



Comparison of plasma homocysteine, hs-crp, fibrinogen, vitamin B12, and folic acid levels in cases with pseudoexfoliative glaucoma and primary open-angle glaucoma

Plasma homocysteine, hsCRP in glaucoma

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Abstract

Aim: This study aims to compare plasma levels of homocysteine, hsCRP, fibrinogen, vitamin B12, and folic acid in cases diagnosed with Pseudoexfoliative Glaucoma (PEXG) and Primary Open-Angle Glaucoma (POAG).

Methods: The study included 47 patients with PEXG and 42 patients with POAG. All participants underwent visual acuity measurement using the Snellen chart, intraocular pressure measurement using Goldmann applanation tonometry, examination of the anterior and posterior segments of the eye, and visual field assessment using the Humphrey Field Analyzer with the 30-2 protocol. To evaluate the biochemical parameters, blood samples were collected from the patients at 09:00 in the morning, following a 12-hour overnight fasting period, considering dietary restrictions and medication use.

Results: The homocysteine levels (14.28 ± 6.93) and hs-CRP levels (6.86 ± 10.29) of the PEXG group were found to be significantly higher compared to the POAG group (9.98 ± 6.21 , 2.95 ± 3.15 , respectively) ($p < 0.01$). There were no statistically significant differences between the PEXG and POAG groups in terms of fibrinogen, folic acid, and vitamin B12 levels ($p > 0.05$). A significant negative correlation was observed between homocysteine and vitamin B12 in PEXG and POAG groups ($p < 0.01$). In the POAG group, a significant negative correlation was found between homocysteine and folic acid ($p < 0.01$).

Conclusion: Glaucoma remains a significant cause of irreversible and preventable blindness worldwide. Vascular diseases are known to play a role in the pathogenesis of glaucoma. Homocysteine, by affecting platelet and coagulation parameters and inducing endothelial dysfunction, can contribute to vascular damage. CRP activates inflammatory processes associated with atherosclerosis in endothelial cells. We believe that homocysteine and CRP could be potential risk factors in the development of glaucoma in PEXG and POAG patients.

Keywords

glaucoma, homocysteine, hs-crp, fibrinogen, vitamin b12, folic acid

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Introduction

Primary open-angle glaucoma (POAG) is a chronic, bilateral, and often asymmetrical optic neuropathy observed in adults, characterized by an open anterior chamber angle, intraocular pressure (IOP) above 21 mmHg, and acquired loss in optic nerve fibers and visual fields. Pseudoexfoliation syndrome is a pathology marked by the chronic accumulation of a gray-white fibro granular material, known as pseudoexfoliation material (PEX), on the anterior lens capsule and/or the pupillary margin.¹ Cases in which exfoliation material accumulates in the eye are defined as pseudoexfoliation syndrome (PES), while those where this accumulation leads to increased intraocular pressure are classified as pseudoexfoliation glaucoma (PEXG).² The etiopathogenesis of both POAG and PEXG has not yet been fully elucidated, and research on the subject is ongoing. It is believed that the pathogenesis of optic nerve damage in POAG is complex and involves multiple factors, such as increased intraocular pressure, neurotoxicity, apoptosis, extracellular matrix changes, and hypoxia secondary to ocular and systemic vascular alterations.³ Anterior segment ischemia, which develops in association with increased extracellular matrix modulators and oxidative stress factors in the aqueous humor, is thought to play a significant role in the pathogenesis of PEXG.⁴

The vascular dysfunction implicated in the etiopathogenesis of glaucoma may develop as a result of vascular endotheliopathy. Systemic inflammation is one of the key causes of vascular endotheliopathy. C-reactive protein (CRP) and fibrinogen are markers of inflammation, and studies have reported elevated levels of these markers in glaucoma. Similarly, increased plasma homocysteine levels have been reported in glaucoma, which is associated with a higher risk of atherosclerosis and contributes to endothelial damage and dysfunction, thereby playing a role in the disease's etiopathogenesis. Folic acid, vitamin B6, and vitamin B12 act as cofactors in homocysteine metabolism, and deficiencies in these factors lead to elevated plasma homocysteine levels.⁵ In the literature, elevated serum homocysteine levels and decreased folic acid levels have been detected in PEXG, and it has been suggested that folic acid supplementation may be beneficial in these cases.⁶ In our study, plasma homocysteine, hs-CRP, fibrinogen, vitamin B12, and folic acid levels were measured and statistically analyzed in cases of POAG and PEXG.

Materials and Methods

Our study included 89 cases diagnosed with PEXG and POAG, followed between February 2008 and March 2009 at the Ophthalmology Clinic of Bakırköy Dr. Sadi Konuk Training and Research Hospital. All patients underwent a comprehensive ophthalmologic examination. Uncorrected and corrected visual acuity was measured using the Snellen chart. Intraocular pressure was measured using a Goldmann application tonometer. Anterior segment examination was performed using a biomicroscope. The iridocorneal angle was evaluated with a Goldmann three-mirror gonioscope. After pupil dilation, the optic disc was assessed. For the evaluation of the visual field, the central 30-2 standard threshold test was performed using a Humphrey Field Analyzer.

The inclusion criteria for the study were as follows: for POAG cases, intraocular pressure of 21 mmHg or higher, or within normal limits through medical or surgical treatment; optic disc cupping upon examination; absence of other pathologies that could cause elevated intraocular pressure; typical glaucomatous visual field loss during visual field testing; and an open iridocorneal angle. For PEXG cases, in addition to the aforementioned criteria, the

presence of pseudoexfoliation material in one or both eyes was required.

Patients with conditions related to hyperhomocysteinemia (such as coronary artery disease, myocardial infarction, peripheral artery disease, chronic kidney failure, uncontrolled arterial hypertension, diabetes mellitus, cerebrovascular disease, chronic alcohol use, a diet rich in B vitamins, or those taking B vitamin supplements, as well as those with ocular vascular diseases [such as retinal vein occlusion, retinal artery occlusion, or ischemic optic neuropathy]), and patients taking vitamins or medications that could affect homocysteine metabolism were excluded from the study.

To assess biochemical parameters, blood samples were collected from patients at 09:00 AM after 12 hours of overnight fasting. Blood samples for homocysteine and hs-CRP were collected into vacutainer gel tubes and for fibrinogen into 3.2% sodium citrate anticoagulant tubes. After appropriate centrifugation, the serum designated for homocysteine analysis was stored at -20°C for later testing. Fibrinogen was measured on the same day from the obtained plasma using a Dade Behring kit on a Cysmex-CA 1500 coagulation analyzer. The hs-CRP test was performed on the same day using a Radim kit on a Delta Seac nephelometer. Once all samples were collected, those for homocysteine testing were thawed and analyzed using an Immulite analyzer with the original kit by chemiluminescence method. Serum vitamin B12 and folic acid levels were also measured using the chemiluminescence method.

Ethical Approval

This study was approved by the Ethics Committee of Bakırköy Dr. Sadi Konuk Research and Education Hospital (Date: 12.03.2009, Decision No: 23).

Statistical Analysis

The findings obtained in the study were analyzed using NCSS 2007 & PAS 2008 Statistical Software (Utah, USA). In addition to descriptive statistical methods (mean, standard deviation), the Student's t-test was used to compare quantitative data between two groups for parameters with normal distribution, while the Mann-Whitney U test was used for comparisons between two groups for parameters without normal distribution. Pearson correlation analysis was applied to examine relationships between parameters with normal distribution, and Spearman's rho correlation analysis was used for parameters without normal distribution. The Chi-square test was used for comparisons of qualitative data. Results were evaluated within a 95% confidence interval, with statistical significance set at $p < 0.05$.

Reporting Guidelines

This study was reported in accordance with the STROBE guideline.

Results

Of the patients included in the study, 51 (57.3%) were female, and 38 (42.7%) were male. The mean age was 65.77 ± 12.98 years. The mean age of the PEXG group was 73.48 ± 9.69 , while the mean age of the POAG group was 57.14 ± 10.58 , with a statistically significant difference in mean age between the groups ($p < 0.001$).

The homocysteine level (14.28 ± 6.93) and hs-CRP level (6.86 ± 10.29) in the PEXG group were found to be significantly higher than those in the POAG group (9.98 ± 6.21 and 2.95 ± 3.15 , respectively) ($p < 0.01$). No statistically significant differences were found between the PEXG and POAG groups regarding fibrinogen, folic acid, and vitamin B12 levels ($p > 0.05$) (Table 1). The correlation between homocysteine vitamin B12 and folic acid was evaluated in

both the PEXG and POAG groups. A significant negative correlation was found between homocysteine and vitamin B12 in both the PEXG and POAG groups (correlation coefficients of 41.1% and 32.4%, respectively) ($p<0.01$) (Table 2). In the POAG group, a significant negative correlation of 44.8% was found between homocysteine and folic acid ($p<0.01$). No statistically significant correlation was observed between homocysteine and folic acid in the PEXG group ($p>0.05$) (Table 3).

Discussion

The pathogenesis of optic nerve damage due to glaucoma is still not fully understood. Although intraocular pressure (IOP) is known as the most important risk factor, vascular risk factors also play a significant role in the pathogenesis of optic nerve damage. Atherosclerosis, vascular dysregulation, and similar conditions lead to the disruption of anatomical and functional structures. These factors cause various problems in blood circulation at the optic nerve head due to abnormal microcirculation and perfusion.⁷ Elevated homocysteine levels are thought to induce smooth muscle cell proliferation through endothelial cell damage and atherosclerosis through platelet activation thrombogenesis. It is suggested that the vascular effects of homocysteine may contribute to the disruption of microcirculation at the optic nerve head in glaucoma.

Epidemiological studies have shown elevated total plasma homocysteine levels in various cardiovascular diseases. Hyperhomocysteinemia has been identified as a risk factor for the development of atherosclerosis, as well as in patients with peripheral vascular, cerebrovascular, and coronary heart diseases.⁸ The incidence of systemic vascular disease is higher in cases with

Table 1. Evaluation of homocysteine, fibrinogen, folic acid, hs-CRP, and vitB12 levels of groups

Variable	Mean ± SD (Median)		P value
	PEXG	POAG	
^a Homocysteine	14.28 ± 6.93	9.98 ± 6.21	.003**
^a Fibrinogen	306.98 ± 68.10	302.04 ± 51.67	.699
^a Folic Acid	6.35 ± 3.43	7.21 ± 3.06	.218
^c HsCRP	6.86 ± 10.29 (4.1)	2.95 ± 3.15 (2.22)	.004**
^c Vit. B12	237.51 ± 353.91 (160)	215.43 ± 111.10 (193)	.175

a Student's t-test c Mann-Whitney U Test **p<0,01

Table 2. Evaluation of the Correlation Between Homocysteine and Vitamin B12 by Groups

Variable	Homocysteine-Vitamin B12	
	r	P value
PEXG	-0.411	.004**
POAG	-0.324	.036*
Total	-0.54	.001**

Spearman's rho correlation analysis was used *p<0.05 **p<0.01

Table 3. Evaluation of the Correlation Between Homocysteine and Folic Acid by Groups

Variable	Homocysteine-Folic Acid	
	r	p
PEXG	-0.139	.35
POAG	-0.448	.003*
Total	-0.33	.002*

Spearman's rho correlation analysis was used *p<0.05

pseudoexfoliation.⁹ Hyperhomocysteinemia has also been identified as a risk factor for non-arteritic ischemic optic neuropathy and central retinal artery occlusion.¹⁰ Similarly, a relationship has been observed between pseudoexfoliation syndrome (PES) and central retinal vein occlusion, as well as branch retinal vein occlusion.¹¹ In studies conducted by Vessani et al. and Leibovitch et al., plasma homocysteine levels were found to be higher in patients with PEXG compared to the control group.^{12,13}

In the study conducted by Altıntaş et al., which evaluated plasma homocysteine and nitric oxide levels, it was found that plasma homocysteine levels were higher in patients with PES and PEXG compared to the POAG and control groups. However, no statistically significant difference was observed between the groups regarding plasma nitric oxide levels.¹⁴ Similarly, in our study, homocysteine levels were found to be statistically significantly higher in the PEXG group compared to the POAG group. In contrast, in the study conducted by Turaçlı et al., plasma homocysteine levels in patients with PES and PEXG were found to be similar to those in the control group.¹⁵ In the study by Puustjarvi et al., which examined homocysteine levels in plasma and aqueous humor, it was found that plasma homocysteine levels were higher in the PEXG group compared to the control group, while the homocysteine level in the aqueous humor was found to be similar to that of the control group.¹⁶ In the study conducted by Bleich et al. plasma and aqueous humor homocysteine levels in patients with PEXG were found to be higher compared to the control group. Additionally, a statistically significant positive correlation was identified between serum and aqueous humor homocysteine levels in this study. This finding suggests an important relationship between homocysteine levels in plasma and aqueous humor, which may indicate the disruption of the blood-aqueous barrier.¹⁷ In the study by Roedl et al., homocysteine levels in tears and blood samples were investigated in patients with PEXG and the control group. They found that plasma and tear homocysteine levels were higher in patients with PEXG compared to the control group. Furthermore, they demonstrated a statistically significant relationship between the homocysteine level in tears and the homocysteine level in plasma within the PEXG group.⁴ However, in the study by Bleich et al. no statistically significant difference was found in aqueous humor homocysteine levels between POAG and PEXG patients.¹⁷

In our study, we found a statistically significant negative correlation between homocysteine and vitamin B12 levels in the PEXG group. Roedl et al. also reported an increase in homocysteine levels and a decrease in vitamin B12 and folic acid levels in the PEXG group.⁶ Jacques et al. demonstrated a significant negative correlation between homocysteine levels in tears and plasma and serum vitamin B12 levels in patients with PEXG.¹⁸ Since it has been shown that administering vitamin B12, folic acid, and vitamin B6 can reduce homocysteine levels in individuals with hyperhomocysteinemia, it is recommended that these parameters be investigated and treatment initiated in those with low levels.¹⁹ Similarly, it has been suggested that B vitamin therapy may mitigate the adverse effects of vitamin B deficiency and reduce elevated homocysteine levels in patients with PEXG.⁶

In the study by Puustjarvi et al., no statistically significant difference was found between the PEXG group and the control group regarding plasma folic acid, serum vitamin B6, and vitamin B12 levels.¹⁶ Cumurcu et al. reported lower folic acid levels and higher homocysteine levels in the PEXG group compared to other groups in their study involving POAG, PEXG, NTG, and the control group. However, they did not find statistically significant differences in plasma homocysteine, folic acid, and vitamin B12

levels among the POAG, NTG, and control groups.²⁰

C-reactive protein (CRP) levels increase in inflammatory reactions occurring in vascular endothelium and smooth muscle, and elevated plasma CRP levels have been associated with vascular endothelial dysfunction and plaque formation. It has been reported that CRP exerts prothrombotic and proinflammatory effects on endothelial cells and that CRP has prothrombotic and pro-inflammatory effects on endothelial cells. Recently, increased levels of this protein have been demonstrated in vascular atherosclerotic events such as coronary syndromes, ischemic stroke, and peripheral vascular diseases.²¹ Although many studies have reported impaired vascular endothelial function in glaucoma patients with NTG, a direct relationship between vascular inflammation and glaucoma has not been established.²²

In the study conducted by Leibovitch et al., hs-CRP levels were found to be higher in patients with normal-tension glaucoma (NTG) compared to the control group; however, they included cases with hypertension, diabetes mellitus, and ischemic heart disease in their study. In contrast, Su et al. reported no significant difference in hs-CRP levels between NTG and POAG cases in their study, where they did not include patients with atherosclerotic diseases. They concluded that excluding patients with systemic diseases would allow for a better determination of the relationship between glaucoma and CRP.²² In our study, which did not include patients with atherosclerotic diseases, we found hs-CRP levels in the PEXG group to be statistically significantly higher than those in the POAG group.

Epidemiological studies have demonstrated that plasma fibrinogen levels are a significant risk factor for coronary heart disease. In their study evaluating the coagulation and fibrinolytic systems, Matsumoto et al. found a significant increase in platelet aggregation in patients with NTG compared to those with POAG; however, they did not find a significant difference between the two groups regarding prothrombin time (PT), activated partial thromboplastin time (APTT), or fibrinogen levels.²³ Matsumoto et al. reported imbalances in coagulation and biochemical measurements in a large group of glaucoma patients.²³ In our study, we did not find a statistically significant difference in fibrinogen levels between the PEXG and POAG groups.

Limitations

The relatively small sample size and the single-center design may limit the generalizability of the findings. Further multicenter studies with larger populations are needed to confirm these results.

Conclusion

Plasma homocysteine levels were found to be higher in PEXG compared to POAG. It was observed that while plasma homocysteine levels increased in both PEXG and POAG cases, there was a decrease in vitamin B12 levels. To prevent ocular and systemic ischemic changes, as well as neurodegeneration in glaucoma, we believe that broader studies are necessary to investigate the effects of factors such as homocysteine, hs-CRP, fibrinogen, vitamin B12, and folic acid in the etiopathogenesis. Furthermore, the efficacy of vitamin B and folic acid supplementation in cases where these parameters are found to be low should also be examined.

Declarations

Animal and Human Rights Statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed Consent

Written informed consent was obtained from all participants before enrollment in the study.

Data Availability

The datasets used and/or analyzed during the current study are not publicly available due to patient privacy reasons but are available from the corresponding author on reasonable request.

Conflict of Interest

The authors declare that there is no conflict of interest.

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None.

Abbreviations

- CRP: C-reactive protein
- hs-CRP: High-sensitivity c-reactive protein
- IOP: Intraocular pressure
- NTG: Normal-tension glaucoma
- PEX: Pseudoexfoliation
- PES: Pseudoexfoliation syndrome
- PEXG: Pseudoexfoliative glaucoma
- POAG: Primary open-angle glaucoma
- PT: Prothrombin time
- Vitamin B12: Vitamin b12

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