Background and Purpose—Elevated plasma levels of homocysteine are associated with an increased risk of deep-vein thrombosis. Through a case–control study, we examined the potential association among homocysteine, folate and vitamin B₁₂, and the common C677→T mutation in the methylene tetrahydrofolate reductase (MTHFR) gene in patients with cerebral venous thrombosis (CVT).

Methods—Forty-five patients with CVT and 90 control subjects were studied. Plasma levels of homocysteine (fasting and after methionine load), folate, and vitamin B₁₂ were measured. Genotyping of the MTHFR gene was also performed. The estimated risk of CVT associated with hyperhomocysteinemia, low vitamin levels, and MTHFR mutation were expressed as odds ratio (OR) and its 95% CI (crude and after adjusting by other independent variables).

Results—The adjusted OR for CVT associated with high (>90th percentile) fasting levels of homocysteine was 4.6 (1.6 to 12.8). The association between low plasma folate values (<10th percentile) and presence of CVT was 3.5 (1.2 to 10.0) after adjustment for confounding factors. There was a higher frequency of MTHFR mutation in patients with CVT (22% versus 10%), but it was not statistically significant (P=0.098). Patients with MTHFR mutation and low folate levels presented the highest homocysteine levels.

Conclusions—High plasma concentrations of homocysteine and low plasma folate levels were associated with an increased risk of CVT in this population in which low socioeconomic conditions and deficient nutritional status may contribute to its relatively high incidence. (Stroke. 2004;35:1790-1794.)

Key Words: cerebral thrombosis ■ sinus thrombosis ■ risk factors ■ coagulation ■ homocysteine

Although cerebral venous thrombosis (CVT) is an uncommon disorder, its diagnosis has become more frequent because of greater clinical awareness and more sensitive neuroimaging techniques. The main progress in CVT study has been focused on identification of thrombophilic factors. Epidemiological studies have suggested that even mild hyperhomocysteinemia (hyper-Hcy) is associated with occlusive arterial vascular disease¹ and venous thromboembolism.²⁻⁷ Little information about the role of homocysteine in CVT is available. Only 1 systematic study on CVT and hyper-Hcy has been published;⁸ recently, Martinelli et al found that hyper-Hcy increases the risk of CVT by ≈4-fold.⁸

Genetic and nutritional factors are important determinants of homocysteine metabolism. The common C677→T mutation in the methylene tetrahydrofolate reductase (MTHFR) gene is associated with a thermolabile variant that has approximately half-normal activity.⁹⁻¹⁰ Approximately 10% to 13% of the white population are homozygous for this mutation. Conversely, because blood levels of folate, vitamin B₁₂, and to a lesser extent vitamin B₉, are related inversely to homocysteine, anyone with a nutritional deficiency of these vitamins is at increased risk of hyper-Hcy.¹¹ The potential interaction between genetic and environmental factors is important in the production of increased homocysteine levels. Consequently, the hereditary metabolic disorder will manifest mainly in individuals with poor nutritional status.¹²

Hyper-Hcy may contribute to the relatively high CVT frequency in Mexico.¹³⁻¹⁴ Although CVT comprises 8% of cases with cerebrovascular disorders in our stroke register (166 of 2045 patients),¹⁵ it represents an uncommon diagnosis in American and European stroke registries. If the prevalence in Mexican population of the thermolabile MTHFR variant is similar to that of the white population, nutritional factor deficiencies associated with poor socioeconomic conditions
may influence development of hyper-Hcy. In the present case–control study, a hypothesis was posed accordingly proposing hyper-Hcy as a risk factor for development of CVT in association with the MTHFR mutation or with a deficient nutritional status resulting from inadequate ingestion of vitamins (folate and B₁₂).

**Patients and Methods**

A total of 45 patients with CVT and 90 healthy control subjects, enrolled from March 1998 to November 2000, participated in this study. The Institutional Review Board of the National Institute of Neurology and Neurosurgery (NINN) of Mexico approved the study. All participants provided informed written consent.

**Cases**

Consecutive cases with diagnosis of CVT admitted to NINN were invited to participate in our study. Cerebral angiography or cranial MRI confirmed CVT diagnosis. From 51 newly diagnosed patients, studies were not performed in 6 patients because of death (n=3) or because of loss of follow-up after hospital discharge (n=3). Laboratory tests were conducted ≥3 months after acute phase of CVT. Only survivors of CVT were recruited; no patients had died after hospital discharge by the 3-month blood collection. There were no patients with known conditions that influenced homocysteine concentration, such as renal or thyroid disease, or who were in anticonvulsant therapy with phenytoin or carbamazepine. Serum creatinine (mean±SD) in the 45 patients was 0.72±0.18 mg/dL.

**Control Subjects**

All controls were free of overt disease according to a questionnaire, including no regular vitamin intake and no known history of thrombosis. Control subjects were recruited from friends or relatives of patients attending our institution for other nonvascular neurological disorders. They were recruited at the same time as cases. To be consistent with the low socioeconomic condition of patients, total family monthly income was used as a marker of socioeconomic status. Because ~90% of patients with CVT were women <50, selection of controls was directed toward recruiting young women. A complete gynecological history was recorded in all women, including parity and use of oral contraceptives.

**Laboratory Tests**

Sampling took place during early morning after overnight fasting. After blood sample extraction, patients and control subjects received a 0.1-g single dose of l-methionine per kilogram of body weight in 200 mL of orange juice. A standardized low-methionine breakfast was given during tests. A second blood sample was taken 4 hours after methionine loading. Plasma total homocysteine (tHcy) was measured with high-performance liquid chromatography with a fluorescence detector. The postload tHcy test was performed in 81 of the 90 control subjects; it was not performed in the remaining 9 for technical reasons. Serum folate and cobalamin were measured with an automated microparticle enzyme immunoassay (Diagnostic Products). For molecular genetics analysis, DNA was extracted from a fraction of a peripheral blood sample using the phenol-chloroform-isoamyl alcohol method. For identification of the C677→T mutation in the MTHFR gene, polymerase chain reaction was performed using 100 ng forward and reverse primer according to Frosst et al. Further thrombophilic tests were performed on CVT patients. Thrombophilia tests included determination of antithrombin III, protein C, protein S, activated protein C (APC) resistance, and presence of factor V Leiden and mutation in the prothrombine gene (G20210A). Also, lupus anticoagulant and IgG and IgM antiphospholipid antibodies were determined.

**Statistical Methods**

Analysis was conducted with SPSS version 10 for Windows. A comparative analysis of baseline variables between patients and controls was performed. The χ² test was used for categorical variables and the Mann–Whitney U test for continuous variables because data were not normally distributed. Hyper-Hcy was defined as levels of fasting homocysteine and methionine postload above the 90th percentile of the homocysteine value distribution in the control population. Low vitamin (folate and B₁₂) levels were defined as those in the <10th percentile of vitamin value distribution in the control group. Estimated risk of CVT associated with hyper-Hcy was expressed as the odds ratio (OR) and its 95% CI. OR (95% CI) was also calculated to assess the association between low folate and vitamin B₁₂ levels and the risk to develop CVT. First, we calculated crude OR by simple cross-tabulation, and then adjusted ORs were obtained to determine the influence of other independent variables through multiple logistic regression analysis.

**Results**

Racial background of all subjects was Mexican mestizo (a mix of European whites, mainly Spaniards, and Amerindians). The most common predisposing factors for CVT was puerperium (51%). Other known predisposing factors for CVT were uncommon (oral contraceptives 2, pregnancy 1, systemic lupus erythematosus 1, recent surgery 1). Thrombophilia tests documented the presence of prothrombine gene mutation in 4 patients, protein S deficiency in 3 patients, protein C deficiency in 3 patients, factor V Leiden mutation in 2 patients, and antithrombin deficiency in 1 patient. Anticardiolipin antibodies were present in 11 patients (concomitant with lupus anticoagulant in 2). Although APC resistance was observed in 7 patients, it was related to factor V Leiden in only 2 patients (1 heterozygote and 1 homozygote). Otherwise, APC resistance was associated with anticardiolipin positivity in 3 patients, contraceptive use in 1, and was the only prothrombotic condition in the remaining patient. A total of 25 patients had anemia (55%). Most patients presented clinically with various combinations of headache (96%), focal neurological deficits (69%), seizures (62%), intracranial hypertension (40%), and consciousness impairment (44%). Superior longitudinal and lateral sinuses were commonly affected (82.2% and 46.7%, respectively). Neuroimaging studies showed a high frequency of venous infarctions (75.5%), including hemorrhagic changes in 49.0%. Most patients were discharged with total recovery (60.0%) or minor neurological symptoms (35.6%); only 2 patients presented moderate neurological impairment (4.5%). Table 1 shows differences between CVT patients and control subjects. There were no differences in socioeconomic status; total family income of most patients and controls corresponds to the lowest income quintile in Mexican population. Obstetrical history (parity, abortions) and current use of oral contraceptives were also similar in both groups. Median levels of tHcy were higher in patients with CVT, both during fasting (P=0.01) and after methionine load (P=0.006). The median serum folate concentration was...
significantly lower in patients with CVT \((P<0.0001)\). Distribution of genotypes of the MTHFR gene showed a trend to higher frequency of TT mutant with CVT patients (22\% versus 10\%). Comparison of patients carrying the MTHFR thermolabile genotype (TT) with those negative for the same condition (CC/CT genotypes) revealed a trend to an association of the TT genotype with CVT, but it was not statistically significant (adjusted OR, 2.2 [95\% CI, 0.86 to 5.98]; \(P=0.098\)).

As Table 2 shows, 17 patients with CVT presented fasting hyper-Hcy (37.8\%) compared with 10\% in the control group. Estimated risk of association between hyper-Hcy and CVT was 5.6 (95\% CI, 2.2 to 14.4) and was maintained independently after adjusting with other independent variables (age, sex, folic acid, vitamin B12, and presence of MTHFR mutation). Subgroup analysis, excluding women with puerperal CVT, revealed that the risk estimate for hyper-Hcy and CVT not related to puerperium

### TABLE 1. Differences Between Patients With CVT and Healthy Control Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients (n=45)</th>
<th>Control Subjects (n=90)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y* ((14–55))</td>
<td>28.0 (16–53)</td>
<td></td>
<td>0.51</td>
</tr>
<tr>
<td>Sex, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>38 (84.4)</td>
<td>67 (74.4)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7 (15.6)</td>
<td>23 (25.6)</td>
<td>0.18</td>
</tr>
<tr>
<td>Socioeconomic status, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total family monthly income</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;150 US dollars</td>
<td>21 (46.7)</td>
<td>37 (41.1)</td>
<td></td>
</tr>
<tr>
<td>150–300 US dollars</td>
<td>16 (35.6)</td>
<td>31 (36.7)</td>
<td></td>
</tr>
<tr>
<td>&gt;300 US dollars</td>
<td>8 (17.8)</td>
<td>20 (22.2)</td>
<td>0.77</td>
</tr>
<tr>
<td>Obstetrical history, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nulliparous</td>
<td>8 (21.1)</td>
<td>18 (26.9)</td>
<td></td>
</tr>
<tr>
<td>Primiparous</td>
<td>16 (42.1)</td>
<td>25 (37.3)</td>
<td></td>
</tr>
<tr>
<td>Multiparous</td>
<td>14 (36.8)</td>
<td>24 (35.8)</td>
<td>0.78</td>
</tr>
<tr>
<td>Abortions</td>
<td>5 (7.5)</td>
<td>2 (5.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>Current oral contraceptive use, %†</td>
<td>2 of 37 (5.4)</td>
<td>4 of 66 (6.1)</td>
<td>1.00</td>
</tr>
<tr>
<td>Baseline homocysteine, (\mu)mol/L*</td>
<td>9.0 (3.04–42.1)</td>
<td>7.0 (2.3–28.7)</td>
<td>0.01</td>
</tr>
<tr>
<td>Homocysteine postload, (\mu)mol/L*</td>
<td>26.7 (10.1–77.1)</td>
<td>21.1 (3.6–49.6)§</td>
<td>0.006</td>
</tr>
<tr>
<td>Folic acid, nmol/L*</td>
<td>5.7 (2.6–12.2)</td>
<td>8.5 (2.3–16.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vitamin B12, pmol/L*</td>
<td>330.0 (97–1515)</td>
<td>430.8 (157–2177)</td>
<td>0.07</td>
</tr>
<tr>
<td>MTHFR genotype, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC (Ala/Ala)</td>
<td>14 (31.1)</td>
<td>26 (28.9)</td>
<td></td>
</tr>
<tr>
<td>CT (Ala/Val)</td>
<td>21 (46.7)</td>
<td>54 (60.0)</td>
<td></td>
</tr>
<tr>
<td>TT (Val/Val)</td>
<td>10 (22.2)</td>
<td>10 (11.1)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

*Values expressed as median (range).
†Percentage calculated on the no. of women of reproductive age. There were only 2 postmenopausal women.
§n=81.

### TABLE 2. Risk Estimate of Association of High Homocysteine Levels and Low Vitamin Concentrations Between Patients With CVT and Healthy Control Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients (n=45)</th>
<th>Controls (n=90)</th>
<th>Crude OR ((95% CI))</th>
<th>(P)</th>
<th>Adjusted OR* ((95% CI))</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting hyper-Hcy ((90th percentile 10.69 \mu)mol/L)</td>
<td>17 (37.8)</td>
<td>9 (10.0)</td>
<td>5.4 (2.2–13.6)</td>
<td>&lt;0.001</td>
<td>4.6 (1.6–12.8)</td>
<td>0.004</td>
</tr>
<tr>
<td>Postload hyperhomocysteinemia ((90th percentile 38.49 \mu)mol/L)</td>
<td>10 (22.2)</td>
<td>8/81 (9.9)</td>
<td>2.6 (0.9–7.2)</td>
<td>0.054</td>
<td>2.2 (0.6–7.4)</td>
<td>0.18</td>
</tr>
<tr>
<td>Low folate levels ((10th percentile 5.11 nmol/L)</td>
<td>16 (35.6)</td>
<td>9 (10.0)</td>
<td>4.4 (1.8–10.8)</td>
<td>0.001</td>
<td>3.5 (1.2–10.0)</td>
<td>0.01</td>
</tr>
<tr>
<td>Low vitamin B12 levels ((10th percentile 242.7 pmol/L)</td>
<td>14 (31.1)</td>
<td>9 (10.0)</td>
<td>4.0 (1.6–10.3)</td>
<td>0.002</td>
<td>5.1 (1.8–14.2)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*Basal and postload hyper-Hcy were adjusted for age, sex, vitamins, and MTHFR mutation. Low vitamin levels were adjusted for age, sex, homocysteine, and MTHFR mutation.
Association between low vitamin values and CVT. A negative correlation with higher levels of tHcy and vitamin B_{12} values (\( \rho = -0.104; P=0.49 \)). Patients with MTHFR mutation and low folic acid level (\( \leq 5.1 \text{ nmol/L} \)) presented the highest tHcy levels, whereas patients with this mutation and adequate folate levels (\( >5.1 \text{ nmol/L} \)) maintained normal homocysteine levels (Figure).

**Discussion**

The main finding of this study was that mild fasting hyper-Hcy revealed an independent association with CVT. This finding was not observed after a methionine load, when adjusted with other independent variables. This discrepancy could be explained because disturbances in the remethylation pathway of homocysteine metabolism, which is dependent of folate, vitamin B_{12}, and MTHFR enzyme activity, are mainly involved in our population, whereas methionine loading is more sensitive for detecting disturbances in the transsulfuration pathway. Hyper-Hcy has proved to be a strong and independent factor associated with ischemic stroke.\(^{3,19}\)

The probable causal link is also observed in young patients and children,\(^{20-22}\) suggesting a thrombogenic rather than an atherogenic effect in these young subjects. Association between high tHcy levels and CVT in this study was observed in the group of patients with CVT not associated with puerperium and the group of women with puerperal CVT.

Results of our study are consistent with the case-control study by Martinelli et al\(^8\) in patients with a first episode of CVT. They found hyper-Hcy (high fasting tHcy or postload increments) in 33 of 121 patients (27%) and 20 of 242 healthy controls (8%; OR, 4.2; 95% CI, 2.3 to 7.5).\(^8\)

A low folate and vitamin B_{12} state was also associated with increased risk of CVT. Although fasting homocysteine correlated negatively with folate, the risk was not reduced by inclusion of homocysteine in the model, implying that increased risk of CVT, accompanying lower folate levels, appears to be mediated in an independent manner. It is recognized more progressively that low levels of folate may play a role in development of cardiovascular disease.\(^{23}\)

Although most studies have focused on homocysteine-lowering effects of folate, benefits of folate, regardless of homocysteine, also have been reported.\(^{24}\) These findings may account for the higher frequency of puerperium-associated CVT within our population because CVT is observed mainly in women with low socioeconomic status with nutritional deficiency. The frequency of low folate levels correlated with a high rate of anemia, which seems to also be mediated by deficient nutritional factors and poor care at delivery.

Martinelli et al\(^{8}\) did not find an association between low vitamin concentrations and CVT, probably because of population differences. Prevalence of oral contraceptive use was high in this Italian study, whereas there was a preponderance of puerperal CVT in this study. Cattaneo et al\(^{25}\) demonstrated recently that vitamin deficiencies are common in patients with deep-vein thrombosis. When the association between vitamin state and the risk of deep-vein thrombosis was investigated, a statistically significant association was found for low levels of vitamin B_{12} but not of vitamin B_{12}. Although our study demonstrated an association of low vitamin B_{12} levels with the risk of CVT, the high frequency of low vitamin B_{12} levels in our study could be an indicator of low
socioeconomic background and subsequent deficient nutritional status of our population.

With respect to the absence of a relationship between MTHFR mutation and CVT in our study, the lack of association could be explained by a large sample size being required to find an association between MTHFR and CVT because of statistical power. A recent meta-analysis by Wald et al revealed a higher risk of deep vein thrombosis in people with the MTHFR mutation.26 Our prevalence of the MTHFR mutation was similar to the white population, which, along with the high frequency of low folate levels, may have implications in development of CVT by promoting hyper-Hcy because there is a need of a higher intake of folate to keep homocysteine at normal levels (Figure).27

Limitations of our study are those inherent in a case–control design. Although cases were classified prospectively and recruited consecutively and controls were chosen from the same source as cases, potential confounding can never be eliminated. However, case–control studies are particularly useful for assessing uncommon disorders such as CVT, for which case–control studies may be the only practical approach. There were some missing variables in our control subjects that could have influenced tHcy levels. Renal function is an important determinant of blood tHcy levels. Although measurement of serum creatinine was not done in our control subjects, they were selected from an apparently healthy population, and the probability of renal dysfunction appears low. Also, purerperal women were not included in our control group, and a diet make-up history was not conducted. These could have been useful to determine their influence on homocysteine and vitamins. The time course of homocysteine and vitamins considering these factors during the postpartum period deserves further investigation in women with puerperal CVT. Finally, although median homocysteine levels are similar in our study compared with other studies, variability is common during this period.24 Further investigation in women with puerperal CVT is required to find an association between tHcy and vitamin levels.

Our findings are consistent with the hypothesis that high blood concentrations of tHcy are associated with increased risk of CVT. Furthermore, low plasma folate levels were also associated highly with an increased risk for CVT in this population in which low socioeconomic conditions and deficient nutritional status may contribute to its relatively high incidence.

References

Hyperhomocysteinemia, Low Folate and Vitamin B₁₂ Concentrations, and Methylene Tetrahydrofolate Reductase Mutation in Cerebral Venous Thrombosis

Carlos Cantu, Elisa Alonso, Aurelio Jara, Leticia Martínez, Camilo Ríos, María de los Angeles Fernández, Irma García and Fernando Barinagarrementeria

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