increased risk of arteriosclerosis and arterial thrombotic events, such as stroke, peripheral vascular disease, and myocardial infarction, at a young age. In some heterozygotes, high plasma levels of homocyst(e)ine can be found only after the oral administration of a large dose of methionine, which metabolically challenges the individual's capacity of converting this amino acid into homocysteine. In contrast to arterial thrombosis, there are very few data concerning heterozygous homocystinuria and venous thrombosis, so no definite conclusions can yet be made regarding hyperhomocyst(e)inemia as a risk factor for venous thromboembolism.

The aim of this study was to determine the prevalence of hyperhomocyst(e)inemia before and after a methionine load in patients who had had at least one verified episode of venous thromboembolism before 40 years of age and had none of the known congenital or acquired hemostatic risk factors for venous thromboembolism.

Methods

Subjects

Consecutive patients referred to our center for juvenile venous thrombosis were enrolled in the study provided they had had at least one episode of venous thrombosis or pulmonary embolism before the age of 40 years, if the time elapsed since the last episode was longer than 3 months, and if they had no congenital or acquired thrombophilic disorder (see below). Eighty patients were enrolled from June 1992 through August 1993 (Table 1). All diagnoses of thrombotic episodes had been confirmed by objective methods: phlebography or compression ultrasonography for deep vein thrombosis, pulmonary scintigraphy or angiography for pulmonary embolism,
TABLE 1. Characteristics of the Patient Population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients Tested (n)</th>
<th>Control (n)</th>
<th>No. of Positive Tests/No. of Patients Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (men/women)</td>
<td>80 (25/55)</td>
<td>80 (25/55)</td>
<td></td>
</tr>
<tr>
<td>Age, y median (range)</td>
<td>33 (18-49)</td>
<td>33 (18-49)</td>
<td></td>
</tr>
<tr>
<td>Age at first episode of venous thromboembolism, y median (range)</td>
<td>29 (16-40)</td>
<td>29 (16-40)</td>
<td></td>
</tr>
<tr>
<td>Time elapsed since last episode, mo median (range)</td>
<td>14 (3-72)</td>
<td>14 (3-72)</td>
<td></td>
</tr>
<tr>
<td>Type of episode</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deep vein thrombosis</td>
<td>50</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Deep vein thrombosis and pulmonary embolism</td>
<td>18</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Cerebral vein thrombosis</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>No. of episodes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One</td>
<td>39/80 (49%)</td>
<td>39/80</td>
<td></td>
</tr>
<tr>
<td>Two or more</td>
<td>41/80 (51%)</td>
<td>41/80</td>
<td></td>
</tr>
<tr>
<td>Patients with predisposing factors at first episode</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone fractures and immobilization</td>
<td>10/80</td>
<td>10/80</td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>5/80</td>
<td>5/80</td>
<td></td>
</tr>
<tr>
<td>Pregnancy</td>
<td>5/55</td>
<td>5/55</td>
<td></td>
</tr>
<tr>
<td>Oral contraceptives</td>
<td>32/55</td>
<td>32/55</td>
<td></td>
</tr>
<tr>
<td>Patients with positive family histories of venous thromboembolism</td>
<td>18/80 (22%)</td>
<td>18/80</td>
<td></td>
</tr>
</tbody>
</table>

and computed tomography or nuclear magnetic resonance and angiography for thrombosis of cerebral veins. All patients had normal prothrombin, activated partial thromboplastin, and thrombin times and normal plasma levels of fibrinogen, protein C, protein S, and antithrombin III. Rarer causes of congenital thrombophilia (plasminogen and heparin cofactor II deficiency, increased histidine-rich glycoprotein, and plasminogen-activator inhibitor) were also excluded. Recently, we also have been able to exclude resistance to activated protein C as a possible cause of juvenile thromboembolism. No patient had lupus anticoagulant, antiphospholipid antibodies, abnormal liver or renal function, or evidence of autoimmune or neoplastic diseases. Fifty-one healthy subjects (20 men, 31 women), median age, 30 years (range, 23 to 45 years), were selected as control subjects from our hospital staff. Blood samples for measuring fasting plasma homocyst(e)ine levels were obtained from all subjects, and post-methionine-load vitamin B, and folate levels also were measured to rule out nutritional deficiencies as causes of increased plasma homocyst(e)ine levels. L-Methionine (3.8 g/m² body surface area) was then administered orally in about 200 mL of fruit juice. Four hours later, a second blood sample was drawn for plasma homocyst(e)ine assay. All subjects remained in the fasting state until the second blood sample had been taken.

Sample Preparation and Analysis

Blood samples for homocyst(e)ine measurements were collected into EDTA-containing evacuated tubes (Vacutainers), immediately placed on ice, and centrifuged within 30 minutes of collection. Plasma samples were stored in aliquots at —80°C until assay. Plasma total homocyst(e)ine levels (free and protein-bound) were determined by high-performance liquid chromatography (Waters Millipore 6000 A pump, Millipore) and by electrochemical detection with a gold and mercury electrode (Bioanalytical System LC44 detector) using the method of Malinow et al. Briefly, 200 μL plasma was diluted with 100 μL distilled water; mixed with 300 μL of 9 mol/L urea, 50 μL of n-amyl alcohol, and 50 μL of a solution of 10% (wt/vol) NaBH₄ in 0.1N NaOH; and incubated in a water bath at 50°C for 30 minutes. The reaction was stopped with 500 μL 20% trichloroacetic acid, and the proteins were separated by centrifugation at 12 000 rpm in an Eppendorf microcentrifuge for 4 minutes. Standards were prepared by the same procedure in pooled normal plasma, but various concentrations of 100 μL L-homocystine (0.5 to 15 μmol/L) were used instead of 100 μL water before reduction and deproteinization. The concentration of homocyst(e)ine was calculated from the height of peaks in the standard curve.

Criteria for Diagnosis of Hyperhomocyst(e)inemia

Hyperhomocyst(e)inemia was diagnosed when fasting plasma homocyst(e)ine levels or post-methionine-load absolute increments of homocyst(e)ine exceeded the upper limit of the normal range (mean±2 SD of values obtained in the control group).

Statistical Analysis

Since plasma homocyst(e)ine levels were not normally distributed, they were transformed logarithmically to approximate normal distribution. Transformed data were analyzed statistically. For descriptive purposes, means±SD are given. The Student’s two-tailed t test was used to compare control and patient groups.

Results

Table 2 shows that mean values of fasting plasma homocyst(e)ine levels of patients were not significantly different from those of control subjects. In contrast, the mean postload increments of plasma homocyst(e)ine were significantly greater in patients than in control subjects. There were no significant differences in any of the data between women and men, either patients or control subjects (not shown). Fasting levels exceeded
the upper limit of the normal range in 7 patients (8.8%) and postload increments, in 14 (17.7%) (Fig 1 and Table 2). Six patients with high fasting levels also had large postload increments (1 patient with a fasting level of 42 µmol/L was not studied after the methionine load). Eight patients with normal fasting levels had large postload increments. On the whole, 15 of the 80 patients (18.8%) had hyperhomocyst(e)inemia according to the definition in this study (Table 2); for all of them the diagnosis was confirmed on at least a second occasion.

Of the 4 patients with cerebral vein thrombosis, 1 had hyperhomocyst(e)inemia; therefore, considering only the patients with deep vein thrombosis and/or pulmonary embolism, 18.4% (14/76) had hyperhomocyst(e)inemia. One patient with a high fasting level and a large postload increment had low serum vitamin B<sub>12</sub> and folate concentrations (not shown). One control subject (1.9%) had a large postload homocyst(e)ine increment.

Of the 15 patients with juvenile thromboembolism and hyperhomocyst(e)inemia, 7 had at least one first-degree relative with a positive history for juvenile venous thromboembolism (deep vein thrombosis in 5 and pulmonary embolism in 2).

**Family Studies**

An attempt was made to contact all 15 patients with high plasma homocyst(e)ine levels and to study the available first-degree relatives. Eight families were studied, four with negative and four with positive clinical histories of venous thromboembolism (Fig 2). Five families (three with negative [families A, B, and C] and two with positive [families E and H] family histories for thrombosis) had at least one first-degree relative with hyperhomocyst(e)inemia and were therefore considered affected. One of the probands with negative family history was the patient with vitamin B<sub>12</sub> and folate deficiency; none of her relatives had hyperhomocyst(e)inemia (family D). The relatives with positive personal histories for thrombosis of the two remaining probands (families F and G) could not be studied in the laboratory.

**Discussion**

This study shows that there is a high prevalence of hyperhomocyst(e)inemia in patients with juvenile venous thrombosis and/or pulmonary embolism that is unexplained by the presence of abnormalities known to be associated with an increased risk for venous thromboembolism. The data also suggest that in some instances the metabolic abnormality is inheritable.

In our patients, the prevalence of hyperhomocyst(e)inemia varied according to the diagnostic criterion used. Previous studies have shown that measurement of plasma homocyst(e)ine increments over fasting levels enables more accurate diagnosis of heterozygous homocystinuria than measurement of fasting homocyst(e)ine levels only. This was confirmed in this study, in which the prevalence of hyperhomocyst(e)inemia in our patients varied between 9% when fasting plasma levels alone were considered and 19% when fasting levels and post-methionine-load increments were considered. Both figures are much higher than the prevalence of heterozygous homocystinuria (0.5% to 1.4%) calculated for the general population.
Hyperhomocyst(e)inemia may be due to heterozygous cystathionine \( \beta \)-synthase deficiency, nutritional deficiencies (resulting in low serum folate or vitamin \( B_12 \) or \( B_6 \) levels), and liver or renal failure. All our patients with hyperhomocyst(e)inemia had normal liver and renal function, and only one had abnormal serum levels of folate and vitamin \( B_12 \). Therefore, the vast majority of our patients with hyperhomocyst(e)inemia are probably heterozygous for cystathionine \( \beta \)-synthase deficiency. The data of our family studies, of probands with relatives available for study, indicate that more than half of these hyperhomocyst(e)inemic probands have inherited abnormalities. In only three of the eight families studied was there no affected first-degree relative of the proband. In one of these cases, the hyperhomocyst(e)inemia of the proband was probably related to her low serum concentrations of folate and vitamin \( B_12 \). In the remaining two cases, insufficient family members were tested because three of the four parents (two of them with positive personal histories for thrombosis) were not available for study. Our family studies also indicate that, in individuals with inherited defects of coagulation inhibitors,\(^{19}\) not all the individuals with hyperhomocyst(e)inemia have thrombotic episodes, suggesting that there must be other triggering factors involved in the occurrence of venous thrombosis.

The mechanisms by which hyperhomocyst(e)inemia may cause venous thrombosis are not well understood. Studies of human endothelial cells cultured in vitro suggest that high concentrations of homocysteine interfere with the natural anticoagulant system\(^{6,8,12}\) and the fibrinolytic system,\(^{11}\) which play essential roles in regulation of thrombus formation.\(^{19}\) It must be noted, however, that most in vitro effects of homocysteine in endothelial cells\(^{6,8,11}\) have been shown for very high homocysteine concentrations, usually at least one order of magnitude higher than the plasma concentrations that can be found in patients with homozygous homocystinuria.

Although the association between hyperhomocyst(e)inemia and juvenile arterial thrombosis is well documented, its association with juvenile venous thrombosis has not been clearly established previously.\(^{13}\) Brattstrom et al\(^{10}\) found a higher prevalence of hyperhomocyst(e)inemia after a methionine load (14%) in 42 patients under 50 years of age with venous thromboembolism than in 42 age- and sex-matched control subjects (5%). At variance with our study, the prevalence of high fasting plasma homocyst(e)rine levels was not higher in patients than in control subjects, family studies were not performed, and the presence of inherited causes of thrombophilia was not ruled out. In another small study, Bienvenue et al\(^{21}\) found that 7 of 23 patients who developed venous thrombosis before 60 years of age had high fasting levels of homocyst(e)rine. Some of these patients, however, also had clinical entities or coagulation abnormalities known to be associated with increased risk of venous thrombosis. Our study of a large series of young patients with none of the abnormalities known to be associated with increased risk of thrombosis shows the usefulness of combined measurements of plasma fasting and post-methionine-load homocyst(e)rine levels for accurate diagnosis of hyperhomocyst(e)inemia. These measurements may be useful in the evaluation of patients with juvenile venous thrombosis, particularly if their family histories suggest the presence of inherited abnormalities.

References

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