

Tumor Necrosis Factor- α and Interleukin-6 Levels in Patients with Primary Open-Angle Glaucoma

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Abstract

Purpose: To investigate the levels of tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) in the aqueous humor and plasma of human eyes with primary open-angle glaucoma, (POAG) and to correlate their concentrations with the severity of glaucoma.

Patients and methods: Thirty five patients with POAG and thirty patients with senile cataract (control group) of matched age and gender were included in the study prospectively. Aqueous humor samples were obtained by paracentesis from glaucoma and cataract patients who were undergoing elective surgery. Aqueous humor and corresponding plasma samples were analysed for TNF- α and IL-6 concentrations by enzyme linked immunosorbent assay.

Results: TNF- α and IL-6 levels were significantly higher in aqueous humor of POAG patients with respect to the comparative group of cataract patients ($P < 0.001$). No significant difference in the levels of TNF- α and IL-6 in plasma of POAG and cataract patients. A positive correlation was found between TNF- α and IL-6 in aqueous humor of POAG patients ($P < 0.001$). Significant correlation was found between TNF- α or IL-6 levels and severity of visual field loss in moderate stage ($P < 0.001$).

Conclusion: Increased levels of TNF- α and IL-6 aqueous humor may be associated with POAG. In addition, TNF- α and IL-6 may be useful proinflammatory cytokines levels in aqueous humor of POAG patients. TNF- α and IL-6 concentrations in aqueous humor are significant with visual field loss in patients with POAG.

Keywords: TNF- α ; IL-6; Aqueous humor; Glaucoma; Cataract

Introduction

Primary open-angle glaucoma is a common neurodegenerative ocular disease in which selective cell death of retinal ganglion cells results in a characteristic clinical pattern of visual field loss and excavated appearance of the optic nerve head. To date, at least 20 genetic foci for POAG have been reported although the underlying patho-physiology of POAG candidate genes remains to be elucidated [1].

Moreover, the marked vulnerability of retinal ganglion cells in glaucoma has not been fully explained. To examine this mechanism, studies have analyzed the contributions of ocular blood circulation [2], oxidative stress [3] and cytokines. Although apoptosis is thought to play an essential role in glaucomatous neurodegeneration [4], the details of the underlying process are unclear.

Tumor necrosis factors are pleiotropic cytokines that are considered to be the primary modifiers of inflammatory and immune reactions. Two forms of TNF, designated TNF- α and TNF- β , have been reported to compete for binding to the same receptors. Interestingly, recent studies have shown that ischemic or pressure-loaded glial cells produce TNF- α , which results in oligodendrocytes death and the subsequent apoptosis of retinal ganglion cells [5].

Proinflammatory cytokines such as TNF- α and IL-6 may play role in mediating inflammation associated with various ocular diseases [6]. TNF- α is known as a potent activator of neurotoxic substances such as nitric oxide, excitotoxins [7].

TNF- α and IL-6 share many common characteristics with VEGF and are also reported to be hypoxia induced [8]. TNF- α and IL-6 gene expression markedly increases in experimental rat retina after transient ischemia [9].

In the present study, we measured the aqueous humor and plasma levels of TNF- α and IL-6, proinflammatory cytokines, in patients with POAG and cataract. In addition, we assessed their relationship with the severity of POAG.

Patients and Methods

This was a case-controlled prospective study. All experiments were carried out in accordance with the tenets of the Declaration of Helsinki (1989) of the World Medical Association. The study was approved by Mansoura university hospital trust ethics committee. All the patients were fully informed of the purpose and procedures of the study and written informed consent was obtained from all individuals.

Patients

Thirty five patients with primary open-angle glaucoma whose ages ranged from 50 to 69 years, attending the ophthalmic center at Mansoura university, Egypt, who were scheduled for glaucoma filtering surgery. The cataract group consisted of 30 patients, whose ages ranged from 52 to 66 years were scheduled for cataract surgery.

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Full ophthalmic examination was done including: assessing visual acuity, slit-lamp anterior and posterior segment biomicroscopy and IOP measurements by Goldmann applanation tonometry. In addition, patients with glaucoma were subjected to gonioscopy using Goldmann three mirror contact lens, full threshold 24-2 program Humphrey visual field analyzer (Humphrey Instruments, San Leandro, CA), and cup/disc ratio. A detailed medical history included age, gender, glaucoma medications, systemic hypertension, systemic medications, and previous ocular surgery were recorded.

The IOP measurements were measured at least five times on different times of the day from 8 AM to 5 PM. Highest and lowest measured IOP values were used to determine IOP diurnal range. The visual field categories were: (I) normal; (II) mild, an arcuate defect; (III) moderate, abnormal in one hemifield and not within 5 degrees of fixation; and (IV) severe, abnormal in both hemifields or within 5 degrees of fixation. Classification of visual field loss was done the basis of the last reliable Humphrey visual field tests before elective ocular surgery.

Exclusion criteria were: (I) patients with any type of glaucoma except POAG such as normotensive glaucoma, ocular hypertensive, angle-closure glaucoma, secondary glaucoma, pigment dispersion, or exfoliation glaucomas, (II) Patients with previous intraocular surgery and laser surgery, (III) Diseases that could influence the TNF- α and IL-6 levels such as diabetes mellitus, kidney diseases, autoimmune diseases, and endocrinopathy, (IV) history of systemic drug therapy (chemotherapeutic agents, corticosteroids, non-steroidal anti-inflammatory drugs), (V) patients with topical use of steroids, prostaglandin analogues and non-steroidal anti-inflammatory drugs.

Inclusion criteria were: POAG patients with elevated intraocular pressure, correlated visual field loss, and glaucomatous optic nerve head changes criteria, and cataract patients had senile cataract, normal IOP and did not receive any topical medications. In all patients, systemic blood pressure should be systolic blood pressure ≤ 140 mmHg and diastolic blood pressure ≤ 90 mmHg.

Sample collection

Aqueous humor sampling: Aqueous humor samples were obtained from each patient requiring either elective glaucoma surgery or cataract surgery. Aqueous humor 0.1 to 0.2 ml was collected at the beginning of surgery through a paracentesis in the peripheral clear cornea using a 27 gauge needle on a tuberculin microsyringe from the central papillary area. Blood contamination or damage to the iris and others intraocular structures were meticulously avoided. Aspiration was stopped as soon as the anterior chamber began to shallow. Aqueous was transferred to a vial and placed in liquid nitrogen immediately upon removal from the anterior chamber. Aqueous humor samples were immediately frozen at -70°C until processing for the subsequent biochemistry techniques. All samples were protected from light.

Blood Sampling: Fasting venous blood samples were withdrawn from the patients and control groups. Each blood sample was collected in EDTA containing tube. The sample was centrifuged at 3000 rpm for 5 minutes at 4°C and the separated non-haemolysed plasma was rapidly frozen at -70°C or storage until the time of assay.

Assay

TNF- α and IL-6 in the aqueous humor and plasma samples were quantitated with sandwich” double antibody” enzyme linked immunosorbent assay (ELISA) Kits (Boehringer, Mannheim, Germany) as described by Meager et al. [10] and Helle et al. [11], respectively.

The assays were processed by using two monoclonal antibodies,

directed against two different epitopes of cytokines, where they essential for receptor binding. These enabled the specific determination of biologically active cytokines in this assay system.

The measuring range of this test system has been shown to be between 5 and 1000 pg/ml, and a value of more than 10 pg/ml usually obtained.

Statistical analysis

All data were analyzed with the SPSS version 15 (SPSS Inc., Chicago, IL, USA). Data were presented as mean \pm SD (standard deviation). Results were compared by using parametric unpaired t test and Chi-square (χ^2) to detect any difference in either demographic or clinical characteristics. Analysis of variance was used to detect any significant difference in both groups. Pearson correlation coefficient was used to test association between variables. P value of less than 0.05 was considered to be significant.

Results

Sixty five eyes of 65 patients, age ranged from 50 to 69 years, were enrolled in the present study according to the inclusion/exclusion criteria as described. There were 35 primary open-angle glaucoma eyes (glaucoma group) and 30 senile cataract eyes (cataract group).

Table 1 summarizes the demographic and clinical features in both groups. The mean age of the POAG group was 61.51 ± 4.91 , whereas the mean age of the control group was 60.35 ± 4.71 and there was no statistically significant difference between the groups ($P = 0.37$). There was no statistically significant difference between the POAG group and the control group regarding gender and IOP [$P = 0.85$, $P = 0.49$, respectively]. As expected, cup/disc ratio and IOP diurnal amplitude were statistically significant.

Table 2 shows ophthalmic characteristics of glaucoma patients, such as visual acuity or cup/disc ratio. Table also includes number of glaucoma eye drops, and the severity of visual field loss were mild ($n = 9$), moderate ($n = 16$), and severe ($n = 10$).

Aqueous humor concentration of TNF- α was highly significant increased in the POAG group when compared with that of the control group [38.3 ± 5.1 pg/ml Vs 11.4 ± 4.9 pg/ml, $P = 0.004$]. Also, aqueous humor concentration of IL-6 was highly significant increased in the POAG group when compared with that of the control group [85.1 ± 45.5 pg/ml Vs 34.6 ± 12.4 pg/ml, $P = 0.005$] (Table 3).

TNF- α concentration in plasma of the POAG group was non-significantly higher when compared with that of the control group [15.8 ± 2.5 pg/ml Vs 10.6 ± 3.1 pg/ml, $P = 0.352$]. Also, IL-6 concentration

Parameters	Group POAG Group	Control group	*P value
No.	35	30	
Age (year)	61.51 ± 4.91	60.35 ± 4.71	$F = 4.51$ $P = 0.37$
Gender			
Male	19 (54%)	16 (53%)	$\chi^2 = 0.036$
Female	16 (46%)	14 (47%)	$P = 0.85$
Cup/Disc ratio	0.76 ± 1.4	0.47 ± 2.5	$F = 3.64$ $P = 0.012^*$
IOP at time of examination	20.5 ± 4.9	18.4 ± 5.1	$F = 3.54$ $P = 0.49$
IOP pulse amplitude**	10.19 ± 3.5	5.45 ± 2.8	$F = 4.51$ $P = 0.016^*$

No. = Number, F = One Way Anova test, χ^2 = Chi-square test

*Significant at $P < 0.05$

**IOP pulse amplitude is the difference between the lowest and highest recorded IOP

Table 1: Demographic and clinical features in the study and control groups.

	Visual field loss			No. eye drops		Cup/disc ratio		VA (Log MAR)	
	Mild	Moderate	Severe	1	2	0.1-0.5	0.6-1.0	0.1-0.5	0.6-1.0
POAG group N=35	25.7%	45.7%	28.6%	71%	29%	62%	38%	68%	32%

No.: Number; IOP= Intraocular Pressure; mmHg: Milimeter Mercury; VA: Visual Acuity; Log MAR= Logarithm of Minimum Angle of Resolution

Table 2: Ophthalmologic characteristics of the POAG patients, expressed as percentage.

Parameter	Group	POAG Group (n=35)	Control Group (n=30)	*P value
		Aqueous humor		
TNF- α (pg/ml)		38.3 \pm 5.1	11.4 \pm 4.9	0.004*
IL-6 (pg/ml)		85.1 \pm 45.5	34.6 \pm 12.4	0.005*
Plasma				
TNF- α (pg/ml)		15.8 \pm 2.5	10.6 \pm 3.1	0.352
IL-6 (pg/ml)		18.6 \pm 2.6	15.5 \pm 4.2	0.241

*significant at p<0.05

Table 3: Aqueous humor and plasma TNF- α and IL-6 levels among the studied patients.

Correlation between studied variables	p	r
Primary open-angle glaucoma		
TNF- α (aqueous) – IL-6 (aqueous)	<0.001	0.216
TNF- α (aqueous) – TNF- α (plasma)	0.465	0.235
TNF- α (plasma) – IL-6 (aqueous)	0.376	0.571
TNF- α (plasma) – IL-6 (plasma)	0.281	0.453
Control		
TNF- α (aqueous) – IL-6 (aqueous)	0.501	0.645
TNF- α (aqueous) – TNF- α (plasma)	0.472	0.463
TNF- α (plasma) – IL-6 (aqueous)	0.621	0.524
TNF- α (plasma) – IL-6 (plasma)	0.395	0.356

Table 4: Correlation between aqueous humor and plasma TNF- α and IL-6 levels among study groups.

Parameters	Mild V.F loss	Moderate V.F loss	Severe V.F loss
Aqueous humor TNF-α (pg/ml)			
r	-0.316	-0.281*	-0.425
p	0.251	<0.001	0.276
Aqueous humor IL-6 (pg/ml)			
r	-0.452	-0.275*	-0.293
p	0.324	<0.001	0.167
Plasma TNF-α (pg/ml)			
r	-0.237	-0.196	-0.326
p	0.451	0.351	0.251
Plasma IL-6 (pg/ml)			
r	-0.189	-0.217	-0.315
p	0.275	0.432	0.361

VF.: Visual Field, TNF- α : Tumor necrosis factor- α ; IL-6: Interleukin-6

*Significant at P< 0.05

Table 5: Correlation co-efficient values between severity of glaucoma stage and both TNF- α and IL-6 in glaucoma patients.

in plasma of the POAG group was non-significantly increased when compared with that of the control group (18.6 \pm 2.6 pg/ml Vs 12.5 \pm 4.2 pg/ml, P= 0.241) (Table 3).

Aqueous humor concentration of TNF- α in the POAG patients was significantly correlated with those of IL-6 [r= 0.216, P<0.001]. No significant correlation was found between aqueous and plasma levels of TNF- α and IL-6 (Table 4). There was no correlation was found in the mild or severe groups.

Table 5 shows the correlation between TNF- α and IL-6 and severity of visual field loss in glaucoma group. Significant correlation was found between TNF- α levels and moderate visual field loss (r= -0.281, P< 0.001). IL-6 levels revealed a significant correlation with moderate visual field loss (r=-0.275, P<0.001).

Discussion

Because intraocular pressure (IOP) alone cannot accurately

predict POAG, it is critical to identify associated risk factors for the development and / or progression of POAG. Approximately, one third of all glaucoma patients have what are considered to be normal IOP. It is now well – established that the visual field deficits associated with all forms of glaucoma are due to retinal ganglion cell apoptosis [12]. Thus, there is much hope that neuro protective agents can one day become an important adjunct or even primary therapy in the management of glaucoma.

TNF- α is a pleiotropic inflammatory cytokine. Tissue ischemia or damage enhances the production of TNF- α , which contributes to the remodeling process during nerve degeneration [13]. In addition to nitric oxide and excitotoxins, TNF- α has neurotoxic effects and functions as an activator. TNF- α concentration in plasma, cerebrospinal fluid, and brain tissue are elevated in CNS disorders, including Alzheimer's disease, multiple sclerosis, Parkinson's disease, and ischemic brain injuries [2]. In a rat cerebral ischemic model, exogenous TNF- α

was found to exacerbate focal ischemic injuries, whereas blocking endogenous TNF- α was neuroprotective [14].

IL-6 is a multifunctional cytokine that act on a wide range of cells. Several reports have demonstrated its major role as a mediator of inflammatory and immune responses [15]. The pleiotropic effects of IL-6 include the stimulation and secretion of immunoglobulin, induction of neuronal differentiation, and activation of acute-phase protein synthesized by liver cells [16].

In the present study, we measured the aqueous humor and plasma levels of TNF- α and IL-6 in patients with POAG and cataract (control). In addition, we correlated their concentrations with the severity of glaucoma.

Our study revealed a statistically significant increase in the aqueous humor level TNF- α in POAG patients compared with control patients but there was no significant increase in the serum level. The significantly higher levels of TNF- α and IL-6 in aqueous humor associated with non-significant serum changes could be attributed to enhanced intraocular synthesis.

Many studies have detailed the relationship between glaucomatous optic neuropathy and TNF- α . In an animal model with high intraocular pressure, elevation of TNF- α precedes the loss of retinal ganglion cells and oligodendrocytes. In addition, these cell losses are observed by administering of TNF- α without the elevated intraocular pressure [17]. TNF- α contributes to this process by adversely affecting oligodendrocytes [18] by increasing the susceptibility of axons to excitotoxicity in the optic nerve head and retinal ganglion cell death [19].

When glial cells are exposed to such stressors as high pressure or ischemia, they are known to excrete TNF- α , which is followed by cellular apoptosis. Additionally, this apoptosis can be prevented using neutralizing anti-TNF- α antibodies [5]. These experiments are supported by immunohistochemistry results with human samples. Yan et al [20] and Tezel et al. [21] found that the expression levels of TNF- α and its receptor TNF-R1 were higher in the inner retinal layers of glaucoma eyes than in control eyes. The increased expression of TNF- α in glaucoma eyes suggests that this cytokine is critically connected with the damaging processes in these tissues.

Intraocular TNF- α is likely secreted from activated macrophages, astrocytes [22], microglial cells [5] and retinal Müller glial cells [23]. These results suggest that intraocular stress, including high pressure and ischemia, stimulates TNF- α production, which may signal the progression of neuronal damage. Post-trauma increases in TNF- α level are known to predict poor prognoses [13]. Because TNF- α is secreted during neuronal modifying and damage, higher TNF- α concentrations in glaucoma patients may suggest that the disease is not fully controlled and that the visual field defect is likely to progress.

Our study revealed a statistically significant increase in the aqueous humor level IL-6 in POAG patients compared with control patients but there was no significant increase in the serum level. However, the increased level of IL-6 in aqueous humor of POAG patients is a cytokine that is synthesized secondary to the higher intraocular pressure.

Normally, cells do not synthesize and secrete IL-6 unless the cells are stimulated by other cytokines or by some physiological event. It has been shown that a wide range of ocular tissues can produce IL-6 in vitro and in vivo, such as cultures of cornea epithelial, stromal and endothelial cells [24], cytokine-stimulated human pigment epithelial cells [25], ischemic retina [9] and hypoxia-induced or cytokine-stimulated vascular endothelial cells and vascular smooth muscle cells

[26]. Therefore, there is a strong possibility that IL-6 in aqueous humor of eyes with POAG comes from intraocular sources, rather than in systemic circulation.

Optineurin gene is induced by TNF- α and interacts with several proteins to regulate apoptosis, inflammation and vasoconstriction. For example, optineurin interacts with adenoviral E3-14.7k protein which protects cells from the cytolytic activity activity of TNF- α [27]. In glaucomatous eyes, the expression of TNF- α and TNF- α receptor-1 was upregulated in the retina and optic nerve head [28]. Yuan and Neufeld [29] reported that the expression of TNF- α and TNF- α receptor-1 appeared to parallel the progression of optic nerve degeneration. In addition, Funayma et al. [30] reported that an interaction between single-nucleotide polymorphisms in the TNF- α gene and those in the optineurin gene increases the risk for development and probably progression of glaucoma in patients with POAG.

We did not find a relationship between intraocular pressure and aqueous humor TNF- α level and POAG. Cordeiro et al. [31] found that a few weeks after certain pressure load conditions were applied, TNF- α was expressed, which was followed by nerve degeneration. The expression of intraocular TNF- α is considered to be the result from the secretion from ocular resident cells including iris, ciliary body [32] and retinal ganglion cells [33]. Previous data shows that TNF- α concentration in vitreous humor are higher than those in aqueous humor [34]. This suggests that some proportion of TNF- α in aqueous humor is the result of diffusion from vitreous humor, which may have resulted in sub threshold concentrations.

The significant increase in aqueous humor TNF- α and IL-6 at the moderate stage of the POAG visual field loss and insignificant increase at end stage of disease may indicate potential secondary consequence. As elevated levels of TNF- α and IL-6 may be accompanied by programmed cell death.

In the current study, there was no correlation between the aqueous humor and plasma levels of TNF- α and IL-6 results suggested that levels TNF- α and IL-6 in aqueous humor were not related to breakdown of blood-retinal barrier and/ or ocular blood. But, the TNF- α level in aqueous humor was significantly correlated with aqueous humor IL-6. This correlation supported that both TNF- α and IL-6 may be associated with glaucoma.

The elevation of intraocular expression of TNF- α and IL-6 may be responsible for causing nerve damage. Our results suggest targeting TNF- α and IL-6 as a mean to inhibit the neurodegenerative mechanisms associated with glaucoma. Further investigation into these mechanisms will elucidate the role of TNF- α in glaucoma neurodegeneration and may provide a new approach to treat glaucoma and improve patient outcomes. However, further investigations are needed to demonstrate these factors in vitreous samples.

Declaration of Interest

None of the authors has a financial or proprietary in any material or method mentioned. The authors alone are responsible for the content of the paper.

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