Plasma Homocysteine and Cysteine Levels in Retinal Vein Occlusion

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PURPOSE. To determine plasma homocysteine and cysteine levels in patients with retinal vein occlusion (RVO) and in healthy subjects and to ascertain whether there are statistically significant differences between patients and control subjects.

METHODS. In this case–control study, the study group consisted of 75 consecutive patients with RVO: 35 had central retinal vein occlusion (CRVO), and 42 had branch retinal vein occlusion (BRVO). Seventy-two apparently healthy age- and sex-matched subjects served as control subjects. Homocysteine and cysteine levels were measured with a new laser-induced fluorescence capillary electrophoresis (CE-LIF) method. Wilcoxon or Student’s t-test was used, when appropriate, to determine differences between groups.

RESULTS. There were no significant differences in median plasma homocysteine between patients with RVO and control subjects, nor were there any statistically significant differences when patients were categorized by type of vein occlusion (CRVO or BRVO). Similarly, there were no significant differences in mean plasma cysteine between patients with RVO and control subjects. However, when categorized by type of vein occlusion, mean plasma cysteine was significantly higher in CRVO patients than in control subjects (P = 0.034).

CONCLUSIONS. This study failed to demonstrate an association between increased plasma homocysteine and RVO. Mean plasma cysteine was significantly higher in patients with CRVO, suggesting that hypercysteinemia may contribute to the pathogenesis of this retinal vascular disorder. (Invest Ophthalmol Vis Sci. 2006;47:4067–4071) DOI:10.1167/iovs.06-0290

Retinal vein occlusion (RVO) is a major cause of visual loss. This condition can involve the central trunk or branches of the retinal venous circulation (CRVO and BRVO, respectively). Elevated plasma homocysteine, which is associated with venous thrombosis and cardiovascular disease,1,2 has been suggested as an important potentially modifiable risk factor for RVO.3–14 In contrast, little is known about the role played by plasma cysteine in RVO. Elevated plasma cysteine has recently been suggested to be a cardiovascular risk factor.15,16 Although less reactive than homocysteine, cysteine shares some of the chemical properties, due to the presence of its sulfhydryl group in the molecule. Cysteine has a general cytotoxicity in vitro and promotes detachment of human arterial endothelial cells in culture. It also exhibits auto-oxidation properties in the presence of metal ions, resulting in the generation of free radicals and hydrogen peroxide.

The purpose of this study was to determine plasma homocysteine and cysteine levels in patients with RVO and apparently healthy subjects and to ascertain whether there are statistically significant differences between patients and control subjects.

METHODS

The present study had a case–control design, recruiting 75 consecutive patients with RVO (CRVO or BRVO) admitted to our institute between April 2003 and November 2005. The duration of visual symptoms, ocular medication, and ocular history were noted. A full ophthalmic evaluation of both eyes was performed, including best corrected visual acuity (BCVA), slit lamp examination, applanation tonometry, fundus biomicroscopy, and fluorescein angiography. Medical conditions, including diabetes, systemic hypertension, cardiovascular status, decreased renal function, relevant drug history, and presence of blood dyscrasias were also recorded. Exclusion criteria included age <18 years, renal failure, and current medication with vitamin B6, B12, or folic acid.

Similar to other studies analyzing the relationship between plasma homocysteine and cysteine levels and vascular occlusive disease, we chose a control group of apparently healthy subjects.16 The control group included 72 subjects, recruited from accompanying relatives or friends of patients or from hospital personnel. Exclusion criteria for control subjects were a history of diabetes, systemic hypertension, cardiovascular or cerebrovascular disease, renal failure, blood dyscrasias, tumors, retinal vascular disorders, age <18 years, and current medication with vitamin B6, B12, or folic acid. All control subjects underwent standard ophthalmal evaluation, including BCVA, slit lamp examination, applanation tonometry, and fundus examination. Control subjects were recruited concurrently during the patients’ recruitment period.

A blood sample was taken from each participant after an overnight fast. Blood for homocysteine and cysteine was collected in an EDTA tube, transported on ice, and immediately centrifuged at 3000g for 10 minutes at 4°C, for plasma and serum separation. Then, the samples were stored at −80°C and analyzed within 1 week. Total plasma thiols were measured by capillary electrophoresis laser-induced detection (P/ACE; Beckman Instruments, Fullerton, CA), as described previously.17 Briefly, 100 μL of standard or plasma sample was mixed with 10 μL of tributylphosphine (10%; TBP), vortexed for 30 seconds and subsequently incubated at 4°C for 10 minutes. After incubation, 100 μL of trichloroacetic acid (10%; TCA) was added, vortexed for 10 seconds, and then centrifuged at 3000g for 10 minutes; 100 μL of supernatant was mixed with 100 μL of 300 mM NaPO4 (pH 12.5) and 25 μL of 5AIF (4.1 mM), incubated at room temperature for 10 minutes, and finally injected in capillary electrophoresis. Analysis of plasma thiols was performed by a masked individual (AZ).

In addition, serum vitamin B12 and folate levels were measured in all patients with RVO (IMX Analyzer; Abbott Laboratories Diagnostics Division, Abbott Park, IL). The IMX B12 assay is based on the micro-particle enzyme immunoassay (MEIA) technology, whereas the IMX folate assay is an ion-capture assay technique. For these immunologic assays, inter- and intra-assay coefficients of variation were <10%. Nor-
RESULTS

The study cohort consisted of 75 patients with RVO (40 men, 35 women; mean age: 63.9 ± 14.5 years; 95% CI: 60.6–67.2). Thirty-three patients (19 men, 14 women; mean age: 63.4 ± 16.2 years, 95% CI: 57.6–69.1) had CRVO and 42 (21 men, 21 women; mean age: 64.4 ± 13.1 years, 95% CI: 60.2–68.5) had BRVO. The patients’ characteristics are reported in Table 1. All patients had similar rates of diabetes, hypertension, hypercholesterolemia, and anticoagulant use, but patients with CRVO had twice the incidence of positive cardiovascular history (angina/myocardial infarction) when compared with those with BRVO; however, this result did not reach statistical significance (P = 0.16).

The control group consisted of 72 subjects (37 men, 35 women; mean age: 63.5 ± 8 years, 95% CI: 61.8–65.5); none had signs of retinal vascular disorders.

Patients and control subjects were well matched for age (RVO patients versus control subjects: P = 0.89, CRVO patients versus control subjects: P = 0.91, BRVO patients versus control subjects: P = 0.72) and sex (RVO patients versus control subjects: P = 0.82, CRVO patients versus control subjects: P = 0.7, BRVO patients versus control subjects: P = 0.95).

Homocysteine levels showed a non-normal distribution; accordingly, statistical analysis was performed with the Wilcoxon test. This result, due to the positively skewed distribution of homocysteine in both patients and control subjects, is consistent with the literature. There were no significant differences in median plasma homocysteine between patients with RVO and control subjects (Table 2), nor were there any statistically significant differences when patients were categorized by type of vein occlusion (CRVO or BRVO).

Cysteine values showed a normal distribution; as a result, statistical analysis was performed using the Student’s t-test. There were no significant differences in mean plasma cysteine between patients with RVO and control subjects (Table 3). However, when categorized by type of vein occlusion, mean plasma cysteine was significantly higher in patients with CRVO than in control subjects (P = 0.034).

Mean plasma vitamin B12 and folate values in patients with RVO, CRVO, or BRVO are shown in Table 4.

DISCUSSION

Homocysteine is a potentially cytotoxic sulfur-containing amino acid produced during the metabolism of the essential amino acid methionine (Fig. 1). Methionine, which comes from dietary animal protein, donates methyl groups to vital transmethylation reactions, which produce important molecules such as creatine and phosphatidylcholine, and allows methylation of DNA, RNA, and neurotransmitters. Homocysteine is an essential intermediate in the transfer of activated methyl groups from tetrahydrofolate to S-adenosylmethionine in the remethylation pathway. It is also an intermediate in the pathway of synthesis of cysteine from methionine (transsulfuration pathway). In the remethylation pathway, homocysteine is remethylated to methionine by transfer of a methyl group from S-5-methyltetrahydrofolate, catalyzed by methionine synthase, an enzyme that requires vitamin B12 as a cofactor. In the transsulfuration pathway, homocysteine is the substrate of the vitamin B6-dependent enzyme cystathionine β-synthase, which catalyzes its condensation with serine to form cystathionine. This is the critical step in the pathway because it is irreversible under physiological conditions. From this point on, homocysteine is committed to follow this pathway. In the last step of the transsulfuration pathway, cystathionine is cleaved by γ-cystathionase, another vitamin B6-dependent enzyme, to form 2-oxoglutarate and cysteine.

There is still no general agreement on the role of hyperhomocysteinemia in RVO. Eleven case-control studies, one population-based case-control study, one retrospective study, and one prospective study have reported an association between hyperhomocysteinemia and vein occlusion. One of these prospective studies, however, did not find a significant association between plasma homocysteine and BRVO. The authors suggested that homocysteine may have a role in the pathogenesis of CRVO, which is clinically different from BRVO.
Homocysteine and Cysteine in Retinal Vein Occlusion

Table 4. Plasma Vitamin B12 and Folate Concentrations

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<thead>
<tr>
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<th>Arithmetic Mean ± SD</th>
<th>95% CI</th>
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<tr>
<td>Vitamin B12 (pg/mL)</td>
<td></td>
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<tr>
<td>RVO patients (n = 75)</td>
<td>325 ± 146</td>
<td>288–361</td>
</tr>
<tr>
<td>CRVO patients (n = 33)</td>
<td>334 ± 131</td>
<td>283–386</td>
</tr>
<tr>
<td>BRVO patients (n = 42)</td>
<td>317 ± 158</td>
<td>264–371</td>
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<tr>
<td>Folate (ng/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVO patients (n = 75)</td>
<td>5.2 ± 2.9</td>
<td>4.5–6</td>
</tr>
<tr>
<td>CRVO patients (n = 33)</td>
<td>4.7 ± 1.6</td>
<td>4.1–5.3</td>
</tr>
<tr>
<td>BRVO patients (n = 42)</td>
<td>5.7 ± 3.5</td>
<td>4.5–6.9</td>
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Normal plasma vitamin B12 concentrations: 179–1162 pg/mL.
Normal plasma folate concentrations: 2.7–34 ng/mL.

FIGURE 1. Path of homocysteine and cysteine metabolism. Methionine from dietary animal protein donates methyl groups to vital transmethylation reactions, producing important molecules such as creatine and phosphatidylcholine, and allows methylation of DNA, RNA, and neurotransmitters.
genotype failed to identify the presence of the thermodilabile polymorphism as a risk factor for RVO.9,10,18,21,25–24

Our study showed that patients with RVO and control subjects had similar plasma homocysteine concentrations, a result consistent with that reported in other studies.18,21–24 We also found no significant differences in mean plasma cysteine between patients with RVO and control subjects. However, when categorized by type of vein occlusion, mean plasma cysteine was significantly higher in patients with CRVO than in control subjects. This result may be explained in part by the patient’s nutritional status. Although CRVO and BRVO patients had similar plasma vitamin B12 and folate levels, we found that patients with CRVO had a lower mean concentration (−17.3%) of folate than did patients with BRVO. As folate is essential for the conversion of homocysteine to methionine in the remethylation pathway, the reduced intake of folate in patients with CRVO may be responsible for homocysteine’s being directed predominantly toward the transsulfuration pathway, thus leading to an increased level of plasma cysteine. Our results are in agreement with a recent study on the relationship between plasma cysteine levels and the risks of vascular disease, which found that hypercysteinemia is associated with decreased plasma folate.16 In contrast, no relationship was found between hypercysteinemia and vitamin B12.16

We are unaware of any previously reported study assessing plasma cysteine levels in patients with RVO. The concentration of total cysteine in serum and plasma from healthy subjects is 200 to 250 μM, which is 20 times higher than the plasma homocysteine level.57 Cysteine shares some of homocysteine’s chemical properties derived from the presence of its sulfhydryl group.28 It is cytotoxic in vitro and promotes the detachment of human arterial endothelial cells in culture.29,30 Cysteine also exhibits auto-oxidation properties in the presence of metal ions,31 resulting in the generation of free radicals and hydrogen peroxide, which promote the activation of the cellular immune system and support superoxide-mediated modification of low-density lipoprotein (LDL).32,33 Therefore, auto-oxidation of cysteine in vitro promotes several processes involved in atherogenesis and thrombogenesis.24–37 The in vivo effects of hypercysteinemia on vascular endothelium may be more relevant than those of hyperhomocysteinemia, because of its higher concentration. Previous studies have demonstrated increased cysteine levels in patients with myocardial infarction,38 cerebrovascular infarction,39 or peripheral vascular disease.40 In addition, recent studies have shown that increased plasma cysteine is a cardiovascular risk factor.15,16 The detection of significantly higher levels of plasma cysteine in patients with CRVO raises the interesting question of whether hypercysteinemia, apart from being a cardiovascular risk factor, also plays a role in the pathogenesis of CRVO. It is noteworthy that, in our study, one-fourth of the patients with CRVO had a positive cardiovascular history, twice the incidence when compared with BRVO patients. The increased levels of plasma cysteine, observed only in the CRVO group, may account for this result.

In conclusion, this study failed to demonstrate an association between increased plasma homocysteine and RVO. Mean plasma cysteine was significantly higher in CRVO patients, thus suggesting that hypercysteinemia may contribute to the pathogenesis of this retinal vascular disorder. Larger studies are needed to confirm our results and elucidate the possible mechanisms by which increased plasma cysteine may cause CRVO.

References


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