·Clinical Research·

Oxidant/antioxidant balance in the aqueous humor of patients with glaucoma

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Abstract

• AIM: To evaluate total antioxidant status (TAS), total oxidant status (TOS), and the oxidative stress index (OSI) of the aqueous humor (AH) in patients with glaucoma.

• METHODS: The prospective study was composed of a study group (*n*=31) and a control group (*n*=31). Fifteen patients in the study group were diagnosed with primary open angle glaucoma (POAG), and 16 patients were diagnosed with pseudoexfoliation glaucoma (PEG). The control group was composed of non-glaucomatous patients with cataracts. AH samples were collected and analyzed for TAS, TOS, and OSI levels.

• RESULTS: Mean AH TAS level was significantly higher in patients with glaucoma than that in the control group (*P*<0.01). Mean TOS and OSI levels tended to increase in patients with glaucoma. No significant differences in TAS, TOS, or OSI levels were observed between patients with POAG and PEG.

• CONCLUSION: High levels of TAS were observed in patients with glaucoma, which was likely a response to the increased oxidative stress observed in these patients.

• KEYWORDS: oxidative stress; primary open angle glaucoma; pseudoexfoliation glaucoma; total antioxidant status; total oxidant status

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INTRODUCTION

Glaucoma is one of the most common causes of blindness worldwide and results in damage to the neuronal layer of the retina, which can lead to optic atrophy and changes in the visual field [1]. It is a degenerative, insidious, and progressive optic neuropathy [1]. High intraocular pressure (IOP), considered the most important risk factor for glaucoma, is the result of increased secretion of intraocular fluid, decreased drainage, or both[1].

Other factors also play a role in the etiology and pathology of the disease, including high glutamate levels [2], alterations in nitric oxide (NO) metabolism [3-4], vascular factors [5-6], and oxidative damage due to increased production of free radicals [7-9]. Free radicals are a consequence of oxidative damage and lead to unfavorable effects on the trabeculum and increased resistance to intraocular fluid drainage [10]. In addition to the increase in free radicals, a decrease in antioxidant potential may also lead to damage in the trabeculum, retinal vascular endothelium, and the retinal ganglion layer, resulting in a rise in IOP, which can damage the optic nerve and promote loss of the visual field[11-12].

In the current study, we evaluated the role of oxidative stress in the pathogenesis of glaucoma. We compared aqueous humor (AH) total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) levels in patients with primary open angle glaucoma (POAG) and pseudoexfoliation glaucoma (PEG) compared to those in patients with cataracts but without glaucoma.

SUBJECTS AND METHODS

Subjects Our study was conducted at the Ulucanlar Eye Research Hospital between February-May 2011 with permission from the Ankara University Faculty of Medicine Department of Medical Ethics in accordance with the Declaration of Helsinki and the World Medicine Association. Patients were given detailed information about the study and signed a written informed consent form to particpate in this prospective study.

Methods

Grouping A detailed patient history, including previous diagnoses and treatments, was taken for each individual. All patients were subjected to a thorough ophthalmological examination consisting of best-corrected visual acuity, IOP measurement with an applanation tonometer, bio-microscopy examination of anterior and posterior segments, and measurement of central corneal thickness with an ultrasonic pachymeter. In addition, patients underwent gonioscopy with a Goldmann three-mirror lens, a perimetric examination with a Humphrey automated visual field analyzer, and a retinal neuronal thickness measurement by optical coherence tomography (SpectralisR, Heidelberg Engineering, Carsbad, CA, USA).

The study group included patients with PEG or POAG who had undergone cataract or glaucoma surgery. The control group comprised patients who applied to our hospital for cataract surgery. Patients >45 years old were diagnosed with PEG if they showed typical pseudoexfoliation material (PEM) on the lens and/or papillary border with IOP ≥22 mm Hg, cup to disc ratio ≥0.3, generalized or partial rim notching on the optic nerve head, peripapillary choroidal atrophy or splinter hemorrhage, and glaucomatous visual field damage according to the Advanced Glaucoma Intervention Study score. Patients with all of these findings, except PEM, were diagnosed with POAG. The control group consisted of the patients with senile, uncomplicated cataract. Patients were excluded from the study for the following conditions: diagnosed with any type of glaucoma other than POAG or PEG, diagnosed with and treated for any systemic disease, age-related macular degeneration, retinal vessel occlusion, or any condition that might be related to free radical damage, or history of any intraocular surgery within 1y.

Aqueous humor sample collection The surgeries for all patients were performed using the rules of antisepsis. The anterior chamber was entered prior to surgery using a 20-G MVR paracentesis knife, and 0.1-0.2 mL of AH was collected, protected from external light, and stored at -80℃.

Biochemical analysis of total oxidant status and total antioxidant Status TAS and TOS measurements in the AH were performed using a colorimetric method first described by Erel[13]. Briefly, a standard hydrogen peroxide solution was oxidized with free radicals to produce a yellow-brown color. Therefore, antioxidants within the sample suppress oxidation and color formation. This reaction was monitored by spectrophotometry, and TAS was deteremined indirectly. Similar to the TAS measurement, oxidization of ferrous iron by oxidants in the sample caused a quantifiable color change allowing for the TOS measurement. Results are expressed as milimolar trolox equivalent per liter (mmol Eqv/L) for TAS and micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Eqv/L) for TOS. OSI was computed by dividing TOS by TAS.

Table 1 Demographic characteristics of patients *n* (%)

Parameters	Glaucoma group (*n*=31)	Cataract group (*n*=31)
Male	23 (74.2)	20 (64.5)
Female	8 (25.8)	11 (35.5)
Mean age (a)	67.1±9.6	67.0±9.8

Table 2 Comparison of AH TAS, TOS and OSI levels between sub-groups

Parameters	Glaucoma group (*n*=31)	Cataract group (*n*=31)	*P*
TAS (Trolox Eqv/L)	2.70±1.57	1.80±0.79	0.006
TOS (μmol H_2O_2 Eqv/L)	31.52±12.79	28.48±10.08	0.304
OSI	14.43±10.12	19,09±11.55	0.097

TAS: Total antioxidant status; Trolox: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Eqv/L: Equivalent/L; TOS: Total oxidant status; OSI: Oxidative stress index.

Table 3 Comparison of AH TAS, TOS and OSI levels between sub-groups

Parameters	POAG (*n*=15)	PEG (*n*=16)	*P*
TAS (Trolox Eqv/L)	2.95±2.0	2.47±0.96	0.692
TOS (μmol H_2O_2 Eqv/L)	31.59±12.7	31.46±13.2	0.937
OSI	13.03±7.7	15.75±12.1	0.906

TAS: Total antioxidant status; TOS: Total oxidant status; Trolox: 6-hydroxy-2, 5,7,8-tetramethylchroman-2-carboxylic acid; Eqv/L: Equivalent/L; OSI: Oxidative stress index; POAG: Primary open angle glaucoma; PEG: Pseudoexfoliative glaucoma.

Statistical Analysis SPSS Versionver. 15.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for all statistical analysis. Descriptive statistics are presented as ratios, arithmetic mean ±standard deviation, and median (range) values. Normality of the continuous data distribution was assessed with the Kolmogonov-Smirnov test, a histogram, and P-P graphics. Two-group comparisons of normally distributed variables in independent groups were evaluated by t-test, whereas the Mann-Whitney U-test was used for non-normally distributed variables. Statistical significance was considered at $P<0.05$.

RESULTS

Sixty-two patients were included [19 females (30.6%) and 43 males (69.4%); mean age, 67.03±9.63y], and each group had 31 patients. Fifteen (48.4%) patients in the study group were diagnosed with POAG, and 16 (51.6%) were diagnosed with PEG. In addition, seven patients (22.6%) had a history of trabeculectomy, and six (19.4%) were pseudophakic. No intergroup difference in sex ($P=0.582$) or age distribution was observed ($P=0.979$) (Table 1). Preoperative IOP in the study group was 22.3±6.4 mm Hg, despite maximum medical treatment, whereas mean IOP in the control group was significantly lower (14.5±3.9 mm Hg, $P=0.001$). Comparisons of the TAS, TOS, and OSI values between the study and control groups are shown in Table 2. TAS values were significantly higher in the study group than in the control group ($P=0.006$), but no significant difference was observed between the TOS and OSI values. No significant difference was observed in the TAS, TOS, or OSI values between the PAOG and PEG sub-groups (Table 3).

DISCUSSION

The most important pathological factor for progression of glaucoma is increased IOP[1]. Other factors, such as decreased antioxidant potential and increased free radicals, may also play an important role in the progression of glaucoma[11-12,14-15]. A balance between free radical production and antioxidant potential occurs under normal physiological conditions, and oxidative stress presents when the antioxidant effect is weakened. Many factors, such as radiation, viruses, cigarette smoke, infection, stress, and toxic products produced by cells, result in oxidative stress [16]. Oxidative damage plays an important role in the pathogenesis of age-related diseases[17-21] and increases with age [22]. Advanced age is a risk factor for glaucoma, and IOP appears to increase with age[23-24]. Patients with glaucoma are genetically more prone to free-radical damage. Malondialdehyde (MDA) is a lipid peroxidation product in the AH of eyes with glaucoma. A previous study found no difference in serum and AH MDA values between glaucomatous and normal eyes [25]. However, TOS levels tended to be higher in the treatment-group eyes in our study compared to eyes from patients in the control group. Another study found that plasma MDA levels in patients with PAOG are significantly higher than those in healthy volunteers[26-27]. These results suggest that possible changes in MDA levels play a role in PAOG pathogenesis. In our study, no significant differences were observed between TOS and TAS levels in the POAG and PEG sub-groups. Similarly, although OSI tended to be higher in patients with PEG, the difference was not statistically significant. Disruption of trabeculae cells can be determined by 8-hydroxydeoxyguanosine (8-OH-dG) levels depending on the DNA damage caused by oxidative stress. One study found that 8-OH-dG levels in the trabeculae of patients with glaucoma were significantly higher than those measured in a control group [28]. Decreased antioxidant potential, as well as increased free radicals, cause damage to trabecular endothelial cells, retinal vascular endothelium, and retinal ganglion cells, which may lead to increases in IOP, optic disc damage, and loss of the visual field [12]. Reduced glutathione (GSH) and glutathione peroxidase are important antioxidant potential markers that eliminate the harmful effects of free radicals. A significant reduction in circulating levels of glutathione may underlie the deterioration of overall antioxidant defense in patients with glaucoma[29].

The role of oxidative damage in the development of cataracts is known [30-32]. GSH is the primary and an essential antioxidant in the lens. A GSH deficiency is a main factor in the formation of cataracts in the lens nucleus [30-32]. In the current study, TAS was significantly higher in the patients with glaucoma than in the controls, suggesting that TAS values increased in response to oxidative stress. A previous study found that blood plasma antioxidant activity is lower and levels of free-radical products are higher in PAOG and senile cataract groups compared to those in healthy volunteers [33]. However, no difference between the glaucoma and cataract groups was observed in terms of oxidative stress or potential antioxidant markers [33]. These findings suggest that oxidative damage may be important in the formation of PAOG and cataracts. A previous study measured AH levels in 90 patients who were undergoing either glaucoma or cataract surgery and found that MDA levels were significantly higher and TAS levels were significantly lower in the glaucoma group [34]. In another study, AH total reactive antioxidant potential levels were lower in the glaucoma group [10]. In the same study, free radical activity increased in the glaucoma group; however, catalase activity was similar between the groups [10]. A shift in the treatment of glaucoma from medications predominantly used to reduce IOP to primarily neuroprotective agents has been observed recently. N-methyl-d-aspartate antagonists, calcium channel blockers, inhibitors of NO synthase, cannabinoids, melatonin, vitamin B12, acetylsalicylic acid, ginkgo biloba, and antioxidants are currently prescribed for this purpose [35]. However, the use of natural antioxidants and vitamin supplementation remains controversial to treat glaucoma and other ocular diseases[36-37]. One of the limitations of the current study was that topical antiglaucoma medications used by the patients were not considered, and some of these medications have antioxidant activities [38-39]. This kind of patient selection was not applied due to ethical considerations. Another point to mention is the selection of patients with cataracts as a control group. In the future, patients with glaucoma can be compared to healthy volunteers if oxidant and antioxidant substances in the AH are measured using a noninvasive technique. Another limitation of our study was the small number of patients. Studies involving a larger number of patients would be expected to achieve more statistically powerful results.

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