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Aqueous Humor Levels of Soluble Fas and Fas-ligand in Patients with Primary Open Angle and Pseudoexfoliation Glaucoma

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ABSTRACT

Background: Glaucoma is one of the most common causes of blindness and is usually associated with elevated intraocular pressure. In patients with primary open angle glaucoma the number of trabecular meshwork cells is decreased. Death of the trabecular meshwork cells may be a result of apoptosis. **Objective:** To investigate the aqueous humor levels of soluble Fas (sFas) and Fas-Ligand (sFasL) in glaucomatous patients. **Methods:** Concentration of sFas and sFasL were measured by ELISA in 41 eyes with glaucoma (21 with pseudoexfoliation and 20 with primary open angle glaucoma) and 39 eyes with cataract as controls. **Results:** The sFas concentration was lower in the primary open angle than the pseudoexfoliation glaucoma and the cataract groups (p=0.002 and p= 0.004, respectively). The sFasL level did not show any significant difference in the three groups. **Conclusion:** A lower level of sFas may provide proper microenvironment for increased apoptosis of trabecular meshwork cells in primary open angle glaucoma.

Keywords: Apoptosis, Open angle glaucoma, Pseudoexfoliation glaucoma, Soluble Fas, Soluble Fas-ligand

INTRODUCTION

Glaucoma is a neurodegenerative disease of the optic nerve and is considered as one of the leading causes of blindness in the world among aging people (1). Glaucoma is usually associated with elevated intraocular pressure (IOP) which is secondary to decreased outflow from trabecular meshwork (TM). One of the prominent findings in TM of patients with primary open angle glaucoma (POAG) is that the number of TM cells is decreased in comparison to age matched controls (2). One of the proposed mechanisms for TM cell loss is the increased rate of cell death with aging (2, 3). Apoptosis is a genetically controlled mechanism that plays an important role in the regulation of cellularity in different tissues by induction of cell death (4).

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Therefore, the increased rate of apoptosis could be considered as a mechanism for TM cell loss in POAG. Agrawal and coworkers (5) showed that human TM cells express Fas receptor and undergo apoptosis after activation of the receptor by monoclonal IgM. Fas death receptor (CD95, APO-1) is a transmembrane cell surface receptor belonging to the tumor necrosis factor receptor family and is one of the main triggers of apoptosis (4, 6). Cross linking of Fas by its ligand, Fas-ligand (FasL), induces apoptosis by triggering a cascade of caspases (4, 6). The Fas-mediated apoptosis can be blocked by binding of soluble Fas (sFas/sCD95) to FasL which prevents the interaction of membranous Fas with FasL (4, 6). Therefore, it could be hypothesized that an increase in the rate of TM cell death is subsequent to decreased levels of sFas or increased level of FasL in glaucoma patients. Thus, the aim of this study is to clarify the possible role of the aqueous humor levels of sFas and sFasL in the pathogenesis of POAG.

PATIENTS AND METHODS

This study was conducted on 41 patients with glaucoma (21with pseudoexfoliation glaucoma and 20 with primary open angle glaucoma) and 39 cases of cataract as control group. This prospective case control study was approved by the local ethics committee. Signed informed consent was obtained from all of the participants.

The glaucoma and control groups were age and sex matched. Before glaucoma or cataract surgery, all participants underwent a complete ocular examination. Intraocular pressure was measured by a calibrated Goldman applanation tonometer and optic nerve head evaluation by stereoscopic method. In all of the glaucoma patients, the visual field was checked by Humphery 24-2 sequence.

The entrance criteria in the POAG group were, IOP≥21mmHg, open iridocorneal angle in gonioscopy, typical glaucomatous optic nerve head and visual field changes. The presence of exfoliative material on the pupillary border and the lens capsule was added to the entrance criteria of POAG for pseudoexfoliation glaucoma (XFG) patients (7). In all of the cataract patients, the IOP was ≤ 20mmHg and optic nerve head cup was ≤ 0.3 without any typical glaucomatous change. In all groups the exclusion criteria included: myopic refractive error exceeding -10.00 diopter, previous ocular surgery or laser application, systemic rheumatologic and inflammatory diseases, ophthalmic conditions other than glaucoma or cataract.

At least 100 µl of aqueous humor samples were obtained prior to entrance to the eye through limbal paracentesis site using a 27-gauge needle on a tuberculin syringe. The samples were stored at -70°c until the analyses of sFas and sFasL levels were performed. The concentration of sFas and sFasL in the aqueous humor samples were determined by an Immunoenzymatic assay using commercially available kits (Euroclone, Italy). For checking the levels of sFas and sFasL, samples were thawed and centrifuged for 5 minutes at 12000g then diluted 4 folds with standard diluent buffer and used in the assays.

Statistical analysis was done by Mann-Whitney U test using SPSS software version 11.5. Findings were considered statistically significant at a P value < 0.05.

RESULTS

The selected demographic data and the levels of sFas and sFasL in the aqueous humor of all participants are shown in Table 1. The mean concentration of sFas in the aqueous humor

of POAG patients (5.17±25.6 pg/ml) was significantly lower than the XFG (30±49.78 pg/ml) and the cataract (28.7±48.93 pg/ml) groups (p=0.002 and p=0.004, respectively). There was no significant difference in the level of aqueous humor sFas between the XFG and the cataract groups (p=0.72). The actual estimated values of sFas in each individual patient are shown in figure 1. The mean concentration of sFasL in the aqueous humor of POAG (9.02±32.54 pg/ml) did not show any significant difference with the XFG (0.99±1.9 pg/ml) and the cataract (2.9±12.54 pg/ml) groups (p=0.44 and p=0.6, respectively). There was no correlation between aqueous humor levels of sFas and vertical cupping in the POAG and XFG groups (p=0.52 and p=0.65, respectively). Also when the concentration of sFasL was correlated with the vertical cupping in the POAG and XFG groups, the results remained insignificant (p= 0.58 and p=0.64, respectively).

Table 1. Demographic, vertical cupping and aqueous humor levels of sFas and sFasL in the pseudoexfoliation glaucoma (group 1), primary open angle glaucoma (group 2) and cataract (group 3) patients

Characteristics	Group 1 (n=21)	Group 2 (n=20)	Group 3 (n=39)	Significance		
				1vs2	1vs3	2vs3
Age (years) Mean±SD	62.4±8.9	61.1±11.1	65.6±11.3	0.45	0.13	0.07
Female:Male	7:14	7:13	15:24	0.83	0.91	0.98
Operated eye (Rt:Lt)	12:9	14:6	19:20	0.59	0.72	0.20
Vertical cupping Mean±SD	0.82±0.15	0.89±0.12	0.24±0.06	0.03	0.0001	0.0001
Soluble Fas (pg/ml) Mean±SD	30±49.78	5.17±25.6	28.7±48.93	0.002	0.72	0.004
Soluble Fas-L (pg/ml) Mean±SD	0.99±1.9	9.02±32.54	2.9±12.54	0.44	0.33	0.60

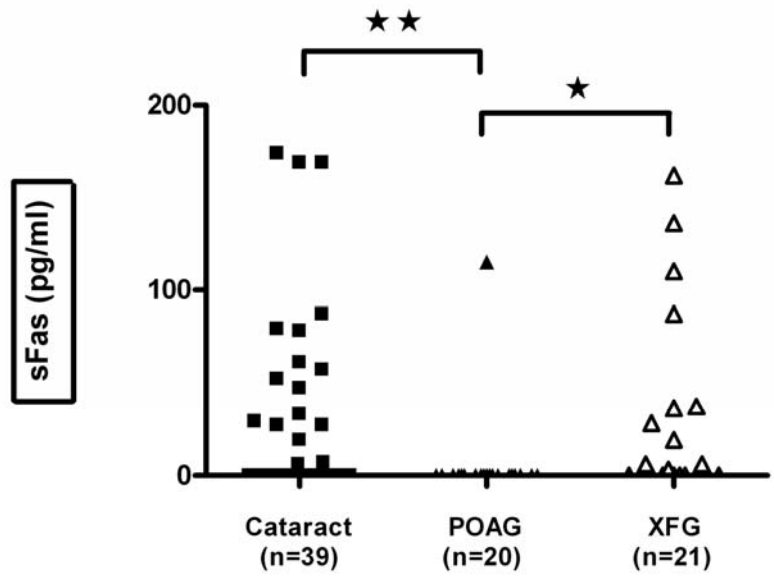

Figure 1. Amount of soluble Fas (sFas) in studied groups. POAG: primary open angle glaucoma XFG: pseudoexfoliation glaucoma, n: number of samples. ★: p=0.002. ★★: p=0.004

DISCUSSION

Agrawal and coworkers (5) showed that human TM cells express Fas receptor and undergo apoptosis after activation of the receptor by monoclonal IgM. Moreover, in histopathologic study of POAG patients, the lower cellularity of TM was reported in comparison to age matched controls (2). In agreements with above mentioned studies, our results provide an indirect evidence for the probability of more apoptosis in POAG compared to control and/or XFG patients. Indeed, the levels of sFas in POAG was significantly lower than the control group ($p=0.004$). The lower aqueous humor levels of sFas in POAG patients could lead to more binding of FasL to Fas and more apoptosis of TM cells, which finally causes the fusion of tubercular beams and increases the outflow resistance (2). On the other hand, the loosened endothelial cells are not replenished due to their limited ability in division (3, 8). The sFas levels in the aqueous humor of XFG group did not show any significant difference with the control group ($p=0.72$), but was significantly higher than the POAG group ($p=0.002$). This result is also compatible with the previous reports showing no difference in the cellularity of TM between XFG and normal eyes (9). Therefore, the most likely cause of changing the pseudoexfoliation syndrome to XFG is the TM obstruction either by pigment or pseudoexfolation material (9).

The FasL is physiologically expressed in immune privileged sites such as testis, placenta and anterior chamber of the eye (10, 11). Membrane bound FasL is cleaved into sFasL by the action of specific serine proteinases such as matrix metalloproteinase 3 and 7 (12). sFasL could induce apoptosis in target cells which express the Fas receptor. Therefore, it could be suggested that TM cell loss in POAG patients may be the result of increased levels of sFasL in the anterior chamber. However, the present study did not provide any evidence for the role of sFasL in the pathogenesis of POAG. In fact, our results showed no statistical difference in the levels of aqueous humor sFasL in POAG group compared to XFG ($p=0.44$) or the control group ($p=0.6$).

Although lower levels of sFas were detected in POAG compared to XFG and the control group, no correlation was obtained between sFas level and vertical cupping ($p=0.52$). This finding may be due to severe glaucomatous damage in most of the participants in this study.

In summary, this is the first report showing the possibility of increased apoptosis, through the Fas-FasL system, in POAG patients compared to controls. Further investigations with techniques such as TUNEL (Terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling) are needed in order to verify the occurrence of apoptosis in TM cells in patients with primary open angle glaucoma.

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