

Relationship of Serum Vitamin B12 and Serum Homocysteine Levels with Pseudo Exfoliation Syndrome

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ABSTRACT

Objective: To evaluate the relationship of serum Vitamin B12 and serum Homocysteine levels with Pseudo Exfoliation Syndrome.

Study Design: Cross-sectional study.

Place and Duration of Study: Department of Ophthalmology, Armed Forces Institute of Ophthalmology, Rawalpindi Pakistan, from Jun to Nov 2022.

Methodology: We included 55 individuals with unilateral Pseudo Exfoliation Syndrome without glaucoma and 50 healthy individuals as controls. All participants were subjected to the assessment of serum Homocysteine levels by high-performance liquid chromatography and Vitamin B12 levels by chemi-luminescent enzyme immunoassay.

Results: Thirty-two (58.18%) individuals out of 55 with unilateral Pseudo Exfoliation Syndrome had increased serum Homocysteine levels (>5umol/L). Of these 32, 25(78.12%) individuals had moderately high serum Homocysteine levels, while 7(21.87%) had severely high serum Homocysteine levels. Twenty-six (47.27%) out of 55 individuals with Pseudo Exfoliation Syndrome had decreased serum Vitamin B12 levels, while the rest 29(52.72%) had normal-range serum Vitamin B12 levels. In comparison, 7(14%) had reduced serum Vitamin B12 levels, while the rest, 43(86%), had normal serum Vitamin B12 levels.

Conclusion: Our study showed that raised Hcy levels were present in individuals with PXS without glaucoma and reduced serum Vitamin B12 levels. Assessment of Hcy level in individuals presenting with PXS may be undertaken as it is a known risk factor for vasculopathies.

Keywords: Homocysteine, Pseudo Exfoliation Syndrome (PXS), Vitamin B12.

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INTRODUCTION

Pseudo-exfoliation syndrome (PXS) is a systemic disorder which involves multiple visceral organs, including the eyes.¹ Ocular findings are characterised by fibrillary deposition involving various anterior segment structures like subconjunctival tissue, pupillary margins, lens anterior capsule, and trabecular meshwork.^{2,3} This disorder is an age-related disease whose severity and risk increase with age. It may affect up to 25% of individuals over 60 years. The most important ocular manifestation of PXS is pseudo exfoliation glaucoma (PXG).⁴ It is the most identifiable cause of open-angle glaucoma worldwide. Up to 50% of PXS individuals who are found to have involvement of trabecular meshwork may progress to glaucoma.⁵ In literature, individuals with PXS with or without PXG have been found to have hyper homocysteinemia.^{6,7}

Homocysteine (Hcy) is a non-proteogenic amino acid produced biologically from the methionine metabolism pathway. Studies have shown hyperhomo-

cysteinemia as a potential risk factor for systemic diseases, including thrombosis, cardiovascular diseases and renal dysfunction.^{8,9} Vitamin B12 and Folate, being enzymatic co-factors, decide the fate of Hcy in the human body. Decreased levels of Vitamin B12 have been considered associated with increased levels of homocysteine.¹⁰ The purpose of this study is to correlate the levels of Hcy and Vitamin B12 in individuals with PXS without glaucoma to keep enrolled individuals under clinical observation for the next few years for the development of PXS.

METHODOLOGY

The cross-sectional study was conducted at the Armed Forces Institute of Ophthalmology, Rawalpindi Pakistan, from June to November 2022 after approval of the Ethical Committee (219/ ERC/20 MAY 2022) with participants' written informed consent. OpenEpi Software was used to calculate the sample size, keeping the reference prevalence of Pseudo-exfoliation syndrome at 1.80%.⁹ As it was a pilot study in the institution, we included all the participants fulfilling the inclusion and exclusion criteria during the study period.

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Inclusion Criteria: Individuals of either gender and any age-group with evident PXF material in one eye confirmed by slit lamp biomicroscopy and no other ocular disease (except refractive error or mild cataract) were enrolled in the Study Group. Individuals without PXF material and ocular disease in any eye were enrolled in the Control Group. Both Groups had healthy neuro-retinal rims with a normal cup-to-disc ratio on dilated funduscopy, within normal limits Visual field 30-2. All these individuals had IOP of <21mmHg recorded on two occasions using Goldmann Aplanation Tonometer.

Exclusion Criteria: Individuals with bilateral ocular PXS, any known ocular disease (except refractive error or mild cataract) and systemic co-morbid like cardiomyopathies, myocardial infarction, hypertension, diabetes mellitus, cerebrovascular stroke, vasculitides and renal/ hepatic dysfunction were excluded from the study. Individuals taking Folate or Vitamin B12 supplementation, lipid-lowering drugs, antidepressants or dopaminergic drugs were also excluded from the study.

A total of 105 eyes of 105 individuals were enrolled to participate in this study, out of which 55 eyes of 55 individuals had unilateral ocular PXS without any clinical or diagnostic signs of glaucoma and 50 eyes of 50 healthy individuals as controls. A detailed history was sought from all the participants to identify any co-morbid and/ or any medication use. All subjects underwent complete ocular examination including gross visual acuity and best corrected visual acuity, slit lamp microscopy of the anterior segment as well as dilated funduscopy using Volk® (VOLK Optical, Ohio, USA) Superfield non-contact lens, IOP assessment with Goldmann Aplanation Tonometer (on two different occasions). Subjects also underwent Humphrey Field Analyser® (Carl Zeiss AG, Oberkochen, Germany) 30-2, and fields with unreliable indices were repeated.

All the subjects enrolled were advised blood sampling for serum Hcy levels and vitamin B12 levels from the Biochemical Department of the Armed Forces Institute of Pathology. These participants reported to the Armed Forces Institute of Pathology, where required blood samples were taken. After the necessary initial steps, blood samples were subjected to biochemical testing. ABBOTT® (Illinois, USA) Architect Plus 1000 SR was used to assess serum Hcy levels, whereas ADVIA® (Siemens Helathineers, Erlangen, Germany) XP CENTAUR was used for serum Vitamin

B12 levels. Reference values of serum Hcy levels used were 5-15 umol/L (desirable), 16-30 umol/L (moderate risk) and >30umol/L (high risk). The reference value of serum Vitamin B12 was 177-686pmol/L.¹¹

Statistical Package for Social Sciences (SPSS) version 24.0 was used for the data analysis. Quantitative variables were expressed as Mean±SD and qualitative variables were expressed as frequency and percentages. Chi-square test was applied to explore the inferential statistics. The *p*-value of ≤0.05 was set as the cut-off value for significance.

RESULTS

A total of 105 patients participated in the study. The mean age of participants is 62±6.75 years. 32 (58.18%) out of 55 individuals with PXS had increased serum Hcy level (>15umol/L). Of these 32, 25(78.12%) individuals had moderately high serum Hcy levels, while 7(21.87%) had severely high serum Hcy levels. In comparison, only 3(6%) individuals out of 50 controls had moderately elevated serum Hcy levels, and the rest 47(94%) controls had desirable serum Hcy levels (Table-I). 26(47.27%) out of 55 individuals with PXS had decreased serum Vitamin B12 levels, while the rest 29(52.72%) had normal-range serum Vitamin B12 levels. In comparison, 7(14%) had reduced serum Vitamin B12 levels, while the rest of 43(86%) had normal serum Vitamin B12 levels (Table II).

Table-I: Serum Vitamin B-12 levels in Diseased and Control Groups (n=105)

Vitamin B12 (pmol/L)	Pseudo-Exfoliation Syndrome	Control	<i>p</i> -value	
Range	1-176	26(24.71%)	7(6.67%)	<0.001
	177-686	29(27.65%)	43(40.95%)	

Table-II: Serum Homocysteine levels in Diseased and Control Groups (n=105)

Homocysteine (umol/L)	Pseudo-exfoliation syndrome	Control	<i>p</i> -value	
Range	5-15	23(21.90%)	47(44.76%)	<0.001
	16-30	25(23.80%)	3(2.85%)	
	≥31	7(6.67%)	-	

DISCUSSION

Pseudo-exfoliation syndrome (PXS) is a systemic disorder of the extracellular matrix involving eyes and other visceral organs like lungs, heart, skin and kidneys.¹⁰ The presence of dandruff-like material in anterior segment structures of the eyes characterises ocular manifestations of PXS. This ocular deposition of pseudo-exfoliative material may lead to secondary

glaucoma, phacodonesis, and zonular dehiscence.¹¹ PXS in 15-30% of individuals will develop PXG later on.¹²

Our study reveals hyper-homocysteinemia and decreased Vitamin B12 levels in a considerable percentage of individuals with PXS without glaucoma. In our study, 58.18% and 47.27% of individuals with PXS were found to have raised Hcy levels. They reduced Vitamin B12 levels, respectively, comparable to the previous studies. Another cross-sectional study revealed significantly elevated levels of serum Hcy in individuals with PXS and PXG.¹³ Tranchina *et al.* studied the level of Homocysteine in patients with PXS and the primary open-angle Group (POAG). It concluded that high Hcy were found PXS Group as compared to the POAG Group.¹⁴ Vessani *et al.* concluded that raised homocysteine levels were more common in individuals with PXS and PXG than the control Group, and patients with PXS may benefit from the measurement of serum homocysteine level.¹⁵

In literature, Homocysteine has been reported to be a known risk factor for vasculopathy.¹⁶ The causes of vasculopathy associated with Homocysteine are vascular endothelial dysfunction, which is a result of free radicals, prothrombotic condition due to an increase in thrombocyte activation and the proliferation in smooth muscle cells.¹⁷ Hyper-homocysteinemia may be a potentiating factor for vasculopathy in addition to PXS.¹⁸ Individuals with PXS with or without glaucoma may benefit by undergoing an assessment of serum homocysteine level and serum Vitamin B12 level.¹⁹⁻²⁰

LIMITATIONS OF STUDY

This study did not consider the dietary intake of Vitamin B12, which may have affected low serum Vitamin B12 levels in the PXS Group.

CONCLUSION

Our study showed that raised Hcy levels were present in individuals with PXS without glaucoma and reduced serum Vitamin B12 levels. Assessment of Hcy level in individuals presenting with PXS may be undertaken as it is a known risk factor for vasculopathies. Further studies are needed to assess the effect of hyper-homocysteinemia correction on the progression of individuals with PXS towards PXG.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

WRB & FH: Data acquisition, critical review, concept, approval of the final version to be published.

SN & UI: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

UNK: & TAK: Critical review, data analysis, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Relationship of Serum Vitamin B12 & Serum Homocysteine Levels

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