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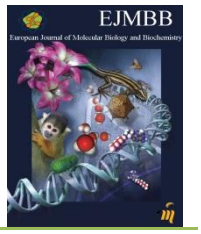

ASSOCIATION OF PLASMA TUMOR NECROSIS FACTOR-ALPHA (TNF-A) LEVELS IN PATIENTS WITH PRIMARY OPEN ANGLE GLAUCOMA

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ABSTRACT

To investigate the association of plasma levels of tumor necrosis factor-alpha (TNF-α) in patients with primary open angle glaucoma (POAG) patients as compared to non-glaucomatous controls. TNF-α concentrations were measured using sandwiched enzyme-linked immunosorbent assay (ELISA) in plasma samples from 39 POAG patients and 36 controls. The assay was performed in duplicate on an automated ChemWell-T ELISA analyzer. The mean (± SD) concentration of plasma TNF-α in POAG patients (1.99 ± 2.32 pg/mL) was significantly increased (P = 0.0144) as compared to controls (0.82 ± 1.62). Similarly, the median levels were also found to be significantly different (P = 0.03). Plasma TNF-α level is significantly elevated in POAG patients as compared to non-glaucomatous control group supporting a key role of systemic TNF- α in glaucoma. The results of these findings however, need to be replicated in larger cohort in our population.

INTRODUCTION

Tumor necrosis factor-alpha (TNF-α) is a pleiotropic inflammatory cytokine associated with tissue ischemia, neuronal damage, and remodeling [1]. TNF-α is found to be elevated in patients with neurodegenerative diseases such as Alzheimer's disease [2], Parkinson's disease [3], and multiple sclerosis [4] suggesting a causative role of TNF-α in neurodegenerative disorders, including glaucoma. TNF-α is expressed in optic nerve and retina of human glaucomatous eyes and parallels the progression of optic nerve damage [5]. Human and animal *in vivo* studies have shown that serum or aqueous levels of TNF-α are elevated in patients with glaucoma and elicit retinal-ganglion cell (RGC) apoptosis suggesting that TNF-α plays a critical role in glaucomatous degeneration [6-8]. The purpose of this study is to compare human plasma levels of TNF-α between primary open angle glaucoma (POAG) patients and non-glaucomatous controls.

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MATERIALS AND METHODS

Study population

The study adhered with the tenets of the Declaration of Helsinki. The Institutional Review Board and the Ethics Committee of our institution approved the study protocols (approval number # 08-657). Informed consent was obtained from all individuals who participated in this study. Saudi Arab participants with clinically diagnosed POAG and ethnically-matched healthy controls were recruited into the study at King Abdulaziz University Hospital (KAUH) in Riyadh, Saudi Arabia. The inclusion and exclusion criteria for POAG patients and controls have been described previously [9]. Blood samples were collected in EDTA (ethylenediaminetetraacetic acid) tubes. The tubes were centrifuged at 5500*x g* for 5 min. and the plasma layer was separated and stored at -80^{o}C until use.

ELISA for TNF-α

TNF-α concentrations were measured with an enzyme-linked immunosorbent assay (Quantikine® ELISA, R & D Systems Inc., MN, USA) in plasma samples from 39 POAG patients and 36 non-glaucomatous controls. The assay uses a monoclonal antibody specific for human TNF-α that has been pre-coated onto a microplate and was performed in duplicate on an automated biochemical analyzer, ChemWell-T (Awareness Technology Inc., FL, USA), as per the manufacturer's instructions (R & D Systems Inc., USA). The reaction was terminated with 50 µL of stop solution (2 N sulfuric acid) and absorbance was measured at 450 nm with wavelength correction set at 540 nm. The concentration of TNF-α level was estimated from the calibrated standard curve and expressed in pg/mL.

Statistical Analysis

Data are presented as mean ± SD and/or ± SEM for TNF-α concentration and other parameters. The Chi-square test was applied to analyze intergroup differences. Student *t*-test was used to compare the intergroup variation in TNF-α levels between normal and glaucoma samples. Nonparametric Mann Whitney U test was used to compare median values between the two study groups. A *p*-value of < 0.05 was considered of statistical significance. Statistical analysis was performed with SPSS version 19.0 (IBM Corp., Armonk, New York, USA).

RESULTS

A total number of 75 subjects that included 39 POAG patients and 36 non-glaucomatous healthy controls were examined in this study. The demographic details and plasma levels of TNF-α in POAG patients and non-glaucoma controls is shown in **Table 1**. The individuals in the POAG patient group were significantly older than the control group ($p < 0.0001$). Both the mean and median plasma levels of TNF-α were significantly elevated in the POAG patient group.

Table 1. Demographic details and plasma levels of TNF-alpha in POAG patients and non-glaucomatous controls

Variables	Controls (N = 36)	POAG (N = 39)	Significance
Gender			
Male (%)	26 (72.2)	25 (64.1)	P = 0.62*
Female (%)	10 (27.7)	13 (33.3)	
Age (years)			
Mean	42.5	58.4	P < 0.0001†
Std. Dev.	10.3	12.9	
TNF-α (pg/mL)			
Mean	0.82	1.99	P = 0.0144†
Std. Error	0.27	0.37	
Std. Dev.	1.62	2.32	
Median (Range)	0 (0 – 8)	0.9 (0 – 6)	P = 0.03‡

* P value calculated by Chi-square test, † P value calculated by Student's *t*-test, ‡ P value calculated by Mann Whitney U-test

DISCUSSION

TNF-α, a cytokine, has been well recognized to play an important role in pro- and anti-apoptotic cellular functions. The apoptotic activity of TNF-α is mediated via TNF- α receptor 1 in vast majority of cell types whereas the TNF-α receptor 2 is found to be neuroprotective involving the caspase-induced and phosphotidylinositol 3-kinase (PI3-kinase) signaling pathways respectively [10].

A number of previous studies have examined the role of oxidative stress and cytokine release in glaucoma. In a mouse model of ocular hypertension induced glaucoma, TNF-α mRNA levels were found to increase followed by loss of oligodendrocytes and RGCs [7]. A similar effect was also observed in normal mice without elevated IOP. Of interest, another study showed that glial cells exposed to elevated IOP and ischemia secreted TNF-α and facilitated death of RGCs [8]. This effect was found to be attenuated in the presence of neutralizing anti-TNF-antibodies. Over-expression of TNF-α in glial cells and TNF-α receptor 1 in retinal ganglion cells have been described in glaucomatous eye [11]. Using ELISA, Swada et al. documented significantly elevated levels of aqueous TNF-α in glaucoma, and more so, in pseudoexfoliation glaucoma compared with cataract [12]. Similarly, aqueous TNF-α level were also found to be elevated in patients with POAG [13]. In addition, similar to our findings of elevated plasma TNF-α observed in POAG patients as compared to normal controls, Huang et al. reported increased TNF-a levels in the serum of patients with severe optic neuropathy as compared to those with mild optic neuropathy [6]. Elevated levels of TNF-α may induce RGC apoptosis by adversely affecting oligodendrocytes, which may increase susceptibility of axons to the excitotoxicity in the optic nerve head. In addition, IOP induced RGC cell death may occur through caspase activation [14] .

POAG is an optic neuropathy associated with progressive apoptotic degeneration of retinal ganglion cells (RGCs). Although the pathophysiology of the disease is not completely understood, elevated intraocular pressure (IOP) is the most important risk factor for the disease. However, presence of glaucomatous damage despite

normal levels of IOP and vice-versa is not uncommon. There is evidence to suggest the role of systemic risk factors as a part of multi-factorial aetiology of open angle glaucoma [15, 16]. In this study we show that the plasma levels of TNF-α is significantly increased in POAG patients as compared to normal non-glaucomatous controls supporting the role of systemic risk factors in pathogenesis of glaucoma. However, this aspect of the study needs to be further validated in a larger sample population.

CONCLUSION

To conclude, increased plasma TNF-α level were shown to be associated with POAG as compared to normal control subjects.

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CONFLICT OF INTEREST: No conflict of interest.

STATEMENT OF HUMAN AND ANIMAL RIGHTS

All procedures performed in human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies performed with animals.

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