Plasma homocysteine and retinal artery occlusive disease:
A case-control study

Figen Narin, PhD*; Nazmi Narin, MD**; Canan Halici, MD*; Ayse Ozturk Oner, MD***; Hakki Dogan, MD***; Musa Karakukcu, MD**

Background: Previous studies have documented that elevated plasma homocysteine is a risk factor for cardiovascular, cerebrovascular and peripheral vascular disease. In a case-control study, we sought to determine whether elevated homocysteine (HCY) is a risk factor for retinal artery occlusive disease.

Patients and Methods: Study subjects consisted of 20 patients (12 male, 8 female) (mean age, 55.8; range 42-70 years) with clinical and objective evidence of retinal vascular occlusive disease and 20 age-matched control subjects (9 males, 11 females) (mean age, 55.3 years; range 50-68 years). Hyperhomocysteinemia was defined as a plasma HCY level >15 µmol/L by HPLC. We also measured concentrations of triglycerides, and total cholesterol, LDL cholesterol, and HDL cholesterol.

Results: The mean plasma HCY level in the patient group was 21.23±9.53 µmol/L (range, 8.00-43.99 µmol/L) compared with 12.59±4.97 µmol/L (range, 6.38 to 22.88 µmol/L) in the control group (P<0.008). There was no correlation between HCY and serum triglycerides or cholesterol levels within each group. We conclude that high plasma HCY level may be a risk factor for retinal artery occlusive disease.

Key words: Retinal artery occlusion, homocysteinemia, atherosclerosis.

Homocysteine (HCY), a sulfur containing amino acid derived from methionine, is essential for a number of vital biochemical processes. The enzyme 5,10-methylene tetrahydrofolate reductase (MTHFR) catalyzes the conversion of HCY to methionine via remethylation. MTHFR requires folic acid as a cofactor. HCY refers to homocysteine and its oxidized forms homocystine and homocysteine-cysteine disulfide. An elevated plasma level of the amino acid has been identified as an independent risk factor for atherosclerosis, including coronary artery disease, peripheral vascular disease, cerebrovascular disease, and venous thromboembolism. Recent studies indicate that 15 to 30 percent of patients with premature occlusive vascular disease have moderately elevated total plasma HCY concentrations (higher than 15 µmol/L). A plasma HCY concentration exceeding 15 µmol/L defines hyperhomocysteinemia.

While retinal vascular occlusive disease and coronary, cerebral and peripheral vascular disease have a number of risk factors in common, including systemic hypertension and smoking, the classic cardiovascular risk factors do not fully explain these relationships. To date, little information exists on the possibility that elevated HCY might increase the risk of retinal vascular disease. The present study was undertaken to assess the relationship between retinal vascular occlusive disease and level of HCY.

Patients and Methods: Our study included 20 patients (12 male, 8 female) with clinical and objective investigational evidence of retinal vascular occlusive disease. The mean age of the patients was 55.8 years (range, 42-70 years). Exclusion criteria were recent major systemic illness, myocardial infarction, angina, vasculitis, renal, hepatic, or thyroid disease, cardiomyopathy, pregnancy, psychiatric illness, chronic alcohol abuse, anticonvulsant therapy, and recent (within in 3 months) exposure to nitric oxide. All retinal arterioles were evaluated by ophthalmoscopy. All patients had central retinal arterial occlusion (CRAO), with no visible evidence of emboli in the retinal arterioles. Twenty age-matched hospital-based control subjects (9 male, 11 female) with a mean age of 55.3 years (range, 50-68 years) had no history or clinical evidence of retinal vascular disease. All controls were patients attending for routine cataract extraction. Exclusion criteria were the same as for the case group. All subjects gave informed consent to participation.

Blood was collected by venipuncture from both patients and controls and analyzed for biochemical parameters including glucose, creatinine, total cholesterol, triglycerides, LDL and HDL cholesterol, and HCY. Cholesterol, triglyceride, HDL and HDL levels were determined using standard laboratory methods on an automatic analyser. For the homocysteine assay, blood was collected by venipuncture in EDTA bottles from patients.
and controls after a standard 12-hour fast. The plasma was stored at -70°C until analyzed. The sample, internal standard and phosphate-buffered saline (PBS, pH 7.4) were mixed. Tris-2-carboxy-ethylphosphine (TCEP) in water-soluble form was added. After incubation at room temperature for 30 minutes, trichloroacetic acid was added to precipitate proteins. Next, the centrifuged supernatant was mixed with NaOH-borate buffer. The sample was incubated at 60°C in water. After cooling at 4°C, 10 μL of the samples were subjected to HPLC (high-pressure liquid chromatography). The flow rate was 0.7 mL/min. L-homocysteine calibrators (5-100 μmol/L) were prepared in PBS, pH 7.4 and in pooled EDTA plasma.5,6 HCY peaks separated with analytic HPLC were provided in 3.3 minutes. HCY concentrations were calculated according to peak levels provided from a fluorescein detector. The variation co-efficient was 3.5%. The cut-off point was found to be 11.675 μmol/L. Hyperhomocysteinemia was defined as a plasma homocysteine level greater than 15 μmol/L by HPLC.

Statistical significance between the groups was assessed by the independent sample t-test. The correlation between HCY, triglyceride, cholesterol, HDL and LDL cholesterol within each group was evaluated by the Pearson correlation coefficient. A P value <0.05 was considered significant. The tests were performed with SPSS, version 9.0.

Results
The difference in mean age between the patients and controls was not statistically significant (P<0.05). The mean values for glucose, serum triglycerides and cholesterol levels were not significantly different between the patients and control subjects. Differences in HCY concentrations were statistically highly significant (Table 1). The odds ratio for increased risk of retinal vascular occlusive disease in the patient group was 1.17 (95%CI, 1.032-1.327). We found no correlation between HCY and serum triglycerides or cholesterol for subjects within each group.

Discussion
For the past 10 years, efforts to combat atherosclerosis have focused on the reduction serum lipoprotein levels. However, many patients with premature artery disease have no detectable lipoprotein abnormalities, and demonstrate little clinical improvement after intensive cholesterol-lowering therapy. The possible role of hyperhomocysteinemia as an independent atherogenic factor in these patients has recently been recognized.7

Elevated HCY concentrations may be associated with an increased risk of atherosclerosis.24 This association was first observed in patients suffering from homocystinuria, a metabolic disorder with grossly elevated HCY as a result of cystathionine-synthase, methylenetetrahydrofolate reductase or other single enzyme deficiencies in methionine metabolism. Heterozygous states of such enzyme deficiency are believed to be a cause of hyperhomocysteinemia when cofactor deficiencies are excluded.14,16

The pathophysiological mechanism of homocysteine-associated arterial and venous thrombosis, where small, medium and large vessels are involved, is not fully elucidated. Clinical and experimental evidence suggests both direct toxicity to endothelial cells and a synergistic interaction between homocysteine and lipoproteins. Various platelet abnormalities have also been reported including increased adhesiveness, decreased survival and increased thromboxane A activity. A prevailing hypothesis is that homocysteine promotes the clotting cascade via several actions; inactivation of protein C, activation of coagulation factor V, increased vascular smooth muscle proliferation and inhibition of thrombomodulin.1,2,3,5

Increased pulse pressure, higher total cholesterol, smoking status, history of coronary by-pass surgery, and increased white blood cell count are important etiologic factors in retinal occlusive disease, but there is limited information on possible hyperhomocysteinemia-associated retinal occlusive disease.9

Hyperhomocysteinemia has been implicated as a cause of retinal artery and vein occlusions. Cahill et al10 reported a series of 87 patients without diabetes, hypertension or cardiac disease. In this study HCY levels were higher in all disease groups than in control groups and this difference was significant in patients with renal artery occlusion and central vein occlusion. Wenzler et al11 described a series of 19 patients with a history of retinal artery occlusion or retinal vein occlusion younger than the age of 50 years. They found that 4 of 19 patients had elevated HCY levels. Bioussé et al12 reported a patient with bilateral vein occlusion who was found to be hyperhomocysteinemic. Vine et al13 described hyperhomocysteinemia as a risk factor for retinal vascular occlusion in 74 patients. Abu El-

| Table 1. Mean concentrations of homocysteine (HCY), glucose, total cholesterol (TC), triglyceride (TG), HDL and LDL cholesterol in patients and controls. |
|---------------------------------|-----------------|-----------------|
| **Patients (n=20)** | **Controls (n=20)** |
| HCY (μmol/L) | Mean (95% CI) | Mean (95% CI) |
| Range | 21.23±9.53* | 12.52±4.97 |
| (16.77-25.88) | 6.38±2.28 |
| Glucose (mg/dL) | 118.50±20.41 | 120.40±18.52 |
| TC (mg/dL) | 170.00±9.10 | 161.52±7.32 |
| TG (mg/dL) | 102.43±8.71 | 76.40±22.42 |
| HDL-C (mg/dL) | 37.41±4.71 | 45.43±9.97 |
| LDL-C (mg/dL) | 112.72±8.8 | 101.40±6.50 |
*P<0.008 vs. controls
Asrar et al. reported that high plasma homocysteine was a risk factor for retinal vascular occlusive disease. Thus, it may be useful to measure homocysteine in the management of these patients. In our patients, we found elevated HCY levels in patients with retinal artery occlusion. Our results are consistent with previous studies.

In older patients, nutritional rather than genetic factors may be important in increasing HCY levels, a probable risk factor, based on case-control studies, for retinal vascular occlusive disease. The ultimate goal of all risk factors research is to identify useful methods of disease and treatment. The clear-cut relationship between plasma HCY levels and vitamin cofactors means that elevated levels can be reliably reduced to normal in most patients by the administration of folate. Folate treatment is found to be effective in patients without folate deficiency. The treatment and monitoring of HCY level may be valuable in the management of patient with retinal artery occlusive disease.

Our findings suggest that elevated HCY is a risk factor for retinal artery occlusion. The next question to be answered is whether HCY-lowering therapy contributes to the prevention of retinal vascular occlusion.

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