

COMPARISON OF PLASMA HOMOCYSTEINE (HCY) LEVELS IN NORMAL POPULATION AND PATIENTS OF PSEUDOEXFOLIATION SYNDROME

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ABSTRACT

Objective: To compare mean plasma homocysteine (HCY) levels (measured in $\mu\text{mol/L}$) of normal population with patients having pseudoexfoliation syndrome (PXS).

Study Design: Cross sectional comparative study.

Place and Duration of Study: This study was carried out at Armed Forces Institute of Ophthalmology, Rawalpindi, from May 2016 to Apr 2017.

Patients and Methods: In this study 60 patients of PXS and 60 healthy subjects of both genders between ages of 40 and 50 years, with best corrected visual acuity of 6/6 on snellen visual acuity chart were included. All the participants were subjected to ophthalmic clinical examination including corrected distance visual acuity and slit lamp examination. Participants in both groups were evaluated for plasma homocysteine levels, by collection into a heparinized tube and later transferred in ice blocks. The samples were 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).

Results: Total 120 participants of both groups were similar with respect to gender and age ($p\text{-value}>0.05$). There were 36 (60%) males and 24 (40%) females in PXS group and in control group there were 29 (48.3%) males and 31 (51.7%) females. The mean age of PXS group was 44.68 ± 3.234 years and for healthy control group it was 45.0 ± 3.13 years. The comparison of homocysteine level between healthy controls and PXS patients showed a highly significant ($p\text{-value}<0.01$) difference between both groups. The mean homocysteine level was (32.14 ± 14.33) in PXS group as compared to healthy controls (14.41 ± 4.51). The comparison of proportions of homocysteinemia showed that the rate of homocysteinemia was significantly ($p\text{-value}<0.05$) greater among patients of PXS. There were 24 (40%) participants in healthy control group who had homocysteinemia in comparison with 58 (96.67%) patients who had homocysteinemia among PXS group.

Conclusion: Plasma HCY levels were raised in patients of pseudoexfoliation syndrome as compared to normal population, hence these patients may be considered for further systemic investigations by a medical specialist.

Keywords: Pseudoexfoliation syndrome, Plasma homocysteine levels.

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INTRODUCTION

Glaucoma is one of the leading causes of painless, gradual and irreversible visual deterioration in the world. The dilemma of the disease is its late diagnosis, and irreversible damage to optic nerve¹. Multiple factors have been evaluated for pathogenesis of glaucoma, including genetics, vascular dysregulation, aqueous humor production and drainage and connective tissue related disorders. Most of the

vascular disorders like hypertension, migraine, hypotension, hypoperfusion and Reynaud's disease have been implicated in pathogenesis of glaucoma. Thus, there is strong evidence that impaired microcirculation and inadequate perfusion of optic nerve head can cause glaucoma^{2,3}.

Pseudoexfoliation syndrome (PXS) is associated with accumulation of fine fibrillary material in anterior segment of the eye, leading to blockage of drainage system of aqueous humor. The changes in the eye become more apparent with age. Anterior lens surface is the most consistent site on which these changes can be

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found⁴. This is considered to be the most common secondary cause of open-angle glaucoma. Due to eventual blockage of outward drainage system, a significant majority of PXS patients develop pseudoexfoliation glaucoma (PXG)⁵.

Homocysteine (HCY) is a product of methionine metabolism which is implicated in diseases of vascular origin. The plasma levels of HCY are found to be elevated in variety of vascular diseases and is considered an important factor for mediation of vascular disorders. The most debilitating of these diseases is the cerebral stroke^{6,7}. The elevation of plasma HCY levels is a well-known independent risk factor for vascular diseases and may contribute to ischemic changes in glaucoma too⁸. Plasma HCY levels are therefore an important marker for determination of vascular etiology of glaucoma. Considering a significant ratio of patients with PXS developing PXG, it is logical to expect elevated HCY levels in patients with PXS^{9,10}.

Multiple studies have identified a higher Plasma HCY levels in patients with PXS. The exact mechanism by which it damages the optic nerve in glaucoma is unknown but speeding up of atherosclerotic changes in the vascular supply of optic nerve may be the cause. However, discrepancy still exists in available literature about the values of plasma HCY levels in patients with PXS¹¹. Turkcu FM et al concluded that mean plasma HCY level in patients with PXS was $15.4 \pm 6.0 \mu\text{mol/L}$ (62% patients having hyper-homocysteinemia), significantly higher compared to normal reference value¹². To our knowledge, no study has evaluated Plasma HCY levels in patients with PXS in Pakistani population. There is need to carry out this study in our population because of difference in results present in available literature. An elevated HCY level can warrant prompt referral to cardiologist for evaluation and treatment of related co-morbid conditions.

MATERIAL AND METHODS

This Cross sectional comparative study was carried out at Armed Forces Institute of

Ophthalmology, Rawalpindi Pakistan from May 2016 to April 2017. The study was started after taking approval from institutional ethics committee. All patients were informed about the study and informed written consent was taken from each patient prior to inclusion in the study. Sample size was calculated by WHO sample size calculator, taking 95% confidence level and 80% power the test, 11.5 pooled standard deviation, 15.8 test value of population mean and 9.9 anticipated population mean. The sample size was found to be 60 participants in each group, and a total of 120 participants were included in the study by non-probability consecutive sampling technique.

Healthy subjects of both genders between age of 40 and 50 years who had no specific symptoms and patients with PXS between 40 and 50 years of age with best corrected visual acuity of 6/6 on snellen visual acuity chart were included in the study. Patients on any topical medication, connective tissue disease, stromal and endothelial disease, those with family history of glaucoma, keratoconus and corneal dystrophy were excluded from the study.

All the participants including controls and PXS patients were subjected to ophthalmic clinical examination including corrected distance visual acuity and slit lamp examination by the single trainee researcher to exclude bias. Participants in both groups were evaluated for plasma HCY levels by using high performance liquid chromatography.

Data was evaluated and analysed using statistical program for social sciences (SPSS) version 17. The quantitative data was summarized with Mean and standard deviation and frequency along with percentages were presented for qualitative variables. Normal population and patients with PXS were compared for age and plasma HCY levels by applying Independent Sample t-test. Chi-square test was applied for the comparison of gender and homocysteinemia status between groups. A $p\text{-value} < 0.05$ was considered significant.

RESULTS

In this study, a total of 120 subjects were included, consisting of 60 participants of normal healthy controls and 60 patients with PXS. These groups were similar with respect to gender and age. In our study sample, there were 36 (60%) males and 24 (40%) females in PXS group and in control group there were 29 (48.3%) males and 31 (51.7%) females with no statistical significant (p -value>0.05) difference between gender distribution of both groups. The mean age of the PXS group was 44.68 ± 3.234 years with a

showed that cases of PXS have significantly higher mean value of homocysteine level (32.14 ± 14.33) as compared to healthy controls in which the mean value was noted to be (14.41 ± 4.51). The comparison of proportions of homocysteinemia showed that the rate of homocysteinemia was significantly greater among patients of PXS. There were 24 (40%) participants in healthy control group who had homocysteinemia in comparison with 58 (96.67%) patients who had homocysteinemia among PXS group, as elaborated in (table-II).

Table-I: Comparison of demographic characteristics of both groups.

Characteristics	Pseudoexfoliaion Status		<i>p</i> -value
	Healthy Controls (n=60)	Cases of PXS (n=60)	
Age of Participants			
Mean \pm SD	45.0 \pm 3.13	44.68 \pm 3.234	0.317**
Gender of participants			
Male	29 (48.3%)	36 (60%)	0.200**
Female	31 (51.7%)	24 (40%)	

**Not significant at 5% level of significance.

Table-II: Comparison of homocysteine level and homocysteinemia in control and pxs groups.

Characteristics	Pseudoexfoliaion Status		<i>p</i> -value
	Healthy Controls (n=60)	Cases of PXS (n=60)	
Homocysteine level			
Mean \pm SD	14.41 \pm 4.51	32.14 \pm 14.33	<0.001*
Homocysteinemia Status			
Absent	36 (60%)	2 (3.33%)	<0.001*
Present	24 (40%)	58 (96.67%)	

* Highly significant at 1% level of significant.

range of 40 to 50 years and the mean age of the healthy control group was 45.0 ± 3.13 years, ranging from 40 to 50 years with no statistically significant (p -value>0.05) difference between mean ages and age intervals (table-I).

No abnormality was found on anterior and posterior segment examination of both eyes on slit lamp biomicroscopy in both groups except for pseudoexfoliation in PXS group patients.

The comparison of homocysteine level between healthy controls and PXS patients showed a highly significant (p -value<0.01) difference between both groups. The results

DISCUSSION

Raised homocysteine level is one of the main risk factors of atherosclerotic vascular disease. Many factors including genetic and environmental factors have great effect on its plasma level. The HCY plasma level can be elevated due to many disorders like vitamin deficiencies, HCY metabolism, systemic arterial hypertension, chronic renal insufficiency with or without malign neoplasms and diabetes mellitus. Some other life style factors like coffee intake and habitual smoking, consumption of alcohol, some medications and physical activity can also affect the HCY levels¹³.

In many studies conducted to assess the consequences of raised level of HCY including both in vitro and in vivo models it was observed that retinal ganglion cells are induced through apoptosis by homocysteine. Many studies have proved that neuronal cells die due to apoptosis expression of Bax, in glaucoma damage due to increased levels, a pro-apoptotic protein has been found¹⁴. Side effects like activation of type II metabotropic glutamate receptors has been observed in many studies and main cause can be considered the neurotoxic effects of homocysteine¹⁵.

The main objective of this present study was to find out the association between homocysteine levels with PXS in our study population. The comparison of homocysteine level between healthy controls and PXS patients showed a highly significant (p -value<0.01) difference between both groups. According to the results the cases of PXS have significantly higher mean value of homocysteine level as compared to healthy controls. These results are in accordance with previous studies who have compared plasma HCY concentrations among patients with exfoliation syndrome and exfoliative glaucoma with healthy control subjects and found HCY levels were higher in both exfoliative groups compared with the control group.

In another study conducted by Puustjarvi T et al, the comparison of plasma HCY levels between patients of PXS and normal controls showed that cases of PXS had significantly raised level of HCY in contrast to normal controls. But there no difference was noted in aqueous humor between both groups¹⁶. Same results were found by Altintas et al, who evaluated total plasma HCY and nitric oxide marker levels in the patients with PXS and the healthy controls. The plasma homocysteine levels were raised significantly in PXS patients in comparison with control group¹⁷.

Our results are in accordance with the previous studies that put forward the association of hyperhomocysteinemia and PXS.

The comparison of proportions of homocysteinemia showed that the rate of homocysteinemia was significantly greater among patients of PXS in which (96.67%) patients had homocysteinemia as compared with healthy subjects in which (40%) participants had homocysteinemia, who did not have any ocular disease but presented with parallel vascular risk profile¹⁸.

Homocysteine (HCY) is an amino acid which serves as an intermediate in methionine metabolism to cysteine. Hyperhomocysteinemia refers to elevated plasma levels of HCY¹⁹. It could result either from genetic defects in enzymes involved in its metabolism, nutritional deficiencies in vitamins cofactors such as vitamin B12 and folic acid or from other factors including chronic medical conditions and drugs²⁰. Both elevated HCY levels and glaucoma were found to be related to increased risk of vascular disease as well as chronic kidney disease²¹.

CONCLUSION

Our study showed that eyes of patients with PXS had higher plasma HCY levels as compared to eyes of control subjects. So the patients of pseudoexfoliation syndrome may be considered for a thorough systemic investigation review for vascular disorders. The results of this study suggest the necessity of measuring the serum levels of HCY, in patients with pseudoexfoliation that may help to prevent further optic vasculopathy.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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