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Research Article

Tumor necrosis factor-alpha in Patients with Pseudo exfoliation Syndrome

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Abstract: The aim of the present study was to determine the level of Tumor necrosis factor-alpha in aqueous humor and serum of patients with pseudo exfoliation syndrome. The materials and methods in this study, we measured the level of Tumor necrosis factor-alpha, in aqueous and serum of patients with pseudo exfoliation syndrome and compared the findings with healthy controls. The Results are Regarding Aqueous humor, the level of Tumor necrosis factor-alpha in PXS group was significantly higher than those of the control group (3.97 ±2.241 vs.2.54 ±0.713 pg/ml, P=0.02). The serum level of Tumor necrosis factor-alpha in PXS group was significantly higher than those of the control group (8.70±1.83 vs.7.03±1.85 pg/ml; p=0.001). In Conclusion the results suggest that level of Tumor necrosis factor-alpha rose in aqueous and serum of pseudo exfoliation syndrome.
Keywords: pseudo exfoliation syndrome, Tumor necrosis factor-alpha

INTRODUCTION

Pseudo exfoliation syndrome (PXS) is one of the most common causes of glaucoma worldwide [1].PXS is a common, age-related, systemic disorder of worldwide significance with an estimated prevalence ranging from 10- 20% in the general population over 60 years of age [2]. Compared to primary open-angle glaucoma (POAG), pseudo exfoliation glaucoma (PXG) has a more serious clinical course and worse prognosis. It is typically associated with high mean levels of intraocular pressure (IOP), greater diurnal pressure fluctuations, marked pressure spikes, high frequency and severity of optic nerve damage, more rapid visual field loss, poorer response to medications, and more frequent surgical interventions [3].Apart from glaucoma development, PXS may be associated with a broad spectrum of other ocular, surgical, and systemic complications including cardiovascular and cerebrovascular diseases[4].The mechanism underlying PXS and its subsequent progression to PXS is still unclear [5].

In PXS, an age-related, complex, generalized disorder of the extracellular matrix, the progressive accumulation of intraocular production of abnormal fibrillar materials in the trabecular meshwork is considered the primary cause of chronic IOP elevation. Though the involvement of various genetic and internal/external stress factors such as immune reactions, inflammation, ischemia, hypoxia, and oxidative stress have been proposed, the exact mechanism of the extracellular matrix changes in PXS is still not well understood [6].

Studies have focused on immunological and inflammatory mediators in the pathogenesis and possible preventive therapies of PXS and PXG. The major targets of interest are cytokines, as recent advances reveal that they play an important role in the pathogenesis of glaucoma and may regulate retinal ganglion cell (RGC) survival or death [7]. Cytokines are secreted proteins that are especially important in regulating inflammatory process.

If we postulate that immune activation is associated with the patho physiology of PXS, then changes in cytokines secretion within the eye might be detectable as changes in the concentration of the cytokines in the aqueous humor of PXS patient. Therefore, in the present study, we measured serum and aqueous level of Tumor necrosis factor-alpha (TNF-α)

in patients with PXS and compared the results with the control group.

METHODS

Thirty patients with PXS and cataract and 30 patients with senile cataract were enrolled in this prospective cross-sectional study. This study was carried out on patients who referred to Nikookari Hospital of Tabriz, Iran, a tertiary educational hospital, between September and December, 2014. This study was approved by the ethics committee of Tabriz University of Medical Sciences. Prior to the participants' enrollment, written informed consent was obtained from all the patients according to the tenets of the Declaration of Helsinki. All of the patients underwent a complete ocular examination including best-corrected visual acuity (BCVA), slit-lamp biomicroscopy, and Goldmann applanation tonometry and dilated fundus exams. The patients with PXS without any glaucomatous damage and significant cataract who were candidates for cataract extraction were enrolled in study.

The control group consisted of 30 patients with senile cataract without any findings indicative of PXS or glaucoma. Patients with BCVA better than 20/40, any signs of ocular inflammation or infection, neuropathy, glaucoma, uveitis, retinopathy or maculopathy were excluded from this study.

Aqueous humor samples (about 200 µl from each patient) were carefully collected at the beginning of the surgery through paracentesis using a 27-gauge needle on a tuberculin syringe under an operating microscope, taking special care to avoid blood contamination. Aqueous humor was immediately cooled at −70 °C and transported to the laboratory to run the assays. Blood samples were obtained after overnight fasting one day before surgery. Serum samples were frozen immediately and stored at −70 °C until they were needed for analysis. Serum and aqueous humor TNF-α (Detection Limit: 0.7pg/ml, Intra assay: CV=6.6 % and Inter assay: CV=4.3 %) concentrations were determined by commercial Enzyme-Linked Immunosorbent Assay (ELISA) kits. All tests were performed according to the manufacturer's instructions after serial dilution. Each plate included standards in parallel with the samples, and all case and control samples were analyzed on the same day for each assay kit.

Statistical Analysis

Statistical analysis was performed using SPSS 17 for Windows (SPSS, Inc, Chicago; IL).The data are presented as mean± standard deviation. The independent *t*-test was used to assess the significance of the difference between the groups. Correlation was estimated using the Pearson correlation coefficient test. Proportions of groups were compared with a chi-square test. The level of statistical significance was set at $P<0.05$.

RESULTS

Sixty patients were enrolled according to the above-mentioned inclusion/exclusion criteria in this study. They were in two groups, namely PXS and control. Thirty patients were recruited in each group. The demographic characteristics and sampling data of the study groups are summarized in Table 1.

Table 1: Demographic and sampling data in study groups

parameter	PXS group(n=30)	Control group(n=30)	p
	Mean (SD)	Mean (SD)	
Age (year)	68.33±9.48	67.27±10.866	0.405‡
Sex*(female/male)	0.578	1.30	0.09
Aqueous TNF-α (pg/ml)	3.97 (2.241)	2.54 (0.713)	0.002‡
Serum TNF-α (pg/ml)	8.70 (1.83)	7.03 (1.85)	0.001‡

Independent t-test.*Values are given as n (%) or Mean (standard deviation).

The mean age of PXS and controls were68.33±9.48and 67.27±10.866respectively.The female/male ratio in PXS and controls was 0.578 and 1.30. There was no significant difference in age and sex ratio between the groups (p= 0.405, p= 0.09).

In Aqueous humor, the level of TNF-α in PXS group was significantly higher than those of the control group (3.97 ±2.241 vs.2.54 ±0.713 pg/ml, P=0.02). The serum level of TNF-α in PXS group was significantly higher than the ones in the control group(8.70±1.83vs.7.03±1.85 pg/ml; p=0.001). In addition, as shown in Table 2, while a significant correlation was observed between level of TNF-α in serum and aqueous humor in PXS group (P=0.04, r=-0.37).

Table 2: Correlation between serum and aqueous cytokines in study groups

parameter	groups	r	p*
TNF-α	PXS	-0.37	0.04
	control	-0.19	0.29

*The Pearson Correlation coefficient test

DISCUSSION

PXS represents a complex, multifactorial and late-onset disease involving genetic, oxidative stress, ischemia, hypoxia constitute and inflammatory factors in the etiopathogenesis [8] .

Inflammatory cytokines have also been shown to be widely expressed by various ocular cell types and to be present in aqueous and vitreous humor with increased levels in patients with uveitis and vitreo retinal disorders [9]. TNF-α is a pro inflammatory cytokine with multiple functions in the immune response. Accumulating studies strongly support the involvement of TNF-α in the etiology of glaucoma [10]. Ischemic or pressure-loaded glial cells could produce TNF-α, which results in oligodendrocyte death and the subsequent apoptosis of RGCs.

TNF-α is up-regulated in several neurodegenerative disorders including multiple sclerosis, Parkinson's disease, Alzheimer's disease [11,12] and in optic nerve microglia and astrocytes in glaucoma patients [13,14]. TNF-α have been found to be significantly associated with PEG in both Pakistani and Iranian populations, suggesting a role of immunological factors in PEX associated neurodegeneration [15,16]. Furthermore, TNF-α was up-regulated as a consequence of increasing IOP. Furthermore, exogenous TNF-α could lead to loss of oligodendrocytes and a delayed loss of RGCs [17]. This research suggests that TNF-α may play a key role in glaucomatous neurodegeneration.

To the best of our knowledge, this is the first study to experimentally measure the serum and aqueous humor level of TNF-α in PXS patient at the same time and in comparison to the control group. The present study demonstrated that the level of TNF-α in serum and aqueous humor in PXS group was significantly higher than those of the control group.

Takai *et al.;* in their study found that the level of TNF-α and IL-1β did not differ in PXG compared with the cataract group [6].However, other cytokine networks may play a critical role in IOP elevation in patients with glaucoma. Because we excluded patients with elevated IOP or glaucoma from our study, the result of our study are not similar to theirs.

Zenkel *et al.;* also found that early stages of PXS were characterized by increased cytokines levels in anterior segment tissues and not late stages of PXS as compared with controls [18].

In a previous study, we found that serum TNF-α level in PXS was significantly higher than the controls, but in that study, we did not have any measure from aqueous level of TNF-α in our patients [8].The present study confirms our previous results by showing that local subclinical inflammatory processes other than systemic issues may have a role in patho physiology of PXS.

Balaiya and coworker in a study demonstrated that TNF-α level in POAG patients was higher than that of controls. Therefore, TNF-α may play a role both in PXS and glaucoma [19]. Sawada et al. in a comparative study between POAG, normal tension glaucoma and PXG demonstrated that TNF-α level was significantly higher than the controls only in PXG. Their findings confirmed that in PXG inflammatory, cytokines have a more important role than other types of glaucoma [20].

Additionally, this study found a significant correlation between TNF-α level in the serum and Aqueous humor of PXS group. Considering the results of the present study and the above-cited ones, such finding could confirm the role of a balance between pro inflammatory and anti–inflammatory cytokines (systemic and local) in the patho physiology and progression of PXS.

The relatively small sample size, measurement of a few cytokines, inclusion of PXS patients regardless of their disease stages, and possible selection bias upon the systemic condition of the participants could be regarded as the limitations of this study. Despite these limitations, the obtained results may provide a basis for further studies on many cytokines in patients at different disease while considering all systemic health views.

CONCLUSION

The major finding of this study is that inflammatory cytokines (systemic and local) play a role in the patho physiology and progression of PXS.

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