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Levels of cytokines in the aqueous humor of eyes with primary open angle glaucoma, pseudoexfoliation glaucoma and cataract

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Abstract

Objective: The focus of this study aimed at measuring multiple inflammatory cytokines levels in the aqueous humor of patients with primary open angle glaucoma (POAG), pseudoexfoliation glaucoma (PEXG) and senile cataract.

Methods: This case control study was conducted at the Research Institute of Ophthalmology, Giza, Egypt in 2016. Aqueous humor (AH) samples were withdrawn from 50 patients (30 POAG, and 20 PEXG) and from 15 patients with senile cataract serving as controls. The levels of IL6, IL8, transforming growth factor β1 (TGFβ1), tumor necrosis growth factor α (TNFα) and serum amyloid A (SAA) were analyzed by ELISA immune-assay. Data were analyzed by SPSS 10, using Pearson Product-Moment Correlation and independent-samples t-test.

Results: The levels of IL8, TGFβ1, TNFα and SAA were significantly higher in POAG and PEXG patients, compared to senile cataract patients. While the levels of IL6, were significantly decreased in both groups of glaucoma patients compared to cataract patients. Significant positive correlations were detected between IL6, IL 8 & TGF β1, IL 8; SAA, IL8 & TGFβ1, SAA in the aqueous humor of different groups.

Conclusion: Thus the assayed cytokines including TGFβ1, TNFα, IL8 and SAA in aqueous humor, play a vital role in IOP elevations in patients with POAG and PEXG.

Keywords: Cytokines, Primary open angle glaucoma, Pseudoexfoliation glaucoma

1. Introduction

Glaucoma can be identified by the progressive optic neuropathy and loss of visual field, due to elevated intraocular pressure (1). The IOP increases in POAG and PEXG, because of the reduction of aqueous humor outflow at the trabecular meshwork (TM) (2). This happens because of an increase of resistance in the aqueous humor outflow due to changes in the amount and quality of the extracellular matrix in the trabecular meshwork (TM) (3). The primary cause of chronic IOP elevation in PEXG is considered to be an age-related, complex, generalized disorder of extracellular matrix ECM and an accumulation of abnormal fibrillar materials in the TM (4). Various genetic internal and external stress factors such as, immune reactions, inflammations, ischemia, hypoxia and oxidative stress are involved in the pathogenesis of glaucoma (5). A number of cytokines including IL6, IL8, TGFβ1, TNFα and SAA have been detected in the aqueous humor (6). Changes in ocular inflammatory cytokines expression can be detected as changes in the concentration of the above cytokines if there is association between an immune reaction and/or chronic inflammation at the TM or anterior segment and elevated IOP in POAG or PEXG (7). The aim of this research was to detect the role of different cytokines in the pathogenesis of glaucoma.

2. Material and Methods

2.1. Research design and participants

This case-control study was conducted in 2016. The study involved 50 patients with glaucoma, 30 patients with POAG and 20 patients with (PEXG) attending glaucoma clinic of the Research Institute of Ophthalmology, Giza,

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Egypt, and 15 patients with senile cataract serving as control subjects. Patients with diabetes mellitus, major systemic illness, myocardial Infarction, hypertension, evidence of renal or hepatic diseases. Also patients with other types of glaucoma were excluded

2.2. Ethics of research

A written informed consent for aqueous humor collection were obtained from all patients in this study. This study was approved by the Ethics Committee of the Research Institute of Ophthalmology (Egypt).

2.3. Examinations

A detailed medical history was obtained to identify patients with risk factors for vascular disease such as, hypertension, diabetes mellitus, hyperlipidemia, smoking status and also the presence of cardiovascular and cerebrovascular disease. Slit lamp bio-microscopy, best corrected visual acuity, and gonioscopy were carried out in order to evaluate the anterior chamber angle, and the intraocular pressure (IOP) was tested using the Goldman applanation tonometer. Visual field (VF) was determined using the Humphrey automated field analyzer and a fundoscopic examination was carried out using a 90-diopter lens with determination of the C/D ratio, optic disc rim, optic disc pallor, lamina cribrosa, blood vessels, hemorrhages and retinal nerve fiber layer defects. Aqueous humor samples (150 µl) were carefully collected at the beginning of glaucoma or cataract surgery through a limbal paracentesis using 0.5 ml syringe with 27-gauge needle on tuberculin syringe under the operating microscope with special care to avoid blood or intraocular tissues contamination. All samples were immediately frozen and stored at -70 °C until assay was performed. Levels of IL6, IL8, TGFβ1, TNFα and SAA were estimated in aqueous humor using enzyme linked immunosorbent assay kits (ELISA).

2.4. Data analysis

Statistical methods: SPSS version 10 (SPSS Inc., Chicago, Illinois, USA) was used for data management and analysis. Data were analyzed using descriptive statistics, Independent-samples t-test, and Pearson Product-Moment Correlation.

3. Results

The concentration of the five cytokines analyzed were shown in Table 1. All cytokines were detected in all the tested samples. The levels of IL8, TGFβ1, TNFα and SAA were significantly higher ($p<0.001$) in glaucoma patients compared to senile cataract patients. Also, IL6 was significantly lower in glaucoma patients compared to senile cataract patients ($p=0.0125$). The correlation between the cytokines concentration are shown in Table 2. Significant positive correlations were found between IL6 and IL8 ($p=0.001$) IL8 and TGFβ1, ($p<0.0001$), IL8 and SAA ($p<0.001$), TGFβ1 and SAA ($p<0.001$) in the aqueous humor of different groups (Table 2).

Table 1. Demographic Data and Levels of Aqueous Humor IL6, IL8, TGFβ1, TNF α and SAA assayed in all the Studied Groups (mean ± SD)

Variables	Groups			
	Cataract (n=15)	POAG (n=30)	PEXG (n=20)	p-value
Age (year) (Mean ± SD)	63	65	67	
Sex (Male/Female)	8/7	15/20	8/12	
IL6 (Pg/ml)	64.3±22.1	15.1±1.5	23±1.4	< 0.05
IL8 (Pg/ml)	1.5±1.2	3.2±1.9	5.6±3.6	< 0.001
TGFβ1 (Pg/ml)	5.4±4.6	26.9±0	67.7±58	< 0.001
TNFα (Pg/ml)	1.5±0.7	1.6±1.2	2.3±1.7	< 0.001
SAA (Pg/ml)	7.2±	85.8	132±	< 0.001

Table 2. The Correlations among Cytokines assayed using Pearson Product-Moment Correlation.

p-value	IL6	IL8	TGFβ1	TNFα	SAA
IL6	-	0.61; p<0.05	-	-	-
IL8	0.61; p<0.05	-	0.72; p<0.001	-	0.81; p<0.001
TGFβ1	-	0.72; p<0.001	-	-	0.74; p<0.001
TNFα	-	-	-	-	-
SAA	-	0.81; p<0.001	0.74; p<0.001	-	

4. Discussion

A potential contributing influence for associated increase of POAG in IOP is the compromised outflow facility of aqueous humor through the TM. An increased accumulation of ECM which occurs in the TM region of POAG eyes is thought to impose greater resistance to the outflow of AH, resulting in increased IOP. (8). Exfoliation syndrome can be characterized by buildup of an abnormal fibrillar ECM material chiefly to the lens, cornea and TM often leading to secondary glaucoma (9). In this study, clear elevation of cytokines including IL8, TGFβ1 and SAA levels, an immune reaction or inflammation, was detected in glaucoma eyes compared to control eyes. IL8, has a role in chemokine functions, as TM cells could be a local source of IL8 secretion (10). The significant positive correlation between IL8 and IOP seen in the current study suggests the role of IL8 in the positive regulation of outflow resistance (11) despite the molecular mechanism of action of IL8 on the TM remaining unclear. TGF-β1 signaling, throughout the body and the eye, acts in various biological processes such as fibrotic responses, ECM turn over, proliferation, apoptosis and modulation of the immune system (12). This study, showed significantly higher levels of TGFβ1 in aqueous-humor from POAG and PEXG patients compared to cataract patients. Many previous results (13, 14), are in agreement with our result. They postulated that TGFβ1 cytokine alters the extracellular matrix ECM metabolism and excess ECM has been proposed to increase aqueous outflow resistance in the trabecular meshwork TM of glaucomatous eye. It has been found that in vitro treatment of cultured human TM cells with TGFβ1 will lead to variations in gene expression, including genes that may contribute to buildup of ECM (15). In addition, a significant positive correlation was found between TGFβ1 and IL8 in the present study, thus the levels of TGFβ1 and IL8 may reflect the status of ECM remodeling and the severity of reduction in aqueous humor outflow facility at the TM in eyes with POAG and PEXG (16). This study detected elevations of SAA in aqueous humor samples from the glaucomatous eyes compared to control group. This is in agreement with Takai et al. who found the same results (17). SAA has a significant part to play regarding inflammation, infections, tissue repair and amyloid deposition (18). A pro inflammatory cytokine-induced up-regulation of SAA biosynthesis in the liver (19), macrophages, smooth muscle cells and endothelial cells (20), is the prime mediator in an acute phase response in inflammation. There is uncertainty of the exact source of SAA detection in the aqueous humor, but many cellular signal transduction pathways (21), such as the extracellular-signal regulated kinase P58, mitogen-activated protein kinase and nuclear factor Kappa β (NFKβ) dependent pathways are activated by SAA (22). This intensifies the production of matrix metalloproteinases, cytokines and cytokine receptor, and brings about the reduction of TNFα, ILB1 and IL8 (23). In PEXG, buildup of irregular extracellular material in the trabecular meshwork, whether in the form of plaque material or EXG material, could be the cause of increased outflow resistance and chromic elevation of IOP (24) The amplified aqueous levels of endogenous matrix metalloproteinases 2 (MMP2) activity could be of significance in the abnormal matrix accumulation found in the juxtacanalicular meshwork of PEXG eyes (25). Pro-inflammatory cytokine IL6 decreased significantly in POAG compared to the control in this study and PEXG patients. TM cells could be a local source of IL6, considering expression of IL6 mRNA in normal donor TM and secretion of IL6 in cultured human TM cells after TGFβ1 treatment have previously been found. TM cell loss with aging can be accountable for functional pathological change in TM tissues in POAG. Considering IL6 is a vital mediator of increased vascular permeability and endothelial dysfunction (17), increased IL6 could be the reason for iris vasculopathy and breakdown of blood-aqueous barriers that are characteristics of PEXG (17). The present study detected significant increase in TNFα concentration in the aqueous humor sample of both POAG and PEXG, compared to control. TNFα was elevated in the optic nerve and retina of the human glaucomatous cadaver eyes, thus TNFα may have a vital role in damaging the retinal ganglion cells rather than increased resistance to aqueous outflow in POAG (28). The significant correlation of TGFβ1, IL8 and SAA levels and the significant correlation of these cytokines and the IOP levels suggested that the cytokine networks play important roles in the IOP elevations in POAG (29). Although the exact mechanism of the interactions among these cytokines are unclear, it was found that adding recombination SAA to cultured TM cells potentially stimulated IL8 secretion (30), thus cytokine like prognostics of SAA may induce an immune reaction - specifically related to IL8 (31). Cross pathways among TGFβ1, TNFα, IL8 and SAA and the exact source of these cytokines could be targets of future investigations of the pathogenesis of IOP levels in glaucoma.

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Conflict of Interest:

There is no conflict of interest to be declared.

Authors' contributions:
All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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