Tumor Necrosis Factor-α Concentrations in the Aqueous Humor of Patients with Glaucoma

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PURPOSE. To investigate the concentration of tumor necrosis factor (TNF-α) in the aqueous humor of patients with glaucoma, including correlations with glaucoma subtypes and intraocular pressure.

METHODS. The study population comprised 84 patients with open-angle glaucoma who were scheduled for filtration or cataract surgery. Glaucoma subgroups included 29 cases of primary open-angle glaucoma (POAG), 28 cases of normal-tension glaucoma (NTG), and 27 cases of exfoliation glaucoma (ExG). Seventy-nine patients with senile cataract were recruited as control subjects. The concentrations of TNF-α in the aqueous humor were measured with an enzyme-linked immunosorbent assay. The percentages of samples positive for TNF-α and the measured concentrations in the glaucoma and cataract groups were compared. In addition, the relationships with the glaucoma subtypes, intraocular pressure, and glaucoma severity were analyzed.

RESULTS. A significantly higher percentage of subjects in the glaucoma group were positive for TNF-α compared with the cataract group (P = 0.011). The mean TNF-α concentrations among the positive cases were not different between the groups (P = 0.689). TNF-α–positive samples were higher in the POAG (15.7%) and NTG (10.7%) subgroups than in the cataract group without significance, but higher in ExG subgroup (29.6%) with significance (P = 0.001). Relationships between the TNF-α concentration and the intraocular pressure or the clinical stage of glaucoma were not observed.

CONCLUSIONS. TNF-α levels were significantly higher in the glaucoma group than in the cataract group, with a particularly large difference observed in those with ExG. The results suggest that TNF-α plays a key role in the progression of glaucoma. (Invest Ophthalmol Vis Sci. 2010;51:903–906) DOI:10.1167/iovs.09-4247

Glaucoma is characterized by visual field defects and an excavated optic nerve head. The disorder affects millions of people and is the second leading cause of bilateral or monocular poor vision in the world. Ocular pressure is known to make significant contributions to glaucomatous neurodegeneration. The recent Advanced Glaucoma Intervention Study provided the first definitive evidence that lowering the intraocular pressure reduces the risk of progression of visual field defects. Nevertheless, we have seen several patients who are being treated appropriately, yet still have progressive visual field defects. Moreover, the marked vulnerability of retinal ganglion cells in glaucoma has not been fully explained. To examine this mechanism, investigators have analyzed the contributions of ocular blood circulation, oxidative stress, and cytokines. Although apoptosis is thought to play an essential role in glaucomatous neurodegeneration, the details of the underlying process are unclear. Of interest, 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. These findings suggest that TNF-α plays a key role in glaucomatous neurodegeneration. Understanding this process also may contribute to the development of novel therapeutic strategies.

In the present study, we examined the effects of TNF-α in the aqueous humor of patients with glaucoma, including potential relationships with clinical stage. We determined the percentage of patients who were positive for TNF-α in the aqueous humor and the concentration in these patients. Furthermore, we compared the results observed in patients with glaucoma with those obtained from those with cataract.

METHODS

All experiments complied with the tenets of the Declaration of Helsinki. The Institutional Review Board and the Ethics Committee of each institute approved the study protocols. All patients were fully informed of the purpose and procedures of the study and written informed consent was obtained from all individuals.

Study Population

We examined 84 eyes of 84 patients with open-angle glaucoma (mean age ± SD, 72.7 ± 9.3 years; men/women, 49/35) who were recruited from the Niigata University Medical Hospital and other related hospitals. We also examined 79 eyes of 79 control subjects (mean age ± SD, 72.7 ± 9.2 years; men/women, 36/43) who were treated at the same facilities and did not have any ocular disease except for senile cataract. Among the 84 patients with glaucoma, 29 (34.5%) received a diagnosis of primary open-angle glaucoma (POAG), 28 (33.3%) received a diagnosis of normal-tension glaucoma (NTG), and 27 (32.1%) received a diagnosis of exfoliation glaucoma (ExG). All enrolled patients were scheduled for filtration surgery or cataract surgery. Aqueous humor samples were obtained from each eye of each patient. The characteristics of the study population are presented in Table 1. At the time of the surgery, none of the patients was being systemically treated with corticosteroids or nonsteroidal anti-inflammatory drugs. In addition, patients with precedent ocular surgery, diabetes, or active inflammatory diseases were excluded. All the participants underwent a comprehensive ophthalmic examination, including visual acuity tests, measurement of intraocular pressure, slit lamp examination, fundus examination, and perimetric examination (Humphrey 30-2; Carl Zeiss Meditec, Tokyo, Japan) of the patients with glaucoma. All patients with glaucoma were being treated with intraocular pressure depressants.

Aqueous Humor Sample Collection

Samples of aqueous humor were collected from patients during elective surgery. Approximately 0.1 mL of aqueous humor was obtained from the anterior chamber of the eye under topical anesthesia with proparacaine hydrochloride. Aqueous humor was obtained from the site of keratoplasty in patients who had undergone penetrating keratoplasty. The aqueous humor was stored at −70°C until analysis. Ethics Committee

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from each subject through a corneal side port with a tuberculin syringe and 27-gauge needle before phacoemulsification or filtration surgery. The collected samples were immediately stored at -70°C and thawed just before the experiment.

**ELISA for TNF-α**

TNF-α concentrations were measured with an enzyme-linked immunosorbent assay. Each sample was measured in triplicate. The accuracy of the ELISA was considered to be high, as the curve index was 0.996 with 0.003 errors. Human TNF-α instant ELISA kits (BMS223INST) were purchased from Bender MedSystems (Vienna, Austria). Briefly, supernatants from the aqueous humor samples were added to the sample wells of a microplate and incubated at room temperature for 5 hours on a microplate shaker at 200 rpm. The microwell contained anti-human TNF-α monoclonal coating antibodies, a lyophilized detection antibody, streptavidin-horseradish peroxidase (HRP), and sample diluents. After incubation, the samples were washed, 100 µL of tetramethylbenzidine HRP substrate solution was added to the wells, and the reaction was terminated with phosphoric acid, and absorbance was measured at 450 nm with a spectrophotometer (model 680, Bio-Rad, Hercules, CA).

**Statistical Analysis**

Data are presented as the mean ± SEM for TNF-α concentrations and as the mean ± SD for the other parameters. The χ² test was applied to analyze intergroup differences. Nonparametric analysis was used to examine the relationships between the percentage of patients positive for TNF-α and patient age or intraocular pressure. P < 0.05 was considered significant (SPSS ver. 14; SPSS, Inc., Chicago, IL).

**RESULTS**

Among the 163 participants, TNF-α was detected in 19 (11.6%). The mean TNF-α concentration ± SEM among the positive cases was 15.9 ± 3.6 pg/mL with a range of 1.7 to 57.6 pg/mL. The percentage of positive cases among the patients with glaucoma was 17.8%, which was significantly higher than that among control subjects (5.0%; P = 0.001, χ² test). For the glaucoma subgroups, TNF-α was detected in 8 (29.6%) of 27 patients with ExG, 4 (13.7%) of 29 patients with POAG, and 5 (10.7%) of 28 patients with NTG. Compared with control subjects, a significantly higher percentage of patients with ExG were positive for TNF-α (P = 0.001, χ² test), whereas results for patients with POAG or NTG were not significant (P = 0.125 and 0.299, respectively; Table 2). There was no significant correlation between the concentrations of TNF-α and the preoperative intraocular pressure (r = 0.165). Also, no significant difference was observed between the average TNF-α concentration and that observed for the various glaucoma subgroups (P = 0.896; Fig. 1).

Nine (10.5%) of 85 men and 10 (12.8%) of 78 women were positive for TNF-α; no significant relationship was observed between sex and TNF-α positivity in the aqueous humor (P = 0.657). The average ages of the positive and negative cases were 75.5 ± 2.6 and 75.4 ± 9.6 years, respectively; no significant difference in the patient ages was observed between positive and negative cases (P = 0.921). The clinical stages of glaucoma were evaluated using the mean deviation (MD) from the perimetric test (Humphrey 30-2; Carl Zeiss Meditec), which did not reveal a relationship with the TNF-α concentration from the positive cases (P = 0.166; Fig. 2).

**DISCUSSION**

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. In addition to nitric oxide and excitotoxins, TNF-α has neurotoxic effects and functions as an activator. Increased TNF-α levels have been associated with a poor prognosis after trauma in the brain, whereas a decrease in TNF-α is known to reduce nerve damage. TNF-α concentrations 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. In a rat cerebral ischemic model, exogenous TNF-α was found to exacerbate focal ischemic injuries, whereas blocking endogenous TNF-α was neuroprotective.

Previous studies have shown the relationships between several ocular diseases and increased TNF-α levels. Diabetics with retinopathy have increased serum levels of TNF-α, which may be predictive of retinopathy in all diabetics. Sugita et al. detected significantly elevated levels of TNF-α and TNF-α receptors in the ocular fluid of patients with active uveitis (19.6 ± 8.5 pg/mL in aqueous humor; 29.8 ± 9.9 pg/mL in vitreous humor) compared with patients with senile cataracts (6.8 ± 6.7 pg/mL in aqueous humor; 9.7 ± 6.7 pg/mL in vitreous humor). Many studies have detailed the relationship between glaucomatous optic neuropathy and TNF-α. In an animal model of 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. TNF-α contributes to this process by adversely affecting oligodendrocytes, which increases the susceptibility of axons to excitotoxicity in the optic nerve head and retinal ganglion cell death.

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. In addition, this apoptosis can be prevented by using neutralizing anti-TNF-α antibodies. These experiments are supported by immunohistochemistry results with human samples. Yan et al. and Tezel et al. found that the expression levels of TNF-α and its receptor TNF-R1 were higher in the inner retinal layers of glaucomatous eyes than in control eyes. Similar findings were reported by Yuan and Neufeld. The authors did not observe TNF-α in the microglial cells of normal eyes, whereas it was abundantly present in glaucomatous eyes. Moreover, these differences were more significant in eyes with NTG or advanced glaucoma. The increased expression of TNF-α in glaucomatous eyes suggests that this cytokine is critically connected with the damaging processes in these tissues.

Our results showed that the percentage of patients with senile cataract who were positive for TNF-α in the aqueous humor was as low as 5.0%. On the other hand, the percentage of patients with glaucoma who were positive for TNF-α was as high as 17.8%. Of note, we believe that the TNF-α concentration in the aqueous humor of normal eyes is below the threshold level of detection of the commercially available kits. Intraocular TNF-α is most likely secreted from activated macrophages, astrocytes, microglial cells, and retinal Müller glial cells, which suggests 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 levels are known to predict poor outcomes. Because TNF-α is secreted during neuronal modifying and damage, higher TNF-α concentrations in patients with glaucoma may suggest that the disease is not fully controlled and that the visual field defect is likely to progress. Considering that, compared with NTG or POAG, ExG is associated with higher intraocular pressure and a faster progression of visual field defects, the higher percentage of ExG patients who were positive for TNF-α is an interesting finding.

In this study, we did not find a relationship between intraocular pressure and TNF-α concentration. Although the average intraocular pressure in the ExG group was the highest observed (25.8 ± 9.3 mm Hg), the relationship with the TNF-α concentration was not significant. Chen et al. examined patients with central retinal vein occlusion and neovascular glaucoma, in whom the average intraocular pressure was 33.5 mm Hg and the range of TNF-α concentrations in the aqueous humor was 13.0 to 15.0 pg/mL. On the other hand, the TNF-α concentration in a patient with phacolytic glaucoma was reportedly below the threshold detection level.

Yan et al. showed increased TNF-α expression levels in the optic nerve head, not only in patients with POAG, but also in those with NTG. Although we did not find a relationship between intraocular pressure and aqueous humor TNF-α levels among the various subtypes of glaucoma, such a correlation is possible. Previous studies have detected increased TNF-α levels after exposure to high intraocular pressure. We believe that TNF-α is expressed at some point after stress loading such that the time at which sampling occurs affects the detected concentration. We also believe that the length of time that the patient experienced high intraocular pressure may affect the results. Some previously published studies support this assumption. Cordeiro et al. 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, and retinal ganglion cells. Data from a prior study showed that TNF-α concentrations in vitreous humor are higher than those in aqueous humor, which suggests that some proportion of TNF-α in aqueous humor is the result of diffusion from vitreous humor, which may have resulted in subthreshold concentrations in some of our samples. Despite these limitations, we were able to show that patients with glaucoma were more likely to have detectable levels of TNF-α in their aqueous humor. Although the elevation of TNF-α was significant only in ExG among glaucoma subtypes, increases were also shown in both POAG and NTG. The elevation of intraocular expression of TNF-α may be responsible for causing nerve damage. Our results suggest targeting TNF-α as a means to inhibit the neurodegenerative mechanisms associated with glaucoma. Further investigation into these mechanisms will elucidate the role of TNF-α in glaucomatous neurodegeneration and may provide a new approach to treating glaucoma and improving patient outcomes.

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