Association of Neovascular Age-related Macular Degeneration and Hyperhomocysteinemia

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• PURPOSE: To assess the relationship between plasma homocysteine levels and exudative neovascular age-related macular degeneration (AMD).
• DESIGN: Cross-sectional study.
• METHODS: A prospective comparative cross-sectional study was conducted in outpatient ophthalmology clinics in a university-affiliated medical institution. The cohort consisted of 59 patients (25 male, 34 female) with a mean age of 78 years (standard deviation [SD] = 8.4) with neovascular AMD who were candidates for photodynamic treatment. Patients were compared for plasma homocysteine levels with 58 patients who had dry AMD (24 male, 34 female) with a mean age of 76.3 years (SD = 8.4) and with a control group of 56 age-matched subjects (27 male, 29 female), with a mean age of 77.3 years (SD = 8.2). A 3-ml venous blood sample was obtained from each participant after an 8-hour fast. Levels of plasma homocysteine were measured by high performance liquid chromatography. The main outcome measure was hyperhomocysteinemia, defined as a plasma homocysteine level above 15 μmol/l.
• RESULTS: Homocysteine levels were higher by 27.9% in the neovascular AMD than in the dry AMD group, and by 21.9% than in the control group (P < .02). Hyperhomocysteinemia was found in 44.1% of the study group, in 22.4% of the dry AMD group, and in 21.4% of the control group (P = .011).
• CONCLUSIONS: This study suggests an association between an elevated plasma level of homocysteine and exudative neovascular AMD but not dry AMD. (Am J Ophthalmol 2004;137:84–89. © 2004 by Elsevier Inc. All rights reserved.)

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A GE-RELATED MACULAR DEGENERATION (AMD) IS the leading cause of legal blindness in the Western world in persons older than 60 years of age.1–4 It has two main presentations, atrophic and exudative. Although the atrophic form, which is slowly progressive, accounts for 90% of all cases, the exudative neovascular form accounts for 88% of all cases of blindness attributable to AMD.1,2 The cause of AMD remains unknown. Recognized risk factors are age5 and heredity.6 Hypertension, high levels of serum cholesterol, and high body mass index are accepted systemic risk factors7,8 and tobacco smoking is an important environmental exposure factor.5

Exudative neovascular AMD involves a degenerative process that leads to proliferation of choriocapillaries into the subretinal space.9 Several authors have recently reported increased levels of plasma vascular endothelial growth factor, von Willebrand factor, and fibrinogen, as well as increased plasma viscosity in patients with AMD. These findings suggest an association of markers of angiogenesis, hemostasis, and endothelial dysfunction with AMD,10 and are supported by the Rotterdam study, which reported an association between AMD and atherosclerosis.11

On the basis of these findings, we hypothesized that neovascular AMD may also be associated with elevated plasma levels of homocysteine, an apparently independent risk factor for atherosclerotic vascular disease, including coronary and cerebrovascular disease.12–14 High levels of plasma homocysteine are toxic to the vascular endothelium by releasing free radicals, creating an environment of hypercoagulability, and modifying the vessel wall.15–18 The aim of the present study was to determine whether hyperhomocysteinemia is involved in AMD.
DESIGN

THIS WAS A PROSPECTIVE, COMPARATIVE CROSS-SECTIONAL STUDY.

METHODS

THE STUDY WAS CONDUCTED IN THE OUTPATIENT OPHTHALMOLOGY CLINICS OF A UNIVERSITY-AFFILIATED MEDICAL INSTITUTION. THE STUDY GROUP CONSISTED OF 59 CONSECUTIVE PATIENTS WITH NEOVASCULAR AMD WHO WERE EXAMINED BY A SINGLE RETINA SPECIALIST (R.A.-S.) OVER A 4-MONTH PERIOD IN THE PHOTODYNAMIC TREATMENT UNIT OF MOR INSTITUTE FOR MEDICAL DATA AND THE RETINAL VASCULAR CLINIC OF RABIN MEDICAL CENTER. A COMPLETE MEDICAL HISTORY WAS OBTAINED, INCLUDING DIABETES, RETINAL DISEASE, HYPERTENSION, HISTORY OF ANGINA PECTORIS, CARDIAC, OR CEREBRAL ARTERIOTHROMBOTIC EVENTS (ATHEROSCLEROTIC CARDIOVASCULAR DISEASE), SMOKING, AND USE OF SYSTEMIC AND OCULAR MEDICATIONS. THE PATIENTS UNDERWENT COMPLETE OCULAR EXAMINATION WITH SLIT-LAMP BIOMICROSCOPY, FUNDUS PHOTOGRAPHY, AND FUNDUS FLUORESCEN ANGIOGRAPHY. ONLY PATIENTS WITH CHOROIDAL NEOVASCULARIZATION (CNV) WERE INCLUDED IN THE STUDY GROUP. SYSTEMIC EXCLUSION CRITERIA WERE RENAL FAILURE, RECENT UNSTABLE ANGINA, MYOCARDIAL INFARCTION OR STROKE, ANEMIA, COLLAGEN OR NEOPLASTIC DISEASE, AND CURRENT SUPPLEMENTAL THERAPY WITH MULTIVITAMINS, FOLIC ACID, VITAMIN B6, OR VITAMIN B12. OCULAR EXCLUSION CRITERIA WERE HISTORY OR PRESENCE OF DIABETIC RETINOPATHY, RETINAL VASCULAR OCCLUSION, AND ANTERIOR ISCHEMIC OPTIC NEUROPATHY, ALL CONDITIONS FOUND TO BE ASSOCIATED WITH ELEVATED PLASMA HOMOCYSTEINE.19

Findings were compared with samples obtained from two groups of patients. The first group included 58 consecutive patients with dry AMD. Those were patients with bilateral soft drusen with or without retinal pigment abnormalities, or geographic atrophy, examined by the same retina specialist (R.A.-S.) in the Outpatient Retina Clinic of Rabin Medical Center during the same time period. The second group (the control group) included 56 age-matched controls without AMD. These were consecutive patients undergoing cataract surgery in the same ophthalmology department during the same time period. A pool of 120 blood samples was available, from which the patients in the control group were matched with the AMD groups for age, sex, and atherosclerotic cardiovascular disease, including history of angina pectoris, cardiac, or cerebral arteriothrombotic events. The latter was chosen, as atherosclerosis is known to be associated with hyperhomocysteinemia.12–14 The same systemic and ocular exclusion criteria were applied to the patients included in the dry AMD group and in the control group without AMD as to the neovascular AMD patients. The study protocol was approved by the Rabin Medical Center Ethics Committee and by the legal department of the Mor Institute for Medical Data. Written informed consent was obtained from all study participants.

A 3-ml venous blood sample was obtained from each participant after an 8-hour fast. The blood was centrifuged and the plasma removed and frozen until all samples were obtained. The blood was then thawed for homocysteine analysis by high-performance liquid chromatography (HPLC) with fluorescent detection.20

As the main outcome measure, hyperhomocysteinemia was defined as a plasma homocysteine level of 15 μmol/l or more.

For statistical analysis, the effect of study group and sex as well as their possible interaction was analyzed jointly using analysis of variance (ANOVA) on logarithmically transformed homocysteine level. The three groups were compared using Tukey pairwise comparisons.

The statistical analysis involved the comparison of proportions outside of externally defined normal ranges using Fisher exact tests. In addition, a similar analysis was performed using internally defined normal range. For that purpose, the mean plus 1.96 standard deviations of the log-transformed homocysteine levels of the control group were used. The statistical analysis was performed using Splus software, version 6.0 (Insightful, Seattle, Washington, USA).

RESULTS

SIXTY-TWO PATIENTS WERE DIAGNOSED WITH NEOVASCULAR AMD. AFTER EXCLUDING THREE PATIENTS OWING TO THE SYSTEMIC AND OCULAR EXCLUSION CRITERIA, THE NEOVASCULAR AMD STUDY GROUP INCLUDED 59 PATIENTS (25 MALE, 34 FEMALE) WITH A MEAN AGE OF 78.0 YEARS (STANDARD DEVIATION [SD] = 8.4 YEARS). THE DRY AMD GROUP INCLUDED 58 PATIENTS (24 MALE, 34 FEMALE) AFTER EXCLUDING 2 PATIENTS OWING TO SYSTEMIC EXCLUSION CRITERIA WITH A MEAN AGE OF 76.3 YEARS (SD = 8.4 YEARS). THE CONTROL GROUP (AGE- AND ARTERIOTHROMBOTIC CARDIOVASCULAR DISEASE-MATCHED PATIENTS WITHOUT AMD) INCLUDED 56 PATIENTS (27 MALE, 29 FEMALE), WITH A MEAN AGE OF 77.3 YEARS (SD = 8.2 YEARS). PATIENT DEMOGRAPHIC CHARACTERISTICS AND MORBIDITY ARE PRESENTED IN TABLE 1. THERE WAS NO STATISTICAL SIGNIFICANT DIFFERENCE BETWEEN THE THREE GROUPS IN AGE AND IN THE RATE OF ARTERIOTHROMBOTIC CARDIOVASCULAR DISEASE AND HYPERTENSION. THERE WAS A SMALLER PROPORTION OF PATIENTS WITH NONINSULIN-DEPENDENT DIABETES MELLITUS IN BOTH AMD GROUPS, COMPARED WITH THE CONTROLS (TABLE 1).

Hyperhomocysteinemia (plasma homocysteine level >15 μmol/l) was found in 26 of 59 blood samples (44.7%) of patients in the neovascular AMD group (13 men and 13 women), in 13 of 58 (22.4%) blood samples in the dry AMD group (six men, seven women; P = .018, Fisher exact test), and in 12 of 56 blood samples (21.4%) of controls (six men and six women; P = .011, Fisher exact test).
There was no difference between the dry AMD and the control groups ($P = 1.00$, Fisher exact test).

Hyperhomocysteinemia was also internally defined using the mean plus 1.96 standard deviations of the log-transformed homocysteine levels of the control group. When backtransformed it amounted to plasma homocysteine level $= 20.6$ nmol/l. Such internally defined hyperhomocysteinemia was found in 12 of 59 blood samples (20.3%) of patients in the neovascular AMD group (six men and six women), in 3 of 58 blood samples (5.1%) in the dry AMD group (one man, two women; $P = .044$, Fisher exact test), and in 2 of 56 blood samples (3.5%) of controls (one man and one woman; $P = .0085$, Fisher exact test). There was no difference between the dry AMD and the control groups ($P = 1.00$, Fisher exact test). Thus, the differences between the three groups are of similar nature whether defining hyperhomocysteinemia externally or internally.

The mean homocysteine levels are presented in Table 2. The mean level was 16.4 nmol/l (SD = 11.9) in the neovascular AMD group, compared with 11.9 nmol/l (SD = 4.1) in the dry AMD group, and with 12.5 nmol/l (SD = 3.5) in the control group ($P < .001$, ANOVA). The distribution of homocysteine levels was studied and found to be approximately normal on the logarithmic scale. The ANOVA of the logarithmically transformed homocysteine level showed that there are statistically significant differences in homocysteine levels among the three groups ($P < .001$). In particular, homocysteine levels were higher in the neovascular AMD group than in the dry AMD and control groups by 27.9% and 21.9%, respectively ($P < .005$ and $P < .02$, respectively, using Tukey pairwise comparison). The homocysteine level in the control group was higher than the dry AMD group by 7% (not statistically significant). Homocysteine levels were higher in male than in female patients by 14% ($P = .01$), with no interaction of group and sex.

Because homocysteine levels were reported to be higher than normal in atherosclerosis, the control group and the neovascular AMD groups were balanced in terms of atherosclerotic cardiovascular disease prevalence. We studied the possible effect of atherosclerotic cardiovascular disease.
Disease on homocysteine levels by introducing it into the ANOVA, comparing the three groups. The effect was not statistically significant \((P = .2)\). Age did not contribute in a statistically significant way to the ANOVA model. We also studied the possible effect of hypertension and diabetes on homocysteine levels, in the above fashion. We found that both hypertension and diabetes had no effect on the homocysteine level \((P = .4\) and \(P = .3\), respectively).

DISCUSSION

The association between AMD and atherosclerosis remains controversial, with many case-control studies reporting a positive association and others failing to confirm these findings.\(^{11,21–23}\) Friedman and associates\(^{24}\) have proposed a model that explains the relationship between neovascular AMD and atherosclerosis. It is based on data that AMD shares both risk factors and pathogenic mechanisms with atherosclerosis, resulting in the deposition of lipid in the sclera and in the Bruch membrane. There is evidence that the scleral lipid results in decreased choriocapillary permeability of choroidal vessels through thickening of the Bruch membrane and decreased perfusion of choroidal capillaries.\(^{25}\)

Atherosclerosis may play a direct role in the development of macular degeneration by affecting the flow and permeability of choroidal vessels through thickening of the Bruch membrane and decreased perfusion of choroidal capillaries.\(^{25}\)

In the choroid, the changes that occur with aging include increased thickness of the Bruch membrane, flattening of the capillaries and narrowing of their lumina, thickening and sclerosis of the precapillary arterioles, and focal choriocapillary dropout. Moreover, in patients with advanced stages of AMD, the decrease in choriocapillary density and diameter is significantly greater than in normal maculae.\(^{25}\) Using fluorescein angiography, Chen and associates\(^{26}\) demonstrated delayed choriocapillary filling in patients with AMD and decreased visual acuity. They suggested that chronic compromise of the choroidal circulation is an important cause of visual impairment in AMD. Arteriosclerosis related to aging is suspected to be the underlying cause of this ischemia.

Yoneya and associates\(^ {27,28}\) examined healthy eyes in subjects older than 50 years of age with indocyanine green angiography. They found that it took longer to fill the choroidal vasculature with dye than in younger patients. On quantitative analysis, the number of choroidal arterioles and the fluorescence intensities in the macular region were significantly reduced with age. Another study on eyes with AMD demonstrated watershed or focal hypofluorescence in the macula, with CNV developing in the areas of hypofluorescence in all the diseased eyes.\(^{29}\) Together, these findings suggest a possible role for ischemia and compensatory choriocapillary neovascularization in patients with AMD.

The present study points to an association of AMD and hyperhomocysteinemia. Homocysteine is a highly reactive amino acid.\(^ {30}\) Epidemiologic studies conducted in the early 1990s have substantiated the presently accepted "normal range" for homocysteine blood levels from 5 to 15 \(\mu\)mol/l in fasting patients. Homocysteine blood levels are sex-related (10%–12% higher in men) and age-related, with gradual elevation with age, especially in the older population. Levels higher than 15 \(\mu\)mol/l are considered to be included in the hyperhomocysteinemic range.\(^ {30,31,32}\) Hyperhomocysteinemia is defined as any value above the 95th percentile or more than 2 standard deviations above the mean values obtained from healthy, fasting control subjects. It is classified as moderate (15–30 \(\mu\)mol/l), intermediate (>30 to 100 \(\mu\)mol/l), or severe (>100 \(\mu\)mol/l).

Several experimental systems have yielded numerous possible mechanisms to account for the vascular effects of homocysteine. Homocysteine has mitogenic activity in vascular smooth muscle cells which could cause arterial wall thickening. It can also induce intracellular release of calcium in these cells, thereby increasing their proliferation and the mass of extracellular matrix.\(^ {30}\) According to another theory, homocysteine causes oxidative injury to endothelial cells and enhances the peroxidation of low-density lipoprotein, thereby promoting the atheromatous process. Increased homocysteine could also augment thrombotic events, as it inhibits the expression of thrombomodulin secreted by the endothelial cells to prevent the activation of protein C. In addition, homocysteine enhances the activity of factors V and VII and the adhesion of platelets to the endothelium. The toxicity of homocysteine to the vascular endothelium may also account for its association with CNV: homocysteine-induced damage to the choriocapillaris endothelium can lead to vascular occlusion and neovascularization.\(^ {15–18}\)

Alternatively, homocysteine may cause thickening of the choriocapillary vessel wall or induce increased mass of extracellular matrix in the choroid, thus promoting ischemia with consequent neovascularization. The increased resistance of choroidal vessels and decreased choroidal perfusion may also cause retinal pigment epithelial atrophy and stimulate the release of vascular endothelial growth factor for neovascularization.

A mildly elevated plasma homocysteine is accepted as an independent risk factor for various pathologies, including vascular diseases and Alzheimer disease.\(^ {12–14,33}\) Dietary factors have been shown to play an important role in the control of homocysteine levels in adults, with additional, but weaker, genetic effects. Specifically, low folate and vitamin B12 have been shown to contribute significantly.
to high plasma homocysteine levels. Moreover, total homocysteine in serum/plasma is increased markedly in patients with cobalamin or folate deficiency, and decreases only when they are treated with the deficient vitamin. A previous study by Seddon and associates has demonstrated a relationship between dietary status and the risk of developing exudative AMD. Therefore, the higher homocysteine plasma level found in our neovascular AMD group may be a marker of nutritional status.

Recent data from the Third National Health and Nutrition Examination Survey showed that AMD does not appear to be associated with homocysteine or its dietary determinants. The authors stated that this may reflect a true lack of association or an inability to detect an association in this population. One such reason could be attributed to the differences in the participants in the fasting state at the time of drawing blood in the mobile examination unit, which could introduce variability in the homocysteine levels. The exclusion of persons with missing homocysteine data (who were more likely to be in the older age groups, in whom AMD is more often seen) could also have biased the results. Respondent biases may have also been introduced, because persons who are visually impaired as a result of AMD would be less likely to have participated in the full examination for the survey. The authors concluded that the results, which are inconsistent across race and age groups, indicate the need for further investigation. In our study, the blood samples were drawn in a fasting state in all patients to prevent variability in the homocysteine levels. To reach the older patients with advanced neovascular AMD and to prevent respondent bias, we performed the study in the photodynamic therapy clinic before treatment. The dry AMD patients were consecutive patients as well, examined at the outpatient retina clinical at the same time period. The compliance rate was good, with all patients agreeing to be tested for homocysteine level. The controls were matched for age and atherosclerotic cardiovascular disease to prevent a bias due to the known positive association among age, atherosclerotic cardiovascular disease, and hyperhomocysteinemia.

In our study, hyperhomocysteinemia was found in 44.1% of the patients with neovascular AMD but only in 22.4% and 21.4% of patients with dry AMD and age-matched controls, respectively. This difference was statistically significant after matching for atherosclerotic cardiovascular disease.

Further prospective studies are needed to examine the possible role of homocysteine in neovascular AMD, and whether supplemental treatment with folic acid, vitamin B6, and vitamin B12 may modify it natural history.

REFERENCES


