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Increased concentration of tumor necrosis factor alpha in the plasma of glaucoma patients

Povišena koncentracija faktora nekroze tumora alfa u plazmi bolesnika sa glaukomom

Marija Trenkić*†, Tatjana Jevtović Stoimenov‡, Jelena Bašić‡, Jelena Vasilijević§‖, Dragana Ristić¶, Milan Trenkić††, Aleksandar Veselinović†, Marija Cvetanović†**

University of Niš, Faculty of Medicine, *Department of Ophthalmology, ‡Institute of Biochemistry, ††Department of Gynecology, Niš, Serbia; †University Clinical Center Niš, Clinic for Ophthalmology, Niš, Serbia; §University of Belgrade, Faculty of Medicine, Department of Ophthalmology, Belgrade, Serbia; ‖University Clinical Center of Serbia, Clinic for Eye Diseases, Belgrade, Serbia; ¶University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; **Military Medical Academy, Clinic for Ophthalmology, Belgrade, Serbia

Abstract

Background/Aim. Changes in the concentration of various mediators of inflammation in blood, aqueous humor, or eye tissues support the role of inflammation in the pathogenesis of open-angle glaucoma (OAG). Inflammatory biomarkers have a great potential for application in clinical practice. The aim of the study was to determine concentrations of tumor necrosis factor (TNF)-α in the plasma of patients with OAG and subjects without glaucoma and examine the correlation between the TNF-α concentration in plasma in glaucoma patients and specific clinical parameters. **Methods.** The study included 87 participants (87 eyes) divided into three groups: 35 subjects (35 eyes) with primary OAG (POAG) with elevated intraocular pressure (IOP) – hypertension glaucoma (HTG) (POAG-HTG), 23 subjects (23 eyes) with pseudoexfoliative OAG (XFG), and 29 subjects in the control group (healthy subjects) matched with the patient groups in terms of age and gender. We performed a complete clinical examination, including standard automated perimetry and determination of changes in the participant's repeated visual field, optical coherence tomography and determination of peripapillary retinal nerve fiber layer (RNFL) thickness. The concentration of TNF-α in participants' plasma was measured using commercial enzyme-linked immunosorbent assay – ELISA. **Results.** The concentrations of TNF-α in the plasma of glaucoma patients (POAG-HTG 2.04 ± 1.98 pg/mL and XFG OAG 2.05 ± 1.48 pg/mL) were significantly higher than in healthy subjects (1.43 ± 2.00 pg/mL, $p < 0.05$). In none of the groups of subjects suffering from glaucoma was there a statistically significant correlation of TNF-α concentration in the plasma with any of the clinical parameters, including IOP, cup/disk ratio, mean deviation, average RNFL, and RNFL in the superior and inferior quadrant. **Conclusion.** The concentration of the pro-inflammatory cytokine TNF-α in the plasma is significantly higher in glaucoma patients compared to non-glaucomatous subjects, and it confirms the role of inflammation in the pathogenesis of glaucoma as one of the non-inflammatory ocular diseases. The concentrations of TNF-α in the plasma of glaucoma patients did not correlate with any of the examined clinical parameters; hence, it cannot be considered a measure of progression and damage in glaucoma.

Key words:
biomarker; exfoliation syndrome; glaucoma, open-angle; tumor necrosis factor-alpha.

Apstrakt

Uvod/Cilj. Promene u koncentraciji različitih medijatora zapaljenja u krvi, očnoj vodici ili tkivima oka, podržavaju teoriju o ulozi inflamacije u patogenezi glaukoma otvorenog ugla (GOU). Biomarkeri inflamacije imaju veliki potencijal za primenu u kliničkoj praksi. Cilj rada bio je da se utvrde koncentracije faktora nekroze tumora alfa (*tumor necrosis factor alpha* – TNF-α) u plazmi bolesnika sa GOU i ispitanika bez glaukoma, i prouči veza između nivoa TNF-α u plazmi bolesnika sa glaukomom i određenih kliničkih parametara. **Metode.** Studijom je obuhvaćeno 87 učesnika (87 očiju) podeljenih u tri grupe: 35 ispitanika (35 očiju) obolelih od primarnog GOU (PGOU), sa povišenim

Correspondence to: Marija Trenkić, University of Niš, Faculty of Medicine, Department of Ophthalmology, Blvd. Dr. Zorana Djindjića 81, 18 000 Niš, Serbia. E-mail: marija.trenkic@medfak.ni.ac.rs

intraokularnim pritiskom (IOP) – hipertenzivni glaukom (HTG) (PGOU-HTG), 23 ispitanika (23 oka) koji boluju od pseudoeksfolijativnog GOU (PEG) i 29 ispitanika u kontrolnoj grupi (zdravi ispitanici), sparenih po starosti i polu, sa obolelima od GOU. Urađen je kompletan oftalmološki pregled, standardna automatizovana perimetrija i određivanje promena u ponovljenom vidnom polju ispitanika, optička koherentna tomografija i određivanje debljine retinalnih peripapilarnih nervnih vlakna (*retinal nerve fiber layer* – RNFL). Koncentracija TNF-α u plazmi ispitanika merena je komercijalnim *enzyme-linked immunosorbent assay*-ELISA testovima. **Rezultati.** Koncentracije TNF-α u plazmi bolesnika sa glaukomom (PGOU-HTG 2,04 ± 1,98 pg/mL i PEG 2,05 ± 1,48 pg/mL) bile su značajno više nego kod zdravih ispitanika (1,43 ± 2,00 pg/mL, $p < 0,05$). Ni u jednoj od grupa ispitanika obolelih od glaukoma nije bilo statistički značajne korelacije koncentracije TNF-α u plazmi sa bilo kojim od ispitanih kliničkih parametara [IOP, *cup/disk ratio*, srednja vrednost devijacije (*mean deviation*), prosek RNFL (RNFL *average*), RNFL superiorni kvadrant i RNFL inferiorni kvadrant]. **Zaključak.** Koncentracija pro-inflamacijskog citokina TNF-α u plazmi je značajno viša kod bolesnika sa glaukomom u poređenju sa ispitanicima bez glaukoma i to potvrđuje ulogu inflamacije u patogenezi glaukoma, kao jednog od neinflamacijskih oboljenja oka. Koncentracija TNF-α u plazmi bolesnika sa glaukomom ne koreliše ni sa jednim od ispitivanih kliničkih parametara, zbog čega ne može biti mera progresije i oštećenja kod glaukoma.

Ključne reči:
biološki pokazatelji; eksfolijativni sindrom; glaukom, otvoreni ugao; faktor nekroze tumora alfa.

Introduction

Glaucoma is a heterogeneous group of progressive optic neuropathies in which complex genetic and other factors lead to retinal ganglion cell (RGC) death [1]. Changes in the concentration of various mediators of inflammation and immune response in blood, aqueous humor (AH), or eye tissues in glaucoma indicate the involvement of inflammation and immune system activity in the pathogenesis of open-angle glaucoma (OAG) [2]. However, the precise mechanism underlying the interaction between lymphocytes, antibodies, and cytokines in glaucoma remains unclear.

Cytokines are low-molecular-weight polypeptides involved in communication between cells [3]. Abnormal production of some cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, soluble IL-2 receptor, IL-6, and the chemokine IL-8, plays a crucial role in the pathogenesis of various inflammatory and autoimmune diseases [3, 4]. Numerous *in vivo* studies have shown that TNF-α, IL-1, IL-6, and IL-8 are significant components in the pro-inflammatory response and intraocular inflammation [4–6]. Moreover, oxidative stress is associated with many systemic inflammatory diseases, free radical production, and lipid peroxidation [7].

TNF-α belongs to the group of pro-inflammatory cytokines. TNF-α is considered a dual-function cytokine that can stimulate cell inflammation and proliferation but also induce the process of apoptosis. Genetic variations, resulting in increased production of this cytokine, are thought to be associated with the potential development of chronic diseases and increased risk of infections and may also affect the prognosis of the disease [8]. TNF-α exerts its biological effects through two TNF receptors (TNF-RI and TNF-RII). TNF-RI (CD120a, p55) is constitutive in most tissues except for erythrocytes and unstimulated lymphocytes and can be activated by both membrane-bound and soluble forms of TNF-α. TNF-RII (CD120b, p75) is exclusively found in immune system cells, and it is activated solely by membrane-bound TNF-α. Although TNF-RII exhibits a five-fold higher affinity for binding TNF-α than TNF-RI, most biological effects are achieved through TNF-RI [9].

TNF-α can initiate the process of apoptosis by external or internal activation. External activation of this signaling pathway, namely, binding of TNF-α to TNF-RI, results in a cascade activation of caspases (-3, -6, -7) through the activation of caspase-8, ultimately leading to cell death *via* apoptosis [10].

TNF-α can lead to the generation of reactive oxygen and nitrogen species by inducing the expression of the genes for inducible nitric oxide synthase and nicotinamide adenine dinucleotide phosphate oxidase. Given the effects of these metabolites on the plasma membrane, their increased concentration can disrupt the permeability of the inner mitochondrial membrane, resulting in the loss of membrane potential and release of cytochrome-c into the cytoplasm (internal pathway of apoptosis activation) [11]. In the presence of adenosine triphosphate, released cytochrome-c binds with apoptotic protease activating factor 1 and pro-caspase-9, forming a complex referred to as the apoptosome. Within the apoptosome, catalytic activation of procaspase-9 to caspase-9 and subsequent cascade activation of caspases (-3, -6, -7) occur, driving the cell to the final stage of apoptosis [10, 11].

Proteins located on the outer mitochondrial membrane regulate the internal pathway of apoptosis activation and control the translocation of certain proteins from the mitochondrial intermembrane space to the cytoplasm. These proteins fall into three categories: proapoptotic (Bak, Bax), proapoptotic facilitator (Bid, Bad, PUMA, Noxa), and antiapoptotic (Bcl-2, Bcl-X1) proteins. Upon activation of the intrinsic pathway of apoptosis, Bak and Bax proteins oligomerize, leading to the formation of pores on the mitochondrial membrane and facilitating the release of cytochrome-c. The antiapoptotic proteins bind to Bax and Bak proteins, preventing the process of apoptosis. Whether a cell will undergo apoptosis through this mechanism or survive depends on the concentration of pro- and antiapoptotic proteins [10]. TNF-α is important, both for numerous physiological and

various pathological conditions in the central nervous system (CNS) and, therefore, in the retina as well [12–15]. TNF-α has been shown to play mostly a harmful role in a wide range of neurodegenerative processes in the retina, such as glaucoma and retinal ischemia. These pathologies are primarily characterized by the loss of RGCs [14, 15]. However, TNF-α may not solely mediate cell death and toxicity; it may also promote cell survival [16].

On the other hand, astrocytes are in close contact with RGCs and provide support to neurons, typically allowing their normal function. However, in specific pathological conditions within the CNS, they are associated with neuronal loss, thereby facilitating RGC death [17–21]. Previous studies have shown that the TNF-α produced and secreted by astrocytes facilitates RGC death in cultures of RGCs and astrocytes [22].

Given that inflammatory biomarkers have a great potential for application in clinical practice and that the role of TNF-α in the glaucoma process has not yet been fully clarified, we decided, as the aim of our paper, to investigate the plasma concentrations of TNF-α in patients with OAG and non-glaucomatous controls and examine the correlation between plasma TNF-α levels of glaucoma patients and their clinical parameters.

Methods

The present study included 87 participants (87 eyes) divided into three groups: 35 subjects (35 eyes) with primary OAG (POAG) with elevated intraocular pressure (IOP) - hypertension glaucoma (HTG) (POAG-HTG), 23 subjects (23 eyes) with pseudoexfoliative OAG (XFG), and 29 subjects in the control group (healthy subjects) matched with the patient groups in terms of age and gender. Prior to the research, all participants were informed about the objectives of the study and signed informed consent to participate according to the Declaration of Helsinki. The Ethics Committee of the Faculty of Medicine in Niš (No. 01-2625-18, from April 8, 2014) and the Ethics Committee of the University Clinical Center Niš (No. 338/43, from January 13, 2015) gave their consent for conducting the research.

We performed a complete clinical examination including collection of demographic characteristics of participants, thorough the following: assessment of family, personal, and ophthalmological history; determination of visual acuity with refraction (Snellen tables); biomicroscopy of the anterior segment of the eye; determination of IOP by Goldmann applanation tonometry; gonioscopy (using a Goldmann three mirrors gonioscope) performed to assess the opening, width, and pigmentation of the chamber angle (according to the Scheie classification system); indirect ophthalmoscopy performed to determine the cup size of the optic nerve head, specifically the cup/disk (C/D) ratio, using a 90D lens, standard automated perimetry (Humphrey Visual Field Analyzer, HFA, USA, Carl Zeiss Meditec, Inc.; Threshold Test 24-2), used to track changes in the participant's repeated visual field as mean deviation (MD); optical coherence tomography (OCT) (Stratus, Carl Zeiss Meditec, Inc., Dublin, CA); measurement of peripapillary retinal nerve fiber layer (RNFL) thickness average (RNFL Avg), in both the superior (RNFL Sup) and the inferior (RNFL Inf) quadrant. Based on the most commonly used criteria for grading glaucoma damage, the Hodapp-Parrish-Anderson classification [23], patients with POAG-HTG and XFG were classified into three groups: a) early defect [MD < -6 decibels (dB)], b) moderate defect (MD < -12 dB), and c) severe defect (MD > -12 dB).

Diagnostic criteria for POAG-HTG included the following: elevated IOP (≥ 22 mmHg); a characteristic arcuate, Bjerrum's scotoma, and/or paracentral scotoma, and/or nasal, Rönne's step observed in Humphrey's computerized vision field, and other corresponding visual field defects; corresponding optic nerve excavation, and/or thinning of the nerve fiber layer on OCT; a finding of an open angle on gonioscopy, and the absence of any secondary cause of glaucomatous optic neuropathy, such as prior trauma, administration of corticosteroids, inflammation, or uveitis. Patients with a history of inflammatory eye diseases, congenital or normotensive glaucoma, inadequate fundus visualization, diabetes mellitus, and systemic factors possibly affecting the examined marker levels were excluded from the study.

The XFG group consisted of patients with already diagnosed XFG based on the established criteria: elevated IOP, visual field alterations, and thinning of the RNFL on OCT, which are also the characteristics for the POAG-HTG group. The XFG patients had pseudoexfoliation on the anterior lens capsule and/or along the pupillary rim.

The control group consisted of healthy subjects without systemic and inflammatory diseases potentially affecting the level of the examined marker and with no family history of glaucoma, matched by gender and age. In these participants, glaucoma was ruled out using the same diagnostic criteria as for the diagnosis of POAG-HTG, i.e., after undergoing the same ophthalmological examinations and procedures. Any participant with suspected normal tension glaucoma or HTG was excluded from further examination.

During the clinical examination of the participants, we collected whole blood samples for subsequent biochemical analyses, using ethylenediaminetetraacetic acid as an anticoagulant. The blood samples were centrifuged at 3,500 rpm for 10 min at a temperature of +4 °C. Subsequently, the plasma was separated and frozen at a temperature of -80 °C.

The concentration of circulating TNF-α in participants' plasma was measured by commercial sandwich enzyme-linked immunosorbent assay (ELISA), based on the competitive binding of polyclonal antibodies specific for TNF-α, following the manufacturer's guidelines (Quantikine ELISA, DTA00C, R&D Systems, Minneapolis, USA). The concentration was determined using a standard curve and reported in pg/mL. The minimum detectable dose was 1.6 pg/mL. As *per* the manufacturer's instructions, there was no significant cross-reactivity or interference with other proteins.

Statistical analysis

We used the methods of descriptive and analytical statistics to process data obtained from the research. Statistical

processing of the results was performed using the SPSS 15.0 software package (SPSS Inc., Chicago, IL, USA). Basic descriptive statistical parameters for qualitative and quantitative evaluation of the obtained results included the following: absolute and relative numbers, arithmetic mean, standard deviation, median, and an interval of variation (minimum and maximum values). We applied the Mann-Whitney *U* test or the Student's *t*-test for independent samples to assess the significance of differences (*p*-value) in tested values between two groups of participants. The Kruskal-Wallis test and analysis of variance (ANOVA) were employed to test the significance of differences among multiple groups, whereas the Student's *t*-test and ANOVA were used when continuous variables exhibited a normal distribution. To test the strength of the association between two continuous variables, we performed a correlation analysis, specifically using Spearman's rank correlation coefficient for a distribution deviating from normal. We conducted a univariate linear regression analysis to test the influence of independent predictor variables on the value of the continuous dependent variable. A value of $p < 0.05$ was used as the threshold for statistical significance.

Results

Table 1 shows the demographic characteristics of the 87 participants, divided into three groups (35 POAG-HTG + 23 XFG + 29 control), and basic clinical parameters of glaucoma (IOP, C/D ratio, MD, RNFL Avg, RNFL Sup, and RNFL Inf).

The results of the study revealed that the average age of all participants was 71.8 ± 8.2 years, with a median of 74 years. The participants' ages ranged from 51 to 88 years. The Kruskal-Wallis test indicated no significant difference in age among the examined groups, as confirmed further by the Mann-Whitney *U* test comparing each group separately. POAG-HTG and XFG were more prevalent in men (54.3% and 56.5%, respectively), while women were more prevalent (51.7%) in the control group. However, there was no significant difference between the groups nor clear dominance of one of the genders within the examined

Table 1

Demographic and clinical characteristics and plasma levels of TNF-α in glaucoma patients and the control group of subjects

Parameter	Group			Statistical analysis
	POAG-HTG (n = 35)	XFG (n = 23)	control (n = 29)	
Age (years)				
mean ± SD	70.9 ± 7.9	73.8 ± 5.8	71.8 ± 9.4	Kruskal-Wallis test
median (min–max)	70.0 (58.0–87.0)	76.0 (59.0–84.0)	74.0 (51.0–88.0)	Mann-Whitney *U* test
Gender, n (%)				
male	19 (54.3)	13 (56.5)	14 (48.3)	
female	16 (45.7)	10 (43.5)	15 (51.7)	
IOP (mmHg)				
mean ± SD	**21.86 ± 7.37**^{c***}	**23.11 ± 11.78**^{c***}	14.76 ± 2.39	Kruskal-Wallis and Mann-Whitney *U* test
median (min–max)	20.00 (10.00–48.00)	20.50 (10.00–56.00)	14.00 (8.00–20.00)	
Cup/Disk ratio				
mean ± SD	0.64 ± 0.20	0.62 ± 0.19	▫	Mann-Whitney *U* test
median (min–max)	0.60 (0.40–1.00)	0.55 (0.40–1.00)		
Mean deviation (dB)				
mean ± SD	-11.73 ± 9.05	-12.95 ± 10.98	▫	Mann-Whitney *U* test
median (min–max)	-8.46 (-0.38– -31.27)	-8.62 (-0.07– -29.69)		
RNFL Avg (μm)				
mean ± SD	78.1 ± 24.4	78.9 ± 21.2	▫	Mann-Whitney *U* test
median (min–max)	80.9 (24.5–143.7)	81.6 (45.8–103.7)		
RNFL Sup (μm)				
mean ± SD	92.4 ± 34.5	100.9 ± 37.3	▫	Mann-Whitney *U* test
median (min–max)	96.0 (26.0–180.0)	104.0 (44.0–161.0)		
RNFL Inf (μm)				
mean ± SD	94.4 ± 37.6	96.4 ± 30.8	▫	Mann-Whitney *U* test
median (min–max)	101.0 (29.0–157.0)	91.00 (57.0–144.0)		
TNF-α (pg/mL)				
mean ± SD	**2.04 ± 1.98**^{c*}	**2.05 ± 1.48**^{c*}	1.43 ± 2.00	Kruskal-Wallis and Mann-Whitney *U* test
median (min–max)	1.82 (0.00–8.41)	1.57 (0.00–6.64)	0.81 (0.00–10.19)	

POAG-HTG – primary open-angle glaucoma - hypertensive glaucoma; XFG – pseudoexfoliative glaucoma; IOP – intraocular pressure; n – number of participants/eyes; SD – standard deviation; dB – decibels; RNFL Avg – retinal nerve fiber layer (RNFL) average; RNFL Sup – RNFL thickness in the superior quadrant; RNFL Inf – RNFL thickness in the inferior quadrant; TNF – tumor necrosis factor; c – vs. control; min – minimum; max – maximum; *$p < 0.05$; ***$p <$ 0.001. Bolded values are statistically significant. ▫The values could not be determined during the sampling period.

groups. The highest IOP value was found in the eyes of participants diagnosed with XFG. Both glaucoma groups exhibited significantly higher IOP values compared to the control group ($p < 0.001$). The values of the C/D ratio were nearly identical in the POAG-HTG and XFG groups. Hence, there was no significant difference in this parameter between POAG-HTG and XFG. Although the absolute value of MD was higher in eyes with XFG, it was not significantly different from the value of this parameter in eyes with POAG-HTG. The distribution of POAG-HTG and XFG patients according to the Hodapp-Parrish-Anderson classification was even, without significant difference. The value of RNFL Avg, RNFL Sup, and RNFL Inf was higher in the eyes of patients with XFG but not statistically significant compared to the group of patients with POAG-HTG.

The lowest value of plasma TNF-α was found in the control group (1.43 ± 2.00 pg/mL), significantly differing from patients with POAG-HTG (2.04 ± 1.98 pg/mL) and XFG (2.05 ± 1.48 pg/mL), $p < 0.05$ (Table 1). Table 1 and Figure 1 present the values of the statistical parameters of TNF-α in the examined groups.

We used Spearman's linear correlation coefficient to examine the relationship between plasma TNF-α concentration and IOP, C/D ratio, MD, RNFL Avg, RNFL Sup, and RNFL Inf (Table 2). No significant correlations of TNF-α concentration with any of these clinical parameters in any of the groups of patients with glaucoma was found.

Univariate linear regression analysis indicated no effect of TNF-α plasma concentration on the values of the examined clinical parameters: IOP, C/D ratio, MD, RNFL Avg, RNFL Sup, and RNFL Inf in patients with POAG-HTG and XFG (Table 3).

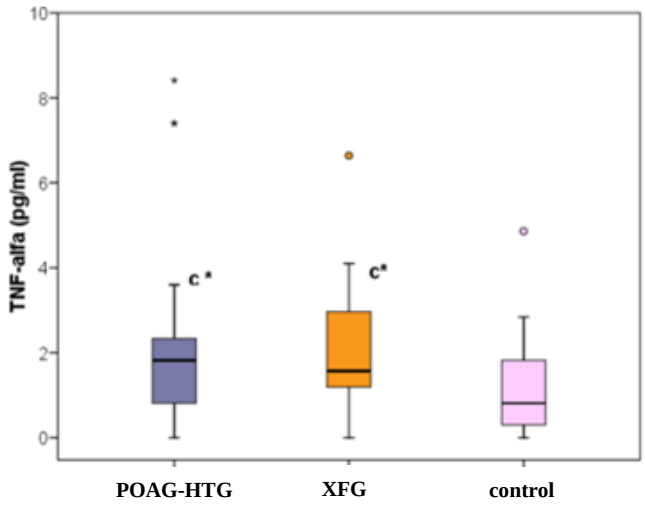

Fig. 1 – Median, minimum, maximum, and 25th and 75th percentile values for TNF-α in the plasma of participants in relation to glaucoma type and control. c – vs. control; * $p < 0.05$ (Kruskal-Wallis and Mann-Whitney *U* test). For abbreviations, see Table 1.

Table 2

Spearman's correlation coefficients of TNF-α and the clinical parameters in groups of patients with glaucoma

Group	TNF-α					
	IOP	C/D	MD	RNFL Avg	RNFL Sup	RNFL Inf
POAG-HTG	-0.03	0.02	-0.02	0.02	-0.03	-0.05
XFG	-0.08	-0.12	0.31	0.29	0.13	0.44

C/D – cup/disk ratio; MD – mean deviation; For other abbreviations, see Table 1.

Table 3

Results of univariate linear regression analysis for patients with POAG-HTG and XFG

Parameter	POAG-HTG				XFG			
	t	*p*	B	95% CI for B	*t*	*p*	B	95% CI for B
IOP	1.05	0.2971	0.52	-0.47–1.50	0.15	0.8846	0.17	-2.15–2.49
C/D	0.19	0.8538	0.00	-0.02–0.03	-0.17	0.4895	-0.03	-0.11–0.06
MD	0.26	0.7973	0.32	-2.20–2.85	1.06	0.3132	2.30	-2.49–7.09
RNFL Avg	-1.16	0.2535	-3.51	-9.64–2.63	-1.16	0.2535	-3.51	-9.64–2.63
RNFL Sup	-0.91	0.3672	-3.26	-10.50–3.98	0.57	0.5801	5.84	-17.19–28.88
RNFL Inf	-1.42	0.1661	-6.13	-14.94–2.67	1.95	0.0834	13.31	-2.16–28.78

***t* – statistical test value; *p* – statistical significance; B – regression coefficient; CI – confidence interval. For other abbreviations, see Tables 1 and 2.**

Discussion

An ischemia or increased pressure on glial cells stimulates the production of TNF-α, as shown in previous studies. This causes oligodendrocyte death and subsequent RGC apoptosis [22, 24]. In addition to nitric oxide and excitotoxicity, TNF-α has a neurotoxic effect and functions as an activator. TNF-α concentrations in plasma, cerebrospinal fluid, and brain tissue are elevated in certain CNS disorders, including Alzheimer's disease, multiple sclerosis, Parkinson's disease, and ischemic brain disorders [25]. Previous studies have demonstrated an association between some ocular diseases and increased levels of TNF-α [26–29]. In animal models with high IOP, an increase in TNF-α concentration led to the loss of RGC and oligodendrocytes. Moreover, this cell loss occurred even if TNF-α was administered without elevated IOP [30]. TNF-α exerts a negative effect on oligodendrocytes, increasing axonal susceptibility to excitotoxicity in the optic nerve head and leading to RGC death [31, 32]. When glial cells are exposed to stressors, such as high pressure or ischemia, TNF-α secretion increases, resulting in apoptosis. The apoptosis can be prevented by the administration of neutralizing anti-TNF-α antibodies [22]. These experiments are supported by the results of immunohistochemical tests on human samples conducted by Yan et al. [32] and Tezel et al. [33], demonstrating the increased level of expression of TNF-α and its receptor TNF-RI in the inner retinal layer of glaucomatous eyes compared to controls. Similar data have been published by Yuan and Neufeld [34]. The increased TNF-α expression in glaucomatous eyes suggests that this cytokine is closely associated with the neurodegenerative process. Numerous studies have investigated serum and local (aqueous) concentrations of TNF-α in XFG patients with conflicting results [35, 36]. However, only a few have examined the serum concentration of TNF-α in POAG-HTG. Currently, there is no evidence of a direct correlation between TNF-α levels in AH and serum or plasma.

In this study, we assessed TNF-α plasma levels in well-defined POAG-HTG and XFG patients and compared them with controls (subjects without glaucoma or any other ophthalmic disease). Our results revealed a significantly higher TNF-α concentration in glaucoma patients compared to healthy subjects in the control group. Although the level of TNF-α is believed to increase with age and can be a precondition for the development of atherosclerosis, diabetes, and Alzheimer's disease in the elderly [37], our control group was carefully matched for age and exhibited the lowest levels of TNF-α. Furthermore, age did not differ between groups; hence, we cannot attribute the increase in TNF-α to age or systemic diseases.

This research was conducted on a larger number of subjects, in all three groups, compared to prior studies [27, 28, 38, 39]. Our findings are in contrast to those of Huang et al. [38], who demonstrated lower serum concentrations of TNF-α in glaucoma patients in comparison to the healthy population. Sarenac Vulovic et al. [35] reported increased levels of TNF-α in the AH of patients with XFG and pseudoexfoliative syndrome but did not report elevated serum levels in the same patients. In contrast, Sorkhabi et al. [40] described an increased level of serum TNF-α as a risk factor for systemic and ocular manifestations in patients with exfoliative syndrome without glaucoma, whereas Kondkar et al. [36] reported elevated systemic levels of the inflammatory marker, TNF-α, in XFG. This suggests that the effects of systemic levels of TNF-α may be different from the effects of local levels of TNF-α in the AH [24, 41–45]. Our research did not establish a correlation of TNF-α concentration in the plasma with any of the examined clinical parameters, such as IOP, C/D ratio, MD, RNFL Avg, RNFL Sup, and RNFL Inf, that would determine the degree of development and damage in glaucoma. This is partially contradicted by the results of Huang et al. [38], as they found lower TNF-α levels in the POAG group of patients with MD < 12 dB than in the group with MD ≥ 12 dB (p = 0.0328) leading to the inference that serum TNF-α is a powerful cytokine playing a significant role in the pathogenesis of glaucoma and glaucomatous neuropathy. Kang et al. [39] demonstrated a higher serum TNF-α concentration in women than men. However, we have not replicated this finding. Our study showed elevated levels of TNF-α in the plasma of patients with POAG-HTG and XPG compared with non-glaucoma controls, indicating that elevated systemic levels of this inflammatory marker may be associated with the pathogenesis of glaucoma. Despite a lack of evidence to confirm whether plasma cytokine changes are directly correlated with AH cytokine levels, an elevated plasma TNF-α level may serve as a possible biomarker for glaucoma screening. Elevated TNF-α in plasma and AH is a clear indicator of an activated anti-inflammatory immune response.

The limitation of this study was the determination of the level of TNF-α in the plasma, which may not directly correlate with the concentration of cytokines in the AH in the anterior segment of the eye or in RGCs, which would require measuring the concentration of TNF-α in AH samples. In addition, further research should be conducted on a larger number of participants to increase the statistical power of the study.

Conclusion

The concentration of the pro-inflammatory cytokine TNF-α in the plasma is significantly higher in glaucoma patients compared to subjects without glaucoma, thus confirming the role of inflammation in the pathogenesis of glaucoma as one of the noninflammatory ocular diseases. The plasma concentration of TNF-α does not correlate with any of the examined clinical parameters; hence, it cannot be considered a measure of progression and damage in glaucoma. Nevertheless, a better understanding of this process may contribute to the development of new biomarkers for early disease detection and therapeutic strategies.

R E F E R E N C E S

1. *Fei ZG, Zeng S*. Glaucoma: etiology, pathogenesis and treatments (Eye and Vision Research Developments). New York: Nova Biomedical Publishers; 2012. 164 p.
2. *Pinazo-Durán MD, Zanón-Moreno V, García-Medina JJ, Gallego-Pinazo R*. Evaluation of presumptive biomarkers of oxidative stress, immune response and apoptosis in primary open-angle glaucoma. Curr Opin Pharmacol 2013; 13(1): 98–107.
3. *Balkwill FR, Burke F*. The cytokine network. Immunol Today 1989; 10(9): 299–304.
4. *Hoekzema R, Murray PI, Kijlstra A*. Cytokines and intraocular inflammation. Curr Eye Res 1990; 9Suppl: 207–11.
5. *Chua J, Vania M, Cheung CM, Ang M, Chee SP, Yang H,* et al. Expression profile of inflammatory cytokines in aqueous from glaucomatous eyes. Mol Vis 2012; 18: 431–8.
6. *Takai Y, Tanito M, Ohira A*. Multiplex cytokine analysis of aqueous humor in eyes with primary open-angle glaucoma, exfoliation glaucoma, and cataract. Invest Ophthalmol Vis Sci 2012; 53(1): 241–7.
7. *Halliwell B*. Reactive oxygen species in living systems: source, biochemistry, and role in human disease. Am J Med 1991; 91(3C): 14S–22S.
8. *Haukim N, Bidwell JL, Smith AJ, Keen LJ, Gallagher G, Kimberly R,* et al. Cytokine gene polymorphism in human disease: online databases, supplement 2. Genes Immun 2002; 3(6): 313–30.
9. *Wajant H, Pfizenmaier K, Scheurich P*. Tumor necrosis factor signaling. Cell Death Differ 2003; 10(1): 45–65.
10. *Schultz R*. Cell circle, programmed cell death, and cancer. In: *Devlin TM*, editor. Textbook of Biochemistry With Clinical Correlations. 6th ed. Hoboken: Willey-Liss; 2006. p. 1020–4.
11. *Li S, Tao L, Jiao X, Liu H, Cao Y, Lopez B*, et al. TNFalpha-initiated oxidative/nitrative stress mediates cardiomyocyte apoptosis in traumatic animals. Apoptosis 2007; 12(10): 1795–802.
12. *Perry SW, Dewhurst S, Bellizzi MJ, Gelbard HA*. Tumor necrosis factor-alpha in normal and diseased brain: Conflicting effects via intraneuronal receptor crosstalk? J Neurovirol 2002; 8(6): 611–24.
13. *McCoy MK, Tansey MG*. TNF signaling inhibition in the CNS: implications for normal brain function and neurodegenerative disease. J Neuroinflammation 2008; 5: 45.
14. *Tezel G*. TNF-alpha signaling in glaucomatous neurodegeneration. Prog Brain Res 2008; 173: 409–21.
15. *Berger S, Savitz SI, Nijhawan S, Singh M, David J, Rosenbaum PS,* et al. Deleterious role of TNF-alpha in retinal ischemia-reperfusion injury. Invest Ophthalmol Vis Sci 2008; 49(8): 3605–10.
16. *Marques-Fernandez F, Planells-Ferrer L, Gozzelino R, Galenkamp KM, Reix S, Llecha-Cano N,* et al. TNFα induces survival through the FLIP-L-dependent activation of the MAPK/ERK pathway. Cell Death Dis 2013; 4(2): e493.
17. *Son JL, Soto I, Oglesby E, Lopez-Roca T, Pease ME, Quigley HA,* et al. Glaucomatous optic nerve injury involves early astrocyte reactivity and late oligodendrocyte loss. Glia 2010; 58(7): 780–9.
18. *Barakat DJ, Dvoriantchikova G, Ivanov D, Shestopalov VI*. Astroglial NF-κB mediates oxidative stress by regulation of NADPH oxidase in a model of retinal ischemia reperfusion injury. J Neurochem 2012; 120(4): 586–97.
19. *Fields RD, Stevens-Graham B*. New insights into neuron-glia communication. Science 2002; 298(5593): 556–62.
20. *Pekny M, Nilsson M*. Astrocyte activation and reactive gliosis. Glia 2005; 50(4): 427–34.
21. *Dvoriantchikova G, Ivanov D*. Tumor necrosis factor-alpha mediates activation of NF-κB and JNK signaling cascades in retinal ganglion cells and astrocytes in opposite ways. Eur J Neurosci 2014; 40(8): 3171–8.
22. *Tezel G, Wax MB*. Increased production of tumor necrosis factor-alpha by glial cells exposed to simulated ischemia or elevated hydrostatic pressure induces apoptosis in cocultured retinal ganglion cells. J Neurosci 2000; 20(23): 8693–700.
23. *Hodapp E, Parrish RK, Anderson DR*. Clinical decisions in glaucoma. St Louis: The CV Mosby Co; 1993. pp. 52–61.
24. *Sawada H, Fukuchi T, Tanaka T, Abe H*. Tumor necrosis factor-alpha concentrations in the aqueous humor of patients with glaucoma. Invest Ophthalmol Vis Sci 2010; 51 (2): 903–6.
25. *Liu T, Clark RK, McDonnell PC, Young PR, White RF, Barone FC,* et al. Tumor necrosis factor-alpha expression in ischemic neurons. Stroke 1994; 25(7): 1481–8.
26. *Zorena K, Myśliwska J, Myśliwiec M, Balcerska A, Hak Ł, Lipowski P,* et al. Serum TNF-alpha level predicts nonproliferative diabetic retinopathy in children. Mediators Inflamm 2007; 2007: 92196.
27. *Doganay S, Evereklioglu C, Er H, Türköz Y, Sevinç A, Mehmet N*, et al. Comparison of serum NO, TNF-α, IL-1beta, sIL-2R, IL-6 and IL-8 levels with grades of retinopathy in patients with diabetes mellitus. Eye (London) 2002; 16(2): 163–70.
28. *Gustavsson C, Agardh CD, Agardh E*. Profile of intraocular tumour necrosis factor-α and interleukin-6 in diabetic subjects with different degrees of diabetic retinopathy. Acta Ophthalmol 2013; 91(5): 445–52.
29. *Sugita S, Takase H, Taguchi C, Mochizuki M*. The role of soluble TNF receptors for TNF-alpha in uveitis. Invest Ophthalmol Vis Sci 2007; 48(7): 3246–52.
30. *Nakazawa T, Nakazawa C, Matsubara A, Noda K, Hisatomi T, She H,* et al. Tumor necrosis factor-alpha mediates oligodendrocyte death and delayed retinal ganglion cell loss in a mouse model of glaucoma. J Neurosci 2006; 26(49): 12633–41.
31. *Coleman M*. Axon degeneration mechanisms: commonality amid diversity. Nat Rev Neurosci 2005; 6(11): 889–98.
32. *Yan X, Tezel G, Wax B, Edward P*. Matrix metalloproteinases and tumor necrosis factor alpha in glaucomatous optic nerve head. Arch Ophthalmology 2000; 118(5): 666–73.
33. *Tezel G, Li LY, Patil RV, Wax MB*. TNF-alpha and TNF-alpha receptor-1 in the retina of normal and glaucomatous eyes. Invest Ophthalmol Vis Sci 2001; 42(8): 1787–94.
34. *Yuan L, Neufeld AH*. Activated microglia in the human glaucomatous optic nerve head. J Neurosci Res 2001; 64(5): 523–32.
35. *Sarenac Vulovic TS, Pavlovic SM, Jakovljevic VLj, Janicijevic KB, Zdravkovic NS*. Nitric oxide and tumour necrosis factor alpha in the process of pseudoexfoliation glaucoma. Int J Ophthalmol 2016; 9(8): 1138–42.
36. *Kondkar AA, Azad TA, Almobarak FA, Kalantan H, Al-Obeidan SA, Abu-Amero KK*. Elevated levels of plasma tumor necrosis factor alpha in patients with pseudoexfoliation glaucoma. Clin Ophthalmol 2018; 12: 153–9.
37. *Brüünsgaard H, Pedersen BK*. Age-related inflammatory cytokines and disease. Immunol Allergy Clin North Am 2003; 23(1): 15–39.
38. *Huang P, Qi Y, Xu YS, Liu J, Liao D, Zhang SS*, et al. Serum cytokine alteration is associated with optic neuropathy in human primary open angle glaucoma. J Glaucoma 2010; 19(5): 324–30.
39. *Kang JH, Wiggs JL, Pasquale LR*. A nested case control study of plasma ICAM-1, E- selectin and TNF receptor 2 levels, and incident primary open-angle glaucoma. Invest Ophthalmol Vis Sci 2013; 54(3): 1797–804.
40. *Sorkhabi R, Ghorbanihaghjo A, Ahoor M, Nahaei M, Rashtchizadeh N*. High-sensitivity C-reactive protein and tumor necrosis factor alpha in pseudoexfoliation syndrome. Oman Med J 2013; 28(1): 16–9.

41. *Balaiya S, Edwards J, Tillis T, Khetpal V, Chalam KV*. Tumor necrosis factor-alpha (TNF-α) levels in aqueous humor of primary open angle glaucoma. Clin Ophthalmol 2011; 5: 553–6.
42. *Chen H, Zheng G, Chen H, Li L, Xu Z, Xu L*. Evaluations of aqueous humor protein markers in different types of glaucoma. Medicine (Baltimore) 2022; 101(41): e31048.
43. *Oribio-Quinto C, Burgos-Blasco B, Pérez-García P, Espino-Paisán L, Sarriá B, Fernández-Vigo JI,* et al. Aqueous Humor Cytokine Profile in Primary Congenital Glaucoma. J Clin Med 2023; 12(9): 3142.
44. *Tong Y, Zhou YL, Zheng Y, Biswal M, Zhao PQ, Wang ZY*. Analyzing cytokines as biomarkers to evaluate severity of glaucoma. Int J Ophthalmol 2017; 10(6): 925–30.
45. *Dammak A, Sanchez Naves J, Huete-Toral F, Carracedo G*. New Biomarker Combination Related to Oxidative Stress and Inflammation in Primary Open-Angle Glaucoma. Life (Basel) 2023; 13(7): 1455.

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