

# The role of the cytokines in the pathogenesis of pseudoexfoliation syndrome

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## INTRODUCTION

The incidence of pseudoexfoliation (PSX) syndrome increases in age with every decade after age 50<sup>[1]</sup>. It was suggested in different studies that most of the patients referred to glaucoma clinics had also PSX syndrome<sup>[1-5]</sup>. PSX syndrome is an age-related syndrome associated with accelerated progression of cataract. It is characterized by production and progressive accumulation of a fibrillar material in bulbus oculi affecting the entire anterior segment structure of the eye<sup>[2,6]</sup>. Glaucoma occurs more commonly in eyes with PSX than in those without it. It is suggested that PSX is the most common identifiable cause of glaucoma<sup>[1,7]</sup>. Patients with PSX are also prone to develop angle-closure glaucoma, while glaucoma in PSX has a more serious clinical progress and worse prognosis than primary open-angle glaucoma<sup>[1,7]</sup>.

Accumulation of white material on the anterior lens surface is the most constant diagnostic feature of PSX. The classic pattern consists of three different zones that become visible when the pupil is fully dilated. Next to the lens, PSX material is most prominent at the pupillary border. Other important findings of PSX are pigment loss from the iris sphincter and its deposition on anterior chamber structures<sup>[1]</sup>. Physicians must take into account the presence of PSX because of the increased risk during intraocular surgery, most commonly zonular dialysis, capsular rupture and vitreous loss during cataract extraction<sup>[7]</sup>.

Despite extensive research, the exact chemical composition of PSX material remains unknown. The protein components of PSX contain both noncollagenous basement membrane components and elastic fiber system<sup>[1,7]</sup>. Typical PSX fibers have been shown electron microscopically in close association with the pre-equatorial lens, the nonpigmented ciliary, the iris pigment, the corneal and the trabecular endothelium with almost all cell types of the iris stroma, such as fibrocytes, melanocytes, vascular endothelial cells, pericytes, and smooth muscle cells<sup>[1,7]</sup>.

PSX has an increased risk for both arterial and venous

## Abstract

• **AIM:** To examine the mechanism of the development of pseudoexfoliation (PSX) syndrome via both cytokine formation and endothelial vasorelaxing and growth factors that will provide us new therapeutic insights for the treatment.

• **METHODS:** This is a cross sectional study included two groups; Group 1: control patients with nuclear cataract ( $n=20$ , aged 51-80 years). Group 2: PSX patients with nuclear cataract ( $n=18$ , aged 50-90 years). Patients with other ophthalmic problems and systemic diseases were excluded. Vascular endothelial growth factor (VEGF), interleukin-6 (IL-6) and interleukin-1 $\beta$  (IL-1 $\beta$ ) and nitrotyrosine levels were determined through serum samples by Enzyme-linked immunosorbent assay (ELISA) method. Nitrite-nitrate levels were measured with photometric endpoint determination.

• **RESULTS:** There were no significant differences between the groups in terms of age, VEGF, IL-1 $\beta$ , nitrite-nitrate and nitrotyrosine. The significant results were the mean IL-6 levels that were higher in PSX group 2 ( $37.68 \pm 29.52$  pg/mL) compared to that in control group 1 ( $15.32 \pm 10.08$  pg/mL) ( $P < 0.001$ ).

• **CONCLUSION:** Several interacting and extending biochemical pathways may lead to the promotion of VEGF and IL-6 expressions. IL-6 which is the only altered marker in our study may indirectly cause an increase of vascular permeability and neovascularization. We suggest inflammation as a factor that can be involved in etiopathogenesis of PSX.

• **KEYWORDS:** pseudoexfoliation syndrome; inflammation; vascular endothelial growth factor; interleukin-6; interleukin-1 $\beta$

**Table 1 Serum VEGF, IL-6, IL-1 $\beta$ , nitrite-nitrate, nitrotyrosine in PSX patients and the control group**

$\bar{x} \pm s$

Groups	Age(a)	VEGF (pg/mL)	IL-6 (pg/mL)	IL-1 $\beta$ (pg/mL)	Nitrite-nitrate ( $\mu$ mol/L)	Nitrotyrosine (nmol/L)
Control (n=20)	66.47 $\pm$ 9.17	434.11 $\pm$ 243.71	15.32 $\pm$ 10.08	4.65 $\pm$ 1.12	4.06 $\pm$ 0.73	40.00 $\pm$ 7.70
PSX (n=18)	72.05 $\pm$ 0.51	509.93 $\pm$ 239.20	37.68 $\pm$ 29.52 <sup>b</sup>	4.37 $\pm$ 0.13	4.07 $\pm$ 0.62	67.50 $\pm$ 47.30

<sup>b</sup> $P < 0.001$  vs control group.

occlusions of the retina [2]. The significant association of PSX, especially with systemic vascular diseases as arterial hypertension, coronary disease, stroke, Alzheimer's disease, dementia, and abdominal aneurysms were demonstrated in different studies [1].

An overproduction and abnormal metabolism of glycosaminoglycans have been proposed as the major changes in PSX [14,7]. The levels of vascular endothelial growth factor (VEGF) which its effects can be mediated via other factors/cytokines and different inflammatory mediators/cytokines such as interleukin-6 (IL-6), interleukin-1 $\beta$  (IL-1 $\beta$ ), nitrite-nitrate and nitrotyrosine were evaluated in order to include the pathogenesis of PSX syndrome. Among those mediators, IL-6 is a multifunctional cytokine that may indirectly cause an increase of vascular permeability and neovascularization by inducing the expression of VEGF, or might directly induce an increase of endothelial permeability and new vessel formation [8]. Besides, nitric oxide (NO) is one of the most important endothelial vasorelaxing factors, providing the homeostatic functions of the eye by regulation of aqueous humor dynamics, neuronal visual processing, local modulation of ocular blood flow, and control of retinal ganglion cell death by apoptosis [9].

In our study, we aimed to examine the mechanism of the development of PSX syndrome via both cytokine formation and endothelial vasorelaxing and growth factors that will provide us new therapeutic insights for the treatment.

## SUBJECTS AND METHODS

**Subjects** Group 1: control patients with nuclear cataract were included 11 females and 9 males ( $n=20$ , age range 51-80 years). Group 2 was PSX group also with nuclear cataract were included 8 females and 10 males ( $n=18$ , age range 50-90 years). Patients with other ophthalmic conditions (*e.g.*, glaucoma, uveitis, progressive other retinal disease) and systemic diseases (*e.g.*, diabetes, arthritis, coronary arterial disease, peripheral vascular disease) were excluded. Informed consent was obtained from patients. Human Ethics Committee rules were fulfilled. All the subjects both in control group and patient group completed a questionnaire giving the following information: age, gender, no smoking habits, no supplements such as vitamins and/or antioxidants. All patients underwent a comprehensive ophthalmic examination and were examined prior to surgery after pupillary dilation for the presence of exfoliation material. Only the patients who exhibited exfoliation material upon the lens or pupil were included. Blood samples were drawn from

patients and controls after an overnight fasting, before the operation then centrifuged at 2.000xg for 10 minutes at 4°C. Serum samples were stored at -70°C for further analyses.

## Methods

**Biochemical parameters** VEGF levels were determined from serum samples, by Enzyme-linked immunosorbent assay (ELISA) method (Quantikine Immunoassay VEGF R&D Systems, Cat No: RRVOO, Lot No: 250498).

Human IL-6 was determined by a solid phase sandwich ELISA (BioSource Immunoassay Kit, BioSource International, Inc, California, USA, Cat No: KHCOO61, Lot No: 064903).

Human IL-1 $\beta$  was also done by the same method with BioSource Immunoassay Kit (BioSource International, Inc, California, USA, Cat No: KHCOOII, Lot No: 064103).

Nitrite-nitrate levels were determined by colorimetric method, photometric endpoint determination, Cat No: 1 746 081, by Roche kit. Assay principle was that nitrate is reduced to nitrite by decreased nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of the enzyme nitrate reductase. The nitrite formed reacts with sulfanilamide and N- (1-naphtyl)-ethylene-diamine dihydrochloride to give a red-violet diazo dye. The diazo dye was measured on the basis of its absorbance in the visible range of 540nm [10, 11].

Nitrotyrosine levels were also analysed by ELISA kit (Hycult biotechnology, Cat No: HK501, Lot No: 5419K18).

**Statistical Analysis** Data were analyzed using SPSS 8.0 statistic program (SPSS, Chicago, IL, USA). Differences in mean values (mean's) between two groups were tested by Mann-Whitney *U*-test. The level of statistical significance was set at  $P < 0.05$ .

## RESULTS

The means and standard deviations for the ages and biochemical parameters for PSX group and control group are listed in Table 1. There were no significant differences between the PSX and control groups in terms of age, VEGF, IL-1 $\beta$ , nitrite-nitrate and nitrotyrosine. The serum VEGF and nitrotyrosine levels were increased in the PSX group when compared to the control group. However, the serum VEGF and nitrotyrosine levels between the PSX group and the control group were not significant. The only significant results were the mean IL-6 levels that were higher in PSX group (37.68  $\pm$  29.52pg/mL) compared to control group (15.32 $\pm$ 10.08pg/mL) ( $P < 0.001$ ) (Table 1).

## DISCUSSION

PSX syndrome is also associated with ocular ischemia, iris hypoperfusion, anterior chamber hypoxia, and with a

decreased ocular and retroocular micro and macrovascular blood flow occurring both in patients with and without glaucoma [12].

The major finding of our study was the increased levels of IL-6 in PSX group compared to control group. Our findings have important implications and led us to support an evolving hypothesis that inflammation is associated with the pathogenesis of PSX.

NO is known as a potent vasodilator and involves in all steps of angiogenesis including dissolution of matrix, endothelial cell migration, proliferation and organization [13]. Changes in the amount of NO may contribute to pathological conditions such as uveitis, retinitis, retinal degeneration, and glaucoma [13, 14]. Retinal ischemia or hypoxia may stimulate the induction of VEGF and VEGF stimulates the production of nitric oxide synthase (NOS). NOS inhibition can also block VEGF-induced vascular permeability in all ocular tissues [15]. Several recent studies have strongly implicated VEGF in the pathogenesis of choroidal neovascularization. VEGF-induced angiogenesis, *in vivo* is significantly attenuated by NOS inhibition [13, 16]. It was demonstrated that the aqueous humor NO levels were lower in PSX subjects [17]. Borazan *et al* [18]. Demonstrated that the aqueous humor and plasma VEGF levels were higher in patients with PSX and pseudoexfoliation glaucoma (PXG) than in controls. Aqueous humor NO levels were higher in patients with PSX and PXG than in controls. However, the plasma NO levels did not differ between the groups.

NO is also known to be a potent inhibitor of cytokine-induced proliferation of microvascular endothelial cells, however there is much evidence that NO is involved in the endogenous regulation of angiogenesis and endothelial integrity mediated by VEGF [16]. Immunological NOS (iNOS) is inducible only in pathological conditions by endotoxins, inflammation, and certain cytokines, such as IL-1, IL-6, tumor necrosis factor. Once induced, iNOS will produce large amounts of NO for long periods of time, that it is converted into NO<sub>2</sub>, nitrite, peroxynitrite and free radicals to induce pathophysiological actions, such as, optic nerve degeneration lesion, which lead to glaucoma, retinopathy, age-related macular degeneration (AMD), myopia, cataracts, uveits and wound healing after surgery [14]. Although, in our study, every subject of both study groups had cataracts, we did not observed any differences in IL-1β, nitrite-nitrate and nitrotyrosine levels between groups that preclude us to put forward any idea about those markers.

Takai *et al* [19] demonstrated that the aqueous humor IL-6 levels were higher in PXG compared with the control and primary open-angle glaucoma (POAG) groups. However, aqueous humor IL-1β, VEGF-A, and tumor necrosis factor-α (TNF-α) levels did not differ between the groups.

Previously, increased proinflammatory cytokines such as

IL-1β, IL-6, IL-8, IL-10, IL-12, and TNF-α in the tears or conjunctival epithelium of glaucomatous eyes treated with topical antiglaucoma drugs have been reported [20-22].

Inflammation is associated with angiogenesis and that neovascularization can cause inflammatory eye diseases. VEGF has been localized in numerous retinal cells including Müller cells, astrocytes, pericytes, vascular endothelial cells, and retinal pigment epithelial cells [23]. IL-6 is also synthesized by a variety of cells within the eye; the sources of IL-6 include the retinal pigment epithelial cells, corneal epithelial cells, keratocytes, iris, and ciliary body [24].

There are different claims about the ocular interaction between IL-6 and VEGF and the role of IL-6 in the pathogenesis of PSX. The following possibilities were suggested both for VEGF and IL-6 that they might directly cause an increase of vascular permeability or IL-6 might indirectly cause an increase of vascular permeability *via* upregulation of VEGF or VEGF alone might cause increases in vascular permeability. IL-6 is reported to induce an ocular inflammatory response that is often accompanied by breakdown of the blood-ocular barrier, as detected by an increased protein concentration in either the aqueous humor or vitreous fluid [24].

In our study, we only observed the statistically significant increased levels of IL-6 in PSX group that we can suggest the indirect effects of IL-6 in order to increase the vascular permeability related with PSX.

Cohen and associates reported that IL-6 might induce angiogenesis indirectly *via* the induction of VEGF expression [8]. The production of both VEGF and IL-6 is known to be upregulated by ischemia, advanced glycation end-product and insulin-like growth factor-1 and it suggests that changes of other cytokines might also promote the expression of VEGF and IL-6 [24].

Although the nature of this relationship is still not well characterized or completely understood, an increase in the numbers of the evidences for the association between PSX and cataract formation have been presented recently. Nuclear cataract is found more frequently in eyes with PSX than in eyes without it [25].

Patients with PSX are much more prone to have complications at the time of cataract extraction. Eyes with PSX dilate lesser and have greater incidences of capsular rupture, zonular dehiscence, and vitreous loss. Pupillary diameter and zonular fragility have been suggested as the most important risk factors for capsular rupture and vitreous loss [1].

Inflammation after cataract extraction is more common in eyes with PSX than in those without it, and a transitory fibrinoid reaction, attributed to breakdown of the blood aqueous barrier, may occur [26].

During ocular surgeries, inflammation could be taken in to

account depending on the increased levels of IL-6 and anti-inflammatory treatment could also be altered. The mechanism of the development of PSX syndrome via both cytokine formation and differences in endothelial vasorelaxing and growth factors will provide us new therapeutic insights for the treatment.

In our study, we only have to be able to include patients that were willing to be in the study, so additional prospective studies and possibly randomized clinical trials can be helpful to the results and hypotheses that require confirmations.

In conclusion, the results of this study suggest that local and chronic inflammation induced by the complement pathways plays a central role in the development of PSX syndrome. Moreover, levels of various inflammatory biomarkers may add clinically relevant, predictive information to known, well-establish risk factors for PSX syndrome.

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