

Plasma homocysteine levels and Vitamin B status in patients with Pseudoexfoliation syndrome

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ABSTRACT

Objectives: To determine and compare the plasma levels of homocysteine and vitamin B (B6, B12 and folate) in patients with Pseudoexfoliation syndrome (PEXS), pseudoexfoliation glaucoma (PEXG), retinal vein occlusion with pseudoexfoliation (PEX+RVO) and in normal individuals.

Methods: The current study was conducted in the Third Eye Clinic, Ankara Numune Training and Research Hospital, Turkey, between August 2004 and February 2005. Twenty cases with PEXS (Group 1), 20 cases with PEXG (Group 2), 16 cases with PEX+RVO (central or branch retinal vein occlusion) (Group 3) and 20 normal individuals (control group) were included in the study. Those who use vitamin supplements or drugs affecting the plasma homocysteine levels were excluded from the study.

Results: Plasma homocysteine levels were 17.6 ± 4.4

mmol/l in Group 1, 18.5 ± 4.5 mmol/l in Group 2, 22.2 ± 6.0 mmol/l in Group 3, and 14.0 ± 3.1 mmol/l in the control group. It was highest in Group 3 ($p<0.001$). The ratio of hyperhomocysteinemia was calculated as 35% (Group 1), 45% (Group 2), 68.7% (Groups 3) and 15% (control). These values were statistically higher in the groups with PEXS than in the control group ($p=0.009$). We did not find any statistically significant difference between the groups with respect to the levels of vitamin B6 and B12 ($p>0.05$), but the level of folate was lowest in Group 3 ($p<0.001$).

Conclusion: Hyperhomocysteinemia is a risk factor for thromboembolic vasculopathy in patients with PEXS and PEXG. Therefore, vitamin B supplementation should be considered in these patients when hyperhomocysteinemia is detected.

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Homocysteine is an amino acid formed in methionine catabolism. It is a toxic product so it has to be metabolized. Vitamin B (B6, B12 and folate) function as co-factors in demethylation, remethylation and trans-sulphuration reactions which take place in relation to the methionine concentration.¹ In recent years, it was reported in many studies that high homocysteine level was an independent risk factor for cardiovascular diseases, cerebrovascular diseases and peripheral vascular diseases.²⁻⁴ In relation with hyperhomocysteinemia, it is now listed among the risk

factors such as hypercholesterolemia and smoking. It was recorded that hyperhomocysteinemia was closely related to vitamin B status and that plasma homocysteine level could be decreased with dietary vitamin B supplementation.^{5,6} Hyperhomocysteinemia was reported in patients with Pseudoexfoliation syndrome (PEXS) and pseudoexfoliation glaucoma (PEXG),^{7,8} but there is no study investigating vitamin B status in these patients according to our knowledge. We aimed to determine and compare the plasma levels of homocysteine and vitamin B (B6, B12 and folate)

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in patients with PEXS, PEXG, retinal vein occlusion with pseudoexfoliation (PEX+RVO) (with or without glaucoma), and in the control group formed from the individuals with a similar age distribution.

Methods. Medical histories of the patients including systemic diseases such as hypertension, diabetes mellitus, cardiovascular disease and cerebrovascular disease and smoking were recorded. Those who use vitamin supplements or drugs affecting the plasma homocysteine levels were excluded from the study. In addition, the patients with renal dysfunction, cancer, ocular inflammatory disease and the patients who were on postmenopausal hormonal replacement were not included in the study. The groups were formed as group 1 from the patients with PEXS (n=20), group 2 from the patients with PEXG (n=20), group 3 from the patients with PEX+RVO (n=16) and the control group from the normal individuals (n=20). Blood samples were taken from each group and immediately forwarded to the laboratory for the determination of the homocysteine and vitamin B. In the beginning of the study, a complete ocular examination including visual acuity, Goldmann applanation tonometry, slit-lamp biomicroscopy, gonioscopy, funduscopy, and perimetry with Humphrey perimeter was performed in all cases. Typical PEX material was seen on the anterior lens capsule in one or both eyes of patients with PEXS. The optic discs were normal and the intraocular pressures (IOPs) were 21 mm Hg or lower in these patients. The visual fields were found to be normal. Glaucomatous cupping was detected and the IOPs were higher than 21 mm Hg in patients with PEXG. Visual field defect was seen in at least one eye. Seven cases from Group 3 had PEXG and 9 had PEXS. Ten of them had branch RVO and 6 had central RVO. The occlusions were diagnosed 8.7 ± 2.2 months (5-12) before the beginning of the study. The control group was formed from the individuals with a similar age distribution. In the control group, IOP values were lower than 21 mm Hg and they had normal optic discs and normal visual fields. There was no evidence of PEX material in slit-lamp biomicroscopic examination.

Enzyme-linked immunosorbent assay was used to measure the plasma total homocysteine levels (tHcy). The normal laboratory values were 16 mmol/l for males and 20.5 mmol/l for females. The higher values were taken as hyperhomocysteinemia. Electrochemiluminescence immunoassay was used for the determination of folate and vitamin B12 levels and high performance liquid chromatography for vitamin B6 level. The normal values were 3-17 ng/ml for folate, 3.6-18 mgr/l for vitamin B6 and 160-800 pg/ml for vitamin B12.

Statistical analysis. Chi-square test was used in the statistical analysis of gender distribution. We used one-way analysis of variance test in the statistical analysis of age distribution and vitamin B levels, and Kruskal Wallis test in the statistical analysis of homocysteine levels. Mann-Whitney U test with Bonferroni correction was used for pair wise comparison. Multiple logistic regression analysis test was applied to determine the risk value for an increase of 1 mmol/l in homocysteine levels in patients with PEXS, PEXG and PEX+RVO.

Results. The mean age was 61.2 ± 5.3 years in Group 1, 61.5 ± 5.8 years in Group 2, 63.5 ± 4.5 years in Group 3 and 62.6 ± 5.8 years in the control group. There were no statistically significant differences between the groups with respect to age and gender distribution ($p > 0.05$). There was no statistically significant difference between the groups with respect to hypertension, diabetes mellitus, peripheral or coronary artery disease, cerebrovascular disease and current smoking ($p > 0.05$). Diabetic retinopathy was not detected in any of the patients with diabetes mellitus. Characteristics of the patients in all groups are shown in **Table 1**. Plasma homocysteine levels were 17.6 ± 4.4 mmol/l in Group 1, 18.5 ± 4.5 mmol/l in Group 2, 22.2 ± 6.0 mmol/l in Group 3 and 14.0 ± 3.1 mmol/l in the control group. It was highest in Group 3 ($p < 0.001$). The ratio of hyperhomocysteinemia was calculated as 35% (group 1), 45% (group 1), 68.7% (group 1) and 15% (control). The ratios were higher in the PEX groups than in the control group ($p = 0.009$). The data related to homocysteine levels are shown in **Table 2** and **Figure 1**. The risk increase with a rise of plasma homocysteine level by 1 mmol/l was found to be 1.31 times in Group 1 (95% confidence interval (CI)= 1.06-1.62), 1.38 times in Group 2 (95% CI=1.1-1.73), and 1.43 times in Group 3 (95% CI=1.13-1.80) in multiple logistic regression analysis test. The mean folate levels was 6.3 ± 1.1 ng/ml in Group 1, 6.1 ± 1.5 ng/ml in Group 2, 4.1 ± 1.1 ng/ml in Group 3 and 8.2 ± 1.2 ng/ml in the control group. There was no statistically significant difference between the first 2 groups ($p > 0.05$). It was lowest in group 3 ($p < 0.001$). The folate levels are plotted in **Figure 2**. The mean levels of vitamin B6 and vitamin B12 were shown in **Table 3**. No statistically significant difference was detected among 4 groups with respect to vitamin B6 and B12 levels ($p > 0.05$). The vitamin B evaluations are given in **Table 3**.

Discussion. Hyperhomocysteinemia was recorded more frequently in patients with PEXS and PEXG in comparison to normal individuals with a similar

Table 1 - Characteristics of study groups.

Characteristics of cases	PEXS (n=20)	PEXG (n=20)	PEX+RVO (n=16)	Control group (n=20)	p value
Age (years)					
Mean±SD	61.2±5.3	61.5±5.8	63.5±4.5	62.6±5.8	0.6*
Range	53-72	52-73	57-71	54-72	
Gender					
Male	8	7	6	9	0.9†
Female	11	13	10	11	
Hypertension	4	5	6	4	0.6†
Diabetes mellitus	1	2	2	3	0.7†
Peripheral or coronary artery disease	2	1	2	2	0.8†
Cerebrovascular disease	-	1	1	-	0.5†
Current smokers	6	8	6	7	0.9†
*One-way analysis of variance test, †Chi-square test. PEXS - Pseudoexfoliation syndrome, PEXG - pseudoexfoliation glaucoma, PEX+RVO - retinal vein occlusion with pseudoexfoliation					

Table 2 - Plasma homocysteine levels and frequency of hyperhomocysteinemia.

Group	Homocysteine levels (µmol/l)* Mean±SD (Range)			Hyperhomocysteinemia† %
	Male	Female	Total	
PEXS	17 ± 4.6 (11.7 - 24)	18.6 ± 4.3 (14.8 - 27.4)	17.6 ± 4.4 (11.7 - 27.4)	35
PEXG	18.2 ± 5.7 (12.4 - 27.7)	19.2 ± 4.5 (13.7 - 24.6)	18.5 ± 4.5 (12.4 - 27.7)	45
PEX+RVO	21.6 ± 6.4 (13.6 - 30.8)	23.2 ± 5.9 (15.2 - 30)	22.2 ± 6 (13.6 - 30.8)	68.7
Control group	13.3 ± 3 (9.8 - 19.6)	14.8 ± 3.3 (11.7 - 22.6)	14 ± 3.1 (9.8 - 22.6)	15
*p<0.001, Kruskal-Wallis test, †p=0.009, Chi-square test, PEXS - Pseudoexfoliation syndrome, PEXG - pseudoexfoliation glaucoma, PEX+RVO - retinal vein occlusion with pseudoexfoliation				

age distribution too.^{7,8} High plasma homocysteine levels were detected in patients with retinal vascular occlusions.⁹⁻¹³ In this study, we aimed to evaluate the relations between PEXS, hyperhomocysteinemia, retinal venous occlusion and vitamin B status. We found higher plasma homocysteine levels in cases with PEXS (with or without glaucoma) in comparison to normal individuals, which is similar to the results of the previous studies.^{7,8} In addition, we formed a new group including patients with PEX+RVO and found the highest ratio of hyperhomocysteinemia in this group. The highest risk value was found in this group in multiple logistic regression analysis test. Findings related to anterior segment ischemia may be seen in PEXS. Iris hypoperfusion and microneovascularizations were shown.¹⁴ Rubeosis was reported in 50% of cases with PEXS in the iris fluorescein angiography study of Friedburg et

al.¹⁵ Ocular blood flow was found to be decreased in eyes with PEX in unilateral PEX cases in the study of Sibour et al.¹⁶ At the same time, the studies stressing the association of PEXS and RVO are impressive.¹⁷ Pseudoexfoliation syndrome is correlated with a history of angina, myocardial infarction, and stroke.¹⁸ Hyperhomocysteinemia may be a separate factor potentializing vasculopathy in addition to PEX. The causes of vasculopathy associated with homocysteine are vascular endothelial dysfunction considered to be a result of free radicals, prothrombotic condition as a result of increase in thrombocyte activation and the proliferation in smooth muscle cells.^{19,20} Hyperhomocysteinemia may have a genetic or nutritional origin. Thermolabile methylene tetrahydrofolate reductase, age, gender, renal failure, some drugs and vitamins may affect the plasma homocysteine levels. Hyperhomocysteinemia

Table 3 - Vitamin B status (mean ± SD; range).

Group	Folate (ng/ml)	B6 (mgr/l)	B12 (pg/ml)
PEXS	6.3 ± 1.1 (3.75 - 8.35)	8.6 ± 1.9 (4.9 - 11.4)	394.2 ± 143.1 (185.5 - 640.5)
PEXG	6.1 ± 1.5 (3.34 - 8.68)	8 ± 1.8 (4.6 - 12.4)	409.5 ± 160.8 (172.7 - 640)
PEX+RVO	4.1 ± 1.1 (2.6 - 6.36)	7.9 ± 1.5 (5.5 - 10.3)	399.2 ± 161.1 (176.5 - 672.4)
Control group	8.2 ± 1.2 (5.6 - 10.35)	8.5 ± 1.9 (5.7 - 12)	435.1 ± 162.5 (170.7 - 731.7)
P-value	<0.001*	0.5*	0.85*

*One-way analysis of variance, PEXS - Pseudoexfoliation syndrome, PEXG - pseudoexfoliation glaucoma, PEX+RVO - retinal vein occlusion with pseudoexfoliation

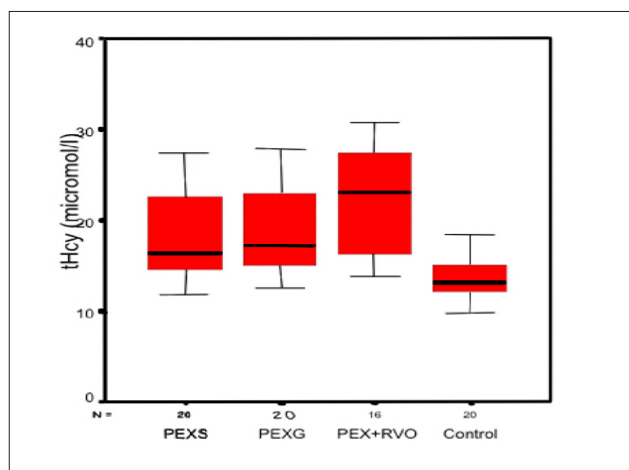


Figure 1 - The plot of homocystein levels in 4 study groups. PEXS - Pseudoexfoliation syndrome, PEXG - pseudoexfoliation glaucoma, PEX+RVO - retinal vein occlusion with pseudoexfoliation.

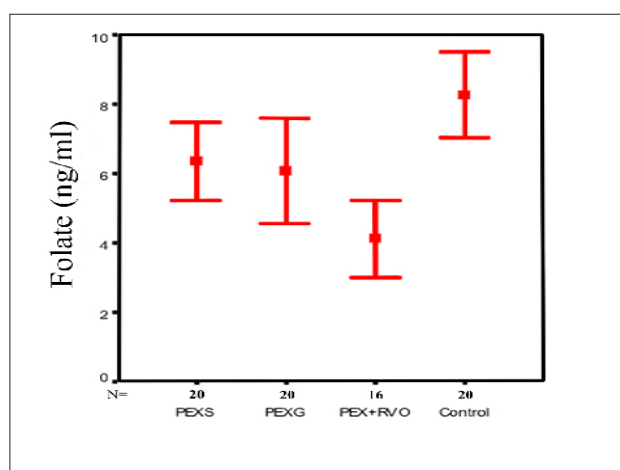


Figure 2 - The plot of folate levels in 4 study groups. PEXS - Pseudoexfoliation syndrome, PEXG - pseudoexfoliation glaucoma, PEX+RVO - retinal vein occlusion with pseudoexfoliation.

is less frequently seen among women than in men.¹ Hormonal or metabolic differences may be the cause of this difference. In our study, the ratio of hyperhomocysteinemia was 45.7% in male and 30.3% in female. The high ratio of hyperhomocysteinemia in female in our study may be related to the fact that most of them were in postmenopausal period and because of that we excluded the cases taking hormonal replacement therapy. Another difference of our study is the evaluation of vitamin B profiles. Lower folate levels were reported in addition to hyperhomocysteinemia in cases with RVO in comparison to the control groups in the study of Weger et al.²¹ Vitamin B profiles were not reported in the studies related to PEX. We found no statistically significant difference between the groups with respect to vitamin B6 and B12 levels, but the folate level was lower in the RVO group associated with PEX than in the other PEX groups and the normal group. This finding is similar to the finding of Weger et al.²¹ In previous studies, an inverse relation was shown between high plasma homocysteine levels

and vitamin B status, and vitamin B supplementation was advised to lower the homocysteine level.²²⁻²⁴ As a result, the risk of vasculopathy due to hyperhomocysteinemia was thought to be decreased. Lee et al showed that cerebral vascular endothelial damage could be decreased with the addition of folate to diet in the experimental hyperhomocysteinemia model in rats.²⁵ The low level of folate in PEXS groups especially the detection of the lowest folate level in PEX+RVO group in our study support vitamin B supplementation including folate. We informed our patients with hyperhomocysteinemia and started vitamin B supplementation after consulting with an internist. If the association of thromboembolic accidents and retinal vein occlusions in PEXS is taken into account, plasma homocysteine levels should be determined and vitamin B supplementation should be considered when hyperhomocysteinemia is detected. Hyperhomocysteinemia is a risk factor which can be easily eliminated because of low cost of vitamin supplementation.

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