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Plasma Homocysteine, Folic Acid, and Vitamin B_{12} Levels in Patients With Pseudoexfoliation Syndrome, Pseudoexfoliation Glaucoma, and Normotensive Glaucoma

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Key Words: *homocysteine; pseudoexfoliation; normotensive glaucoma; folic acid; vitamin B_{12}.*

Summary. *Objective. The aim of this study was to evaluate the levels of plasma homocysteine (Hcy), vitamin B_{12}, and folic acid in patients with pseudoexfoliation glaucoma (PEXG), pseudoexfoliation syndrome (PEXS), PEXS plus normotensive glaucoma (NTG).*

Material and Methods. In total, 24 patients with PEXG, 35 patients with PEXS, 18 patients with PEXS plus NTG, and 35 control subjects were enrolled into study. Their Hcy levels were measured by high performance liquid chromatography (HPLC); the levels of vitamin B_{12} and folic acid were measured by a competitive electrochemiluminescence immunoassay.

Results. Higher plasma Hcy levels and lower folic acid and vitamin B_{12} levels were found in all 3 patients' groups compared with the control group (all $P<0.001$, expect for folic acid in the PEXG group, $P=0.03$). Although plasma Hcy levels in the PEXG and PEXS groups were similar, the PEXS plus NTG group had significantly higher Hcy levels compared with these groups ($P=0.019$ and $P=0.032$, respectively).

Conclusions. Our study showed that there was an association between hyperhomocysteinemia and PEXS either with or without glaucoma. The patients with PEXS plus NTG had higher plasma Hcy levels than the patients with PEXS or PEXG and the healthy controls. The treatment of hyperhomocysteinemia by taking low-cost vitamin B_{12} and folic acid preparations may prevent additional vascular problems.

Introduction

The pathogenesis of optic nerve damage in glaucoma is complex and remains poorly understood. While intraocular pressure (IOP) is thought to be the most important risk factor for glaucoma, there is a growing body of evidence that vascular risk factors may also play an important role (1). Factors leading to vascular dysregulation may contribute to the pathogenic process (2); therefore, impaired microcirculation and abnormal perfusion may cause ischemia of the optic nerve head (3).

Homocysteine (Hcy) is a highly reactive amino acid that is synthesized during the metabolism of methionine. The elevation of plasma Hcy levels is a well-known independent risk factor for vascular diseases and may contribute to ischemic changes and oxidative stress (2). Vitamin B_{12} and folic acid, which are required as cofactors in the Hcy metabolism, are nongenetic determinants of Hcy levels in plasma; an alteration in either of these cofactors may lead to elevated Hcy plasma levels (4).

Pseudoexfoliation syndrome (PEXS) is associated with the extracellular deposition of the fibrillar material in the outflow pathway of the trabecular meshwork (5). This is considered to be the most common identifiable cause of open-angle glaucoma (6). An active involvement of the trabecular meshwork in this matrix process may lead to the development of glaucoma in about half of the patients who have PEXS (7), but the exact mechanisms underlying the development of PEXS and the subsequent progression to pseudoexfoliation glaucoma (PEXG) remain unknown (8).

In literature, higher levels of plasma Hcy in PEXG and PEXS patients have been reported (9–12). In addition, studies have looked at whether patients with normotensive glaucoma (NTG) have elevated Hcy levels as well (10, 13–15). However, to the best of our knowledge, the plasma Hcy levels of patients with PEXS plus NTG have not been studied. Therefore, the aim of our study was to evaluate the plasma levels of Hcy, folic acid, and vitamin B_{12} in patients with PEXG, PEXS, and also PEXS plus NTG and to compare these findings with those of healthy subjects.

Material and Methods

The study involved 112 participants, including 24 patients with PEXG, 35 patients with PEXS, 18

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patients with PEXS plus NTG in one or both eyes, and 35 control subjects. The subjects were divided into 4 groups: PEXG, PEXS, PEXS plus NTG, and control. All patients were newly diagnosed and had no previous history of antiglaucomatous treatment. The study was conducted in accordance with the Declaration of Helsinki, all the subjects gave their informed consent, and the Local Ethics Committee approved the study.

A detailed medical history was obtained from all the enrolled subjects in order to determine whether they had known or suspected diabetes mellitus, systemic hypertension, coronary artery disease, cerebrovascular disease or were receiving any drug therapy at that time. All the patients were referred to the same cardiologist (C.K.) for the further evaluation of possible systemic diseases.

Each patient underwent a complete eye examination, including best-corrected visual acuity, perimetry (Humphrey Field Analyzer; HFA II, model 750, Humphrey instruments, San Leandro, CA, 30–2 full threshold program), slit-lamp examination of the anterior segment, gonioscopic evaluation of the anterior chamber angle, pachymetry, IOP measurement by Goldmann applanation tonometry, and fundoscopy.

PEXS diagnosis was made on seeing the exfoliation material on the anterior lens capsule or the edge of the pupil before or after dilatation during the biomicroscopical examination.

The patients with IOP of 21 mm Hg or more, glaucomatous damage in the optic disc, and glaucomatous damage during a visual field examination were diagnosed as having PEXG. The glaucomatous visual field was considered when one of the following criteria was fulfilled on 2 consecutive examinations: abnormal results of the glaucoma hemifield test; a band of 3 or more nonedge points approved with a<5% probability of normality, one of which should have $P<1\%$ and none should be contiguous with the blind spot; or a corrected pattern standard deviation of <5% when the visual field was otherwise normal (16).

The diagnostic criteria for NTG were glaucomatous changes in the optic disc (focal or concentric atrophy, neuroretinal rim loss, peripapillar hemorrhage), IOP of 21 mm Hg or lower, and glaucomatous changes in a visual field test (16).

The control subjects did not have any history of ocular diseases (except refractive error, cataract). The patients with a retinal lesion other than glaucoma that might affect the visual field examination, any ocular disease similar to corneal opacity, any inflammatory or compressive disease that might affect the optical nerve, a history of eye surgery and a thromboembolic, hepatic, or neurological disease that might affect Hcy levels, smoking history and those who were given medications (statins, antidepressants, dopaminergic drugs, phenytoin) were excluded from the study.

Biochemical Assessment. The blood samples taken for the measurement of Hcy, vitamin B_{12}, and folic acid levels were collected into a heparinized tube and transferred in ice blocks. The samples were then centrifuged at 4000 rpm for 5 minutes and stored at –20°C until the biochemical assay. Hcy levels were determined by high performance liquid chromatography (HPLC) (Shimadzu Corporation, Kyoto, Japan). The levels of vitamin B_{12} and folic acid were measured by a competitive electrochemiluminescence immunoassay (Immulite 2000-BIODPC, Los Angeles, CA, USA). The reference ranges for plasma Hcy, vitamin B_{12}, and folic acid were 5–14 μmol/L, 145–980 pg/mL, and 2.7–34.0 ng/mL, respectively, according to the manufacturer's instructions. Hyperhomocysteinemia was defined when a plasma Hcy level was greater than 14 μmol/L.

Statistical Analysis. Statistical analysis was performed using the SPSS software, version 15.0. The Mann-Whitney *U* test was used to compare each of the patients' groups with the control group and also to compare the patients' groups to each other. The Kruskal-Wallis test was used to compare the mean age among the groups. Qualitative variables were compared using the chi-square test. A *P* value of <0.05 was considered to be statistically significant.

Results

Table 1 shows the demographic and clinical data of the subjects. There were no significant differences between the groups in terms of age, gender, systemic hypertension, and coronary artery disease ($P>0.05$). The laboratory data of the subjects are given in Table 2. Significantly higher plasma Hcy levels and lower folic acid and vitamin B_{12} levels were found in all 3 patients' groups compared with those of the control group (all $P<0.001$, except $P=0.03$ for the folic acid level in the PEXG group). There were no significant differences in the mean plasma folic acid, and vitamin B_{12} levels between the patient groups. Although the plasma Hcy levels in the PEXG and PEXS groups were similar, the PEXS plus NTG group had significantly higher Hcy levels compared with the 2 patients' groups ($P=0.019$ and $P=0.032$, respectively).

Hyperhomocysteinemia was present in 12 (50%) of the 24 patients with PEXG, 22 (62%) of the 35 patients with PEXS, 17 (94%) of the 18 patients with PEXS plus NTG, and 1 (12%) of the 35 control patients.

Discussion

In this study, increased plasma Hcy levels and decreased folic acid and vitamin B_{12} levels were found

Table 1. Demographic and Clinical Data of the Groups

	PEXG (n=24)	PEXS (n=35)	PEXS+NTG (n=18)	Control (n=35)	*P* Value
Age, mean (SD), years	67.0 (6.9)	67.6 (7.4)	68.3 (9.4)	69.6 (6.5)	>0.05*
Gender, n (male/female)	10/14	20/15	10/8	18/17	>0.05†
Hypertension, n (%)	6 (25)	10 (28.5)	5 (27.7)	9 (25.7)	>0.05†
CAD, n (%)	3 (12.5)	5 (14.2)	2 (11.1)	4 (11.4)	>0.05†

PEXG; pseudoexfoliative glaucoma, PEXS; pseudoexfoliative syndrome, NTG; normotensive glaucoma, CAD; coronary artery disease.
*Kruskal-Wallis test (for quantitative data), †chi-square test (for qualitative data).

Table 2. Laboratory Data of the Groups

	PEXG (n=24)	PEXS (n=35)	PEXS+NTG (n=18)	Control (n=35)
Homocysteine, μmol/L	15.4 (6.0)*	15.8 (5.6)*	19.8 (5.5)‡	9.9 (3.5)
Folic acid, pg/mL	8.2 (3.6)†	6.3 (3.2)*	7.0 (4.4)*	10.7 (4.7)
Vitamin B_{12}, ng/mL	232.2 (104.8)*	241.4 (141.6)*	227.2 (104.0)‡	372.8 (138.8)

Values are expressed as mean (standard deviation).
PEXG; pseudoexfoliative glaucoma, PEXS; pseudoexfoliative syndrome, NTG; normotensive glaucoma.
*$P<0.001$ compared with the control group (Mann-Whitney *U* test); †$P<0.05$ compared with the control group (Mann-Whitney *U* test); ‡$P<0.001$ compared with the control group (Mann-Whitney *U* test) and $P<0.05$ compared with both PEXG and PEXS groups (Mann-Whitney *U* test).

in the patients with PEXS, PEXG, and PEXS plus NTG as compared with the healthy controls. In addition, the PEXS plus NTG group had the highest level of plasma Hcy among the groups, and nearly all of the patients (94%) in this group had hyperhomocysteinemia. To the best of our knowledge, this is the first study that evaluated plasma Hcy, folic acid, and vitamin B_{12} levels in patients with PEXS plus NTG.

PEXS presents clinically as small whitish deposits of the fibrillar and granular material in the anterior segment of the eye. It may also be found in the extraocular tissues, including the skin, heart, lungs, liver, kidneys, gall bladder, and cerebral meninges (17, 18). Although the pathogenic mechanism of PEXS and its clinical importance still are not exactly clear, some studies have shown decreased orbital blood flow in patients with PEXS (19–23). Ocular and systemic vascular disorders, especially central retinal vein occlusion, aortic aneurysms, and cerebrovascular and cardiovascular disease, were seen commonly in patient with PEXS and high Hcy levels (21, 24–28).

Hyperhomocysteinemia causes an increase in the expression of elastolytic matrix metalloproteinase (MMP) 2 and 9 in the vessel wall, as well as the expression of the human tissue activator of MMP-2 and MMP-9 (29). The structural association of exfoliation fibers with the components of the elastic system (i.e., zonular fibers) has been reported, along with the similar histochemical staining properties of the exfoliative material and zonules and the ultrastructural indications for the development of degenerating elastic microfibrils into the exfoliative material (8). These findings support the hypothesis that Hcy plays a role in the mechanism involved in the formation of the pseudoexfoliation material.

Our results are in accordance with the previous studies that put forward the association of hyperhomocysteinemia and PEXS, with or without glaucoma (9–12). Vessani et al. (9) have compared plasma Hcy concentrations among patients with exfoliation syndrome, exfoliative glaucoma, and normal-tension glaucoma and healthy control subjects and shown that Hcy levels were higher in both exfoliative groups compared with the control group. The levels were higher in those with normal-tension glaucoma, but not significantly different from those of the controls. Leibovitch et al. (10) have demonstrated the prevalence of hyperhomocystinemia in patients with PEXG to be significantly increased compared with subjects who did not have ocular disease, but they had a similar vascular risk profile. Puustjärvi et al. (11) have shown that plasma Hcy levels were significantly higher in the patients with PEXS than in the normal controls, but the Hcy concentration in the aqueous humor did not differ between the 2 groups. Altıntas et al. (12) evaluated total plasma Hcy and nitric oxide marker levels in the patients with PEXS, PEXG, and primary open-angle glaucoma (POAG) and the healthy controls. The mean plasma homocysteine level was statistically significantly elevated in the PEXS and PEXG groups, but not in the POAG group when compared with the control group. In addition, multiple logistic regression analysis performed in this study showed that an elevation in the plasma Hcy concentration was a significant risk factor for PEXS and PEXG, but was not a significant risk factor for POAG.

Vitamin B_{12} and folic acid are the most common environmental cofactors for Hcy. Deficiencies in vi-

tamin B_{12} and folic acid cause the majority of cases of hyperhomocysteinemia (30). The levels of folic acid (15, 31) and vitamin B_{12} (31, 32) have been found to be lower in patients with PEXG, which is similar to our results. A study by Lobo et al. has reported that folic acid, vitamin B_{12}, and vitamin B_6 supplementation can reduce Hcy levels by 30% (33).

The exfoliative process has been reported as a risk factor for optic nerve head damage, independent of IOP (34–37). In our previous study, we showed that there were glaucomatous findings in a significant proportion of the normotensive patients with PEXS (38).

This study showed that the plasma Hcy levels in the patients with PEXS plus NTG were higher than in the patients with PEXS and PEXG. However, the results concerning the plasma Hcy levels in NTG are conflicting. No relationship was found between the elevated Hcy levels and NTG (10, 13–15). On the other hand, Clement et al. (39) demonstrated that in their study population, elevated plasma Hcy occurred in NTG at comparable levels and concluded that elevated plasma Hcy seemed to be associated with glaucoma in these patients. The results of our study were in agreement showing that the patients in the PEXS plus NTG group had the highest plasma Hcy levels among all the groups. Therefore, we may infer that high Hcy levels increase the risk of NTG progression in patients with PEXS, independent of IOP. Vascular, neurotoxic, and apoptotic factors might play a role in this progression (40).

Conclusions

Our study showed that plasma Hcy levels were increased and those of folic acid and vitamin B_{12} were reduced in the patients with PEXS, PEXG, and PEXS plus NTG. The most remarkable finding was that the patients who had PEXS plus NTG had higher plasma Hcy levels than the patients with PEXS and PEXG and the healthy controls and hyperhomocysteinemia was more common among them. However, it is not clear whether the elevated Hcy levels contribute to the pathogenesis of the pseudoexfoliation material and to the development of normotensive- or high-pressure glaucoma in these patients, but we suggest that the measurements of plasma Hcy might be useful in order to understand the pathogenesis of PEXS plus NTG. Hyperhomocysteinemia is readily reversible in most patients, when they take low-cost vitamin B_{12} and folic acid preparations. This treatment may also reduce other vascular problems in patients with pseudoexfoliation.

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Statement of Conflict of Interest

The authors state no conflict of interest.

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