European Journal of Ophthalmology / Vol. 15 no. 6, 2005 / pp. 764-767

Homocysteine, vitamin B12, and folic acid in age-related macular degeneration

M. NOWAK[1], E. ŚWIETOCHOWSKA[2], T. WIELKOSZYŃSKI[3], B. MAREK[1], B. KOS-KUDŁA[4], B. SZAPSKA[2], D. KAJDANIUK[1], J. GŁOGOWSKA-SZELÁG[1], L. SIEMIŃSKA[1], Z. OSTROWSKA[2], H. KOZIOŁ[5], J. KLIMEK[5]

[1]Pathophysiology Division, Department of Pathophysiology and Endocrinology
[2]Department of Clinical Biochemistry
[3]Department of Chemistry
[4]Endocrinology Division, Department of Pathophysiology and Endocrinology
[5]Ophthalmology Clinic, Medical University of Silesia, Zabrze - Poland

PURPOSE. *Hyperhomocysteinemia is considered an independent risk factor for atherosclerosis and thrombosis. The purpose of this study was to evaluate plasma homocysteine, red blood cell folate, plasma folate, and plasma vitamin B12 concentration in patients with exudative age-related macular degeneration (ARMD).*
METHODS. *The participants of this study included 30 patients aged 60 to 71years (mean age 66.2±3.6) with exudative ARMD. Plasma homocysteine levels were determined by high performance liquid chromatography (HPLC). Red blood cell folate, plasma folate, and plasma vitamin B12 concentration were determined using a standard kit (Dualcount Solid Phase No Boill radioassay kit for B12/folic acid, DPC Diagnostic, USA) by radioassay method.*
RESULTS. *The plasma concentration of Hcy (14.88±6.23 µmol/L) in ARMD patients was significantly increased (p<0.0001) compared with the control group (8,.72±3,.34 µmol/L). We found not a significant decrease of the plasma vitamin B12 concentration in the ARMD group (476.88±220.91 pg/mL) compared with the control group (527.08±208.97 pg/mL). Red blood cell folate (158.44±56.30 ng/mL) and plasma folate (6.5±3.4 ng/mL) in ARMD patients were also not significantly decreased when compared with the control group (183.86±59.33 ng/mL and 7.93±5.05 ng/mL).*
CONCLUSIONS. *Hyperhomocysteinemia might be one of the risk factors for the exudative form of ARMD. (Eur J Ophthalmol 2005; 15: 764-7)*

KEY WORDS. *Age-related macular degeneration, Homocysteine, Vitamin B12, Folic acid*

Accepted: May 8, 2005

INTRODUCTION

Age-related macular degeneration (ARMD) is a primary degenerative disorder of the central retinal area with loss of visual acuity, most often apparent in people over 50 years of age (1).

The late stage of ARMD includes both dry (geographic) and wet (disciform, exudative) form. In the exudative form, retinal pigment epithelium (RPE) detachment and choroidal neovascularization is present (2).

Apart from age, the risk factors of ARMD are genetic predisposition (3, 4), cigarette smoking (5), post-

1120-6721/764-04$15.00/0

The results of this study were presented as a poster at the 37th Annual Scientific Meeting of the European Society for Clinical Investigation; April 2–5, 2003; Verona, Italy

menopausal estrogen decrease (6), excessive exposure to sunlight (7), oxidative stress (8, 9), and low macular pigment level (10, 11). Recent articles discuss vascular diseases and blood lipid disturbances as risk factors for ARMD (12-15).

Homocysteine (Hcy) is an intermediate product of the methionine metabolism. Folic acid, vitamin B12, and vitamin B6 deficiencies as well as reduced enzyme activities inhibit metabolism of the Hcy, thus increasing the Hcy concentration.

Recent studies have shown a relationship between elevated levels of Hcy and vascular disease including cerebrovascular accidents and myocardial infarctions (16, 17). Elevated Hcy plasma is considered an independent risk factor for atherosclerosis and thrombosis (18). Moore et al found that Hcy is toxic to neurons of the ganglion cell layer and that apoptosis of the ganglion cells is faster in simultaneous elevation of Hcy and glutamate (19).

The purpose of this study was to determine plasma homocysteine, red blood cell folate, plasma folate, and plasma vitamin B12 concentration in patients with exudative ARMD.

METHODS

The participants of this study included 30 patients aged 60 to 71years (mean 66.2±3.6) with exudative ARMD. Exclusion criteria were diseases other than ARMD associated with neovascularization, retinal detachment, severe ocular trauma, high myopia, and intraocular inflammation.

The control group consisted of 20 patients aged 55 to 70 years (mean 65.8±5.2) without ophthalmologic complications or family history of ARMD reporting to an ophthalmologic outpatient clinic for routine tests and for glasses correction. No patients were on antioxidant micronutrients supplementation and all lived in the same industrial area. Because of the small number of patients in studied groups we excluded diabetic patients and those with a current or past history of smoking. Both groups of patients were matched by age and sex.

The eye examination included visual acuity, biomicroscopy, ophthalmoscopy using either a 90-diopter lens or a direct ophthalmoscopy after papillary dilation, photography, and fluorescein angiography of the retina. Exudative ARMD was found in eyes with serous or hemorrhagic detachment of the RPE, hemorrhage, or a grey subretinal fibrous membrane involving the macula (2).

In the morning hours (after 8- to 12-hour fasting) blood specimens for biochemical examination were collected. Plasma homocysteine levels were determined by high performance liquid chromatography (HPLC) method with fluorescence detection. Red blood cell folate, plasma folate, and plasma vitamin B12 concentration were determined using a standard kit (Dualcount Solid Phase No Boill radioassay kit for B12/folic acid, DPC Diagnostic) by radioassay method.

Statistical analysis was carried out using the Statistica 5.0 Pl program with Student t-test for normal distribution

TABLE I - PLASMA HOMOCYSTEINE, RED BLOOD FOLATE, PLASMA FOLATE, AND VITAMIN B12 CONCENTRATIONS IN PATIENTS WITH AGE-RELATED MACULAR DEGENERATION (ARMD) AND THE CONTROL GROUP

	ARMD (n=30)	**Control (n=20)**	**p value**
Hcy (µmol/L)	14.88±6.23	8.72±3.34	<0.0001
Vitamin B12 (pg/mL)	476.88±220.91	527.08±208.97	NS 0.061
Plasma folate (ng/mL)	6.5±3.40	7.93±5.05	NS 0.2502
Red blood cell folate (ng/mL)	158.44±56.30	183.86±59.33	NS 0.36

Values are means ± standard deviation
Hcy = Homocysteine

of the results in the study groups or Mann-Whitney U test in other than normal distribution, assuming $p<0.05$ as statistically significant.

The project was carried out with the permission of The Bioethical Board of the Medical University of Silesia (NN 013-275/01/02). All subjects gave a formal consent before participating in the study, and research followed the tenets of the Declaration of Helsinki.

RESULTS

The results obtained in the biochemical investigations in the ARMD and control patients are presented in Table I.

Plasma concentration of Hcy in patients with ARMD was significantly increased ($p<0.0001$) compared with the control group. Plasma vitamin B12, red blood cell folate, and plasma folate concentration in patients with ARMD were decreased when compared with the control group, although not significant.

DISCUSSION

Hyperhomocysteinemia is the result of a disturbed methionine metabolism. It may result from reduced enzyme activity and/or vitamin B12, B6, and folate deficiency (18).

Hyperhomocysteinemia is believed to play an important role in the development of age-related and several neurodegenerative disorders. Elevated Hcy levels have been recognized as a risk factor for venous thrombosis (20, 21). Previous studies documented that an elevated plasma Hcy level is also a risk factor for central retinal vein occlusion (22-25).

The authors have found that patients with retinal venous occlusive disease have higher levels of homocysteine, which may serve as a modifiable risk factor.

Heuberger et al (26) have not found a correlation between total serum homocysteine, red blood cell folate, and serum cyanocobalamin level and ARMD. They concluded that ARMD does not appear to be associated with homocysteine or its dietary determinants. Consistent with our results, Chan et al (16) found that plasma Hcy levels of patients with cardiovascular disease did not correlate to their plasma vitamin B status. It is possible that most known forms of vascular damage or injury are due to homocysteine mediated oxidative stress.

Atherosclerotic vascular disease, due to its influence on the choroidal circulation, has been hypothesized as a possible pathogenetic factor for development of ARMD (27).

In a recent study, Vingerling et al (28) found that macular degeneration was associated with atherosclerosis determined by the presence of plaques in the carotid bifurcation and common carotid artery.

Data from the National Health and Nutrition Examination Survey also showed that persons with a positive history of atherosclerotic vascular disease had a higher prevalence of ARMD (29). The atherosclerotic process could play an important role in ARMD development by affecting the flow of choroidal vessels, but the mechanism for this process is unclear (13). This can be the reason for ischemia of the avascular macular zones of the retina and disturbances in balance of angiogenic and antiangiogenic factors, which might consequently lead to neovascularization of the macular region.

A possible explanation for the elevated Hcy level in patients with ARMD is decreased concentration of vitamin B12 and folate and consequent reduced enzymes activities that inhibit the breakdown of Hcy.

CONCLUSIONS

Hyperhomocysteinemia might be one of the risk factors for exudative ARMD. Further studies are necessary to define the usefulness of Hcy concentration measurement in patients with ARMD.

ACKNOWLEDGEMENTS

The project was carried out with the permission of The Bioethical Board of the Medical University of Silesia NN 013-275/01/02.

The authors have no proprietary interest in any aspect of the article.

Reprint requests to:
Mariusz Nowak, MD
Department of Pathophysiology and Endocrinology
Medical University of Silesia
pl. Traugutta 2, Poland
41-800 Zabrze pl.
nowak-mar@wp.pl

REFERENCES

1. Hyman L. Epidemiology of eye diseases in the elderly. Eye 1987; 1: 330-41.
2. The International ARM Epidemiological Study Group. An international classification and grading system for age-related maculopathy and age-related macular degeneration. Surv Ophthalmol 1995; 39: 367-74.
3. Silvestri G, Johnston PB, Hughes AE. Is genetic predisposition an important risk factor in age-related macular degeneration? Eye 1994; 8: 564-8.
4. Kimura K, Isashiki Y, Sonoda S, Kakiuchi-Matsumoto T, Ohba N. Genetic association of manganese superoxide dismutase with exudative age-related macular degeneration. Am J Ophthalmol 2000; 130: 769-73.
5. Klein R, Klein BE, Linton KL, DeMets DL. The Beaver Dam Eye Study: the relation of age-related maculopathy to smoking. Am J Epidemiol 1993; 137: 190-200.
6. The Eye Disease Case-Control Study Group. Risk factors for neovascular age-related macular degeneration. Arch Ophthalmol 1992; 110: 1701-8.
7. Cruickshanks KJ, Klein R, Klein BE. Sunlight and age-related macular degeneration. The Beaver Dam Eye Study. Arch Ophthalmol 1993; 111: 514-8.
8. Nowak M, Świçtochowska E, Wielkoszy_ski T, et al. Changes of the blood antioxidants and several lipid peroxidation products in women with age related macular degeneration. Eur J Ophthalmol 2003; 13: 281-6.
9. Beaty S, Koh HH, Phil M, Henson D, Boulton M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. Surv Ophthalmol 2000; 45: 115-34.
10. Beatty S, Murray IJ, Henson DB, Carden D, Kob HH, Boulton ME. Macular pigment and risk for age-related macular degeneration in subjects from a northern European population. Invest Ophthalmol Vis Sci 2001; 42: 439-46.
11. Bone RA, Landrum JT, Mayne ST, Gomez CM, Tibor SE, Twaroska EE. Macular pigment in donor eyes with and without ARMD: a case-control study. Invest Ophthalmol Vis Sci 2001; 42: 235-40.
12. Hyman L, Schachat AP, He Z, Leske C. Hypertension, cardiovascular disease, and age-related macular degeneration. Arch Ophthalmol 2000; 118: 351-8.
13. Ross RD, Barofsky JM, Cohen G, Baber WB, Palao SW, Gitter KA. Presumed macular choroidal watershed vascular filling, choroidal neovascularization, and systemic vascular disease in patients with age-related macular degeneration. Am J Ophthalmol 1998; 125: 71-80.
14. Smith W, Mitchell P, Leeder SR, Wang JJ. Plasma fibrinogen levels, other cardiovascular risk factors, and age-related maculopathy. Arch Ophthalmol 1998; 116: 583-7.
15. Nowak M, Świçtochowska E, Marek B, et al. Changes in the lipid metabolism in women with age-related macular degeneration. Clin Exp Med 2005; 4: 183-7.
16. Chan SJ, Chang CN, Hsu JC, Lee YS, Shen CH. Homocysteine, vitamin B(6), and lipid in cardiovascular disease. Nutrition 2002; 18: 595-8.
17. Laraqui A, Bennouar N, Meggouh F, et al. Homocysteine, lipoprotein (a): risk factors for coronary heart diseases. Ann Biol Clin (Paris) 2002; 60: 549-57.
18. Stanger O, Herrmann W, Pietrzik K, et al. DACH-LIGA homocysteine (German, Austrian, and Swiss homocysteine society): consensus paper on the rational clinical use of homocysteine, folic acid and B-vitamins in cardiovascular and thrombotic diseases: guidelines and recommendations. Clin Chem Lab Med 2003; 41: 1392-403.
19. Moore P, El-sherbeny A, Roon P, Schoenlein PV, Ganapathy V, Smith SB. Apoptotic cell death in the mouse retinal ganglion cell layer is induced in vivo by the excitatory amino acid homocysteine. Exp Eye Res 2001; 73: 45-57.
20. Fernandez-Moreno MC, Castilla-Guerra L, Castella-Murillo A, et al. Hyperhomocysteinemia- related cerebral venous thrombosis. Rev Neurol 2003; 37: 1040-3.
21. den Heijer M. Hyperhomocysteinaemia as a risk factor for venous thrombosis: an update of the current evidence. Clin Chem Lab Med 2003; 41: 1404-7.
22. Cahill MT, Stinnett SS, Fekrat S. Meta-analysis of plasma homocysteine, serum folate, serum vitamin B(12), and thermolabile MTHFR genotype as risk factors for retinal vascular occlusive disease. Am J Ophthalmol 2003; 136: 1136-50.
23. Abu El-Asrar AM, Abdel Gader AG, Al-Amro SA, Al-Attas OS. Hyperhomocysteinemia and retinal vascular occlusive disease. Eur J Ophthalmol 2002; 12: 495-500.
24. Brown BA, Marx JL, Ward TP, et al. Homocysteine: a risk factor for retinal venous occlusive disease. Ophthalmology 2002; 109: 287-90.
25. Vine AK. Hyperhomocysteinemia: a new risk factor for central retinal vein occlusion. Trans Am Ophthalmol Soc 2000; 98: 493-503.
26. Heuberger RA, Fisher AI, Jacques PF, et al. Relation of blood homocysteine and its nutritional determinants to age-related maculopathy in the third National Health and Nutrition Examination Survey. Am J Clin Nutr 2002; 76: 897-902.
27. Klein R, Klein BEK, Jensen SC. The relation of cardiovascular disease and its risk factors to the 5-year incidence of age-related maculopathy. Ophthalmology 1997; 104: 1804-11.
28. Vingerling JR, Dielemans I, Bots ML, Hofman A, Grobbee DE, de Jong PT. Age-related macular degeneration is associated with atherosclerosis. The Rotterdam Study. Am J Epidemiol 1995; 142: 404-9.
29. Goldberg J, Flowerdew G, Smith E, Brody JA, Tso MOM. Factors associated with ARMD: analyses of data from the first NHANES. Am J Epidemiol 1988; 128: 700-10.