Original article

A study on plasma homocysteine level in age-related macular degeneration

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Abstract

Introduction: Age-related macular degeneration (AMD) related to adverse vascular changes is the most frequent cause of irreversible visual impairment in the elderly. Elevated plasma concentrations of serum homocysteine have been shown to increase the risk of vascular disease. **Objective:** To assess the relationship between plasma homocysteine level and age related macular degeneration. **Materials and methods:** A case control study was conducted in a tertiary eye care hospital with 32 diagnosed AMD patients. The patients were compared for plasma homocysteine levels with a control group of 32 patients without AMD. A 1.5 ml of fasting venous blood sample was obtained from each participant. Plasma homocysteine level was measured by high performance liquid chromatography. The main outcome measure was hyperhomocysteinemia, defined as a plasma homocysteine level above 15 μmol/l. **Results:** Hyperhomocysteinemia was found in 10 blood samples (83.3 %) of patients in the wet AMD group, in 16 (80 %) blood samples in the dry AMD group, and in 12 blood samples (37%) of controls. The mean ± SD homocysteine level in the AMD group was 16.86 ± 3.52 μmol/L, while in the non-AMD control group it was 14.53 ± 4.08 μmol/L. This difference was statistically significant (p-value = 0.0186). In the individual analysis, it was also found out that the homocysteine level differed significantly between cases and controls in only the wet variety of AMD. **Conclusion:** Hyperhomocysteinemia was significantly associated with the wet AMD variety but not with the dry AMD. Thus, homocysteine by oxidative stress and vascular dysfunction can be an important risk factor in the pathogenesis of AMD.

Keywords: Dry AMD, wet AMD, homocysteine

Introduction

Age-related macular degeneration (AMD) is the most common cause for visual impairment in individuals of more than 50 years of age. It is the major challenge of the new millennium in the developing countries as the size of the elderly population continues to rise due to better medical facilities and increased life expectancy. AMD is found to be second only to cataract as the cause of severe visual loss in Asian countries. Three population-based studies, namely the Beaver Dam Eye Study (Klein et al, 1992), Blue Mountain Eye study (Mitchel et al, 1995) and the Rotterdam Study (Vingerling et al, 1995) report the prevalence rates of AMD to be 1.7 % in the US, 1.4 % in Australia and 1.2 % in Netherlands respectively.

In AMD, macular degenerative changes have typically been classified into two clinical forms, dry

Received on: 12.12.2012 Accepted on: 09.04.2013
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or wet, both of which can lead to visual loss. In the dry (non-exudative) form, visual loss is gradual. Ophthalmoscopy reveals yellow subretinal deposits called drusen, or retinal pigment epithelial (RPE) irregularities, including hyperpigmentary or hypopigmentary changes. Large drusen become confluent and evolve into drusenoid RPE detachments. These drusenoid RPE detachments often progress to geographic atrophy and less frequently to neovascular AMD. Geographic atrophy involving the centre of the macula leads to vision loss. In the wet (exudative) form, vision loss can occur suddenly, when a choroidal neovascular membrane (CNVM) leaks fluid or blood into the subpigment epithelium or subretinal space. Serous RPE detachments with or without coexisting choroidal neovascularization (CNV) are also classified as a wet form. Exudative serous RPE detachments often, but not always, advance to the neovascular stage.

Homocysteine (Hcy), an intermediary amino acid formed during the conversion of methionine to cysteine, is rapidly auto-oxidized in plasma, forming homocystine, mixed disulfides and homocystine thiolactone. Potent reactive oxygen species, including superoxide anion and hydrogen peroxide, are produced during the auto-oxidation of homocysteine. An elevated homocysteine level has been shown to induce vascular injury, aiding in atherothrombogenesis, and this has been considered an independent risk factor for the development of vascular diseases. (Zarbin et al, 2004; Ambai et al, 2003). 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 the extracellular matrix. 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 (McCully et al, 1969; Stamler et al, 1993; Upchurch et al, 1997; Jakubowsk,1997). Alternatively, homocysteine may cause thickening of the choriocapillary vessel wall or induce an increase in the mass of the 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 the vascular endothelial growth factor for neovascularization (Axer Siegel et al, 2004).

Homocysteine can be converted to methionine with folate and vitamin B12 via the major pathway or with choline and betaine via the minor pathway. Epidemiologic studies conducted in the early 1990s have substantiated the presently accepted "normal range" for homocysteine blood levels from 5 to 15 μmol/l in fasting patients. Homocysteine blood levels are sex-related (10 % – 12 % higher in men) and age-related, with a gradual elevation with age, especially in the older population.

The exact cause of AMD remains unknown. AMD is a multifactorial disease of ageing and several theories of pathogenesis have been proposed including oxidative ocular damage (Delcourt et al, 1999; Cai et al, 2000) and ocular perfusion abnormalities (Friedman et al, 1995; Harris et al, 1999). Atherosclerotic vascular disease has been suspected as a risk factor for the development of AMD in the epidemiologic studies by Delaney et al (1982) and Hyman et al(1983). Risk factors for atherosclerotic disease, such as smoking, hypercholesterolemia, decreased estrogen

exposure, and high intakes of fat or cholesterolhave also been associated with AMD in the studies by Smith et al (2000) and Cho et al (2001).

On the basis of these findings, it can be hypothesized that AMD may also be associated with elevated plasma levels of homocysteine, an apparently independent risk factor for atherosclerotic vascular disease, as evidenced by Clarke et al (1991) and Rosenberg et al (1999). 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. It is possible that changes in the vasculature and antioxidant status as a result of hyperhomocysteinemia may increase the risk of AMD. The aim of the present study was to determine whether hyperhomocysteinemia is involved in AMD.

Materials and methods

The study was conducted in the Ophthalmology and Biochemistry department of a tertiary eye care hospital in eastern India. The study group consisted of 32 consecutive patients with AMD who were examined by a single retina specialist. The control group included 32 age-sex and atherosclerotic cardiovascular disease-matched patients without AMD. The same systemic and ocular exclusion criteria were applied to the persons included in the control group. After obtaining detailed medical history of diabetes, renal disease, hypertension, history of angina pectoris, cardiac or cerebral atherothrombotic events (atherosclerotic cardiovascular disease), smoking, and use of systemic and ocular medications, complete ocular examination with slit-lamp biomicroscopy, fundus photography and fundus fluorescein angiography were performed in all subjects. The exclusion criteria included presence of renal failure, recent unstable angina, myocardial infarction or stroke, anemia, collagen or neoplastic disease and current supplemental therapy with multivitamins, particularly folic acid, vitamin B6 and vitamin B12. Ocular exclusion criteria were diabetic retinopathy, retinal vascular occlusion and anterior ischemic optic neuropathy, as these conditions are found to be associated with elevated plasma homocysteine. The study protocol was approved by the Medical Ethics Committee of the hospital. Written informed consent was obtained from all the study participants.

A 1.5-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.

The statistical analyses were carried out by using the Statistical Package for Social Sciences soft-ware 13.0 for Windows package software (SPSS, Inc., Chicagoil). The unpaired t-test was used for the variables. P values less than 0.05 were considered as statistically significant.

Results

Thirty-two patients diagnosed with AMD were included in this study as cases. So, the study group included 32 patients (14 male, 18 female) with a mean ± SD age of 67.44 ± 6.54 years (range, 54-79). There were 20 patients (9 male, 11 female) with dry AMD and 12 patients (5 male, 7 female) with wet AMD. The control group included 32 patients (14 male, 18 female), with a mean ± SD age of 66.48 ± 5.91 years (range, 54-79). Proper matching was done between cases and controls with regard to age and sex, atherosclerotic cardiovascular disease and hypertension. There was a smaller proportion of patients with noninsulin-dependent diabetes mellitus in the AMD group, compared with the controls. Hyperhomocysteinemia (plasma homocysteine level >15 µmol/l) was found in 10 blood samples (83.3 %) of patients in the neovascular AMD group, in 16 (80 %) blood samples in the dry AMD group and in 12 blood samples (37%) of the controls. The mean ± SD homocysteine level in the AMD group was 16.86 ± 3.52 µmol/L, while in the non-AMD control group it was 14.53 ± 4.08 µmol/L. The standard error of

mean was 0.621 in the AMD group and 0.7446 in the non-AMD group. The p value found using the unpaired t test (value 2.42) with 60 degree of freedom and a 95 % confidence interval was 0.0186.This difference by conventional criteria is considered to be statistically significant and is shown in a tabulated form in Table 1. An analysis was made to find out the relation of homocysteine level in both groups of AMD patients. It was found out that the homocysteine level differed significantly between cases and controls in only the wet variety of AMD (Table 2 & 3).

Table 1. Homocysteine level and AMD

	AMD group	Non AMD group
No.of patients	32	32
Mean Hcy level	16.86	14.53
SD	3.52	4.08
SEM	0.621	0.7446
Min.value	8	7
Max.value	22.3	21.3
t-test=2.42; DF=60; C.I.=95%; p-value=0.0186		

Table 2. Homocysteine level and dry AMD

	AMD group	Non AMD group
No.of patients	20	32
Mean Hcy level	15.99	14.53
SD	3.37	4.08
SEM	0.75	0.7446
Min.value	8	7
Max.value	19	21.3
t-test=1.326; DF=48; C.I.=95%; p-value=0.1911		

Table 3.Homocysteine level and wet AMD:

	AMD group	Non AMD group
No.of patients	12	32
Mean Hcy level	18.325	14.53
SD	3.39	4.08
SEM	0.97	0.7446
Min.value	11.3	7
Max.value	22.3	21.3
t-test=2.848; DF=40; C.I.=95%; p-value=0.0069		

Discussion

The association between AMD and atherosclerosis remains controversial, with many case-control studies by Vingerling et al (1995) and Goldberg et al (1988) reporting a positive association and others failing to confirm these findings. Friedman et al (2000) 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 a decreased choroidal blood flow, as well as in an elevation of the choriocapillary pressure, and the lipids in the Bruch membrane result in basal deposits and drusen and in calcification and fragmentation of the membrane. The hypothesis proposes that it is the combination of elevated choriocapillary pressure, vascular endothelial growth factor and breaks in calcified Bruch membranes that cause CNV. 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 (Ramrattan et al, 1994). 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 the normal maculae. Using fluorescein angiography, Chen et al (1989) demonstrated delayed choriocapillary filling in patients with AMD and decreased visual acuity. They suggested that the 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. Another study by Axer-Siegel et al (2004) demonstrated an association of elevated plasma level of homocysteine

and exudative neovascular AMD in a cohort of 59 patients.

The present study points to an association of AMD and hyperhomocysteinemia. In this study, the blood samples were drawn in a fasting state in all patients to prevent variability in the homocysteine levels. The AMD patients were consecutive patients examined at the outpatient retina clinic. 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 relation among age, atherosclerotic cardiovascular disease and hyperhomocysteinemia. In our study, hyperhomocysteinemia was found in 83.3 % of the patients with neovascular AMD, in 80 % of patients with dry AMD and in 37 % of age-matched controls. This difference between the total cases and controls was statistically significant after matching for atherosclerotic cardiovascular disease. The results varied significantly while comparing the wet AMD cases with the controls.

Conclusion

Based on the results of our study and supportive theories of previous studies, we can conclude that homocysteine by oxidative stress and vascular dysfunction can be an important risk factor in the pathogenesis of AMD and that supplemented treatment with folic acid, vitamin B6 and vitamin B12 can modify the natural disease process of AMD.

Acknowledgement

Department of Biochemistry, RG Kar Medical College, Kolkata-4, India is specially acknowledged.

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Source of support: nil. Conflict of interest: none