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LABORATORY STUDY

Role of oxidative stress enzymes in open-angle glaucoma

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Abstract

Purpose **To investigate the role of oxidative stress and lipid peroxidation in the pathogenesis of primary open-angle glaucoma (POAG).**

Materials and methods **The activities of myeloperoxidase (MPO), catalase (CAT), and the levels of plasma malondialdehyde (MDA) were measured in 40 (15 men and 25 women) patients with POAG and 60 (30 men and 30 women) healthy controls.**

Results **There was no significant difference in the activities of CAT and MPO between the POAG patients and the controls. However, the plasma MDA level was significantly higher in patients than the controls.**

Conclusion **The results of this preliminary study suggest that the possible alterations of plasma MDA levels may be associated with the pathogenesis of POAG, but further research is needed to understand the role of oxidative damage in this important disorder of aging.**

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Introduction

Primary open-angle glaucoma (POAG), the most common form of glaucoma, is a slowly progressive atrophy of the optic nerve, characterized by loss of peripheral visual function and is usually associated with elevated intraocular pressure. Glaucoma is the second leading cause of vision loss in the world. The number of people with primary glaucoma is estimated at nearly 66.8 million by the year 2000, with 6.7 million of them suffering from bilateral blindness.[1] The molecular basis of POAG is mostly unknown. Several theories of its pathogenesis have been proposed, including mechanic and ischaemic ones.

Oxidation–reduction mechanisms have special importance in the eye. Oxidative damage can result in a number of molecular changes that contribute to the development of glaucoma, cataract, and other eye diseases.[2,3] If the free radical theory of aging is applied to the eye, an altered antioxidant/oxidant balance should be evident for age-related ocular diseases, such as age-related macular degeneration, cataract, and glaucoma.[4]

All of the studies investigating the relation between POAG, oxidant stress, and antioxidant systems were carried out at the tissue level. We could not find any study concerning systemic antioxidant enzyme level in published reports. Owing to this, the exact role of the levels of blood antioxidant substances in disease development and progression remains to be fully elucidated.

In this study, myeloperoxidase (MPO) and catalase (CAT) enzyme activities that belong to the antioxidant defence system, and malondialdehyde (MDA), the by-product of lipid peroxidation, plasma levels were measured in POAG patients. It was investigated whether the spoilt balance between oxidant stress–antioxidant defence systems occurring at the cell level was reflected on the blood level or not.

Materials and methods

This study was performed with 40 POAG patients whose ages range from 40 to 83 years (15 men and 25 women) attending Mersin University Faculty of Medicine Department of Ophthalmology. A total of 60 healthy subjects (30 men and 30 women; age range: 45–73 years), who visited our hospital for an annual check-up, were considered as the control group.

Cases with chronic diseases such as diabetes mellitus, systemic hypertension, chronic anaemia, thyroid function disorders, liver and renal dysfunction, inflammatory arthritis, heart failure, and patients under drug treatment were excluded from the study. All of the subjects were nonsmokers and none of them was consuming alcohol. Only POAG cases were included in the study, and primary angle closure glaucoma, normotensive glaucoma, and ocular hypertension cases were excluded from the study. POAG was diagnosed with elevated intraocular pressure, visual field loss, and glaucomatous optic nerve head changes criteria. The follow-up period of the cases in the study group ranged from 6 months to 20 years. None of the study group and control cases had any other age-related eye disease. During the examination, Lens Opacities Classification System II, which was described by Chylack Jr *et al*[5] and which uses photographic standards for grading cataract type and severity, was used to grade lens status with slit-lamp examination. Both lenses of all cases were graded as having no nuclear, posterior subcapsular, cortical opacities, or having grade I or II opacities

After all subjects gave their informed consent, venous blood samples (10 ml) were taken into ethylenediaminetetraacetic acid-containing test tubes.

Determination of MPO

The determination of sera MPO activity depends on the reduction of *o*-dianozidine. Reduced *o*-dianozidine was measured by spectrophotometer at 410 nm.[6]

Determination of CAT

CAT activity was measured according to the method that was defined by Beutler.[7]

Principle

CAT catalyses the breakdown of H_2O_2 to H_2O and O_2. The rate of decomposition of H_2O_2 by CAT is measured spectrophotometrically at 230 nm, since H_2O_2 absorbs light at this wavelength.

Determination of MDA

The MDA level, as an index of lipid peroxidation, was determined by thiobarbituric acid (TBA) reaction according to Yagi. The principle of the method depends on measurement of the pink colour produced by interaction of the barbituric acid with MDA elaborated as a result of lipid peroxidation. 1,1,3,3-Tetraethoxypropane was used as the primary standard.[8]

Statistical method

CAT and MPO enzyme activities, and MDA levels are given as mean $\pm$ standard deviation. It was evaluated by ANOVA as to whether there was a significant difference in terms of these variables by forming a general linear model by taking age and gender as covariates. Statistical tests were performed by the SPSS 9.0. P-value < 0.05 was considered statistically significant.

Results

The mean age of study group and the control group were 57.3 ± 1.9 and 57.4 ± 1.08 years respectively. Men formed 37.5% in the study group and 50% in the control group. The mean ages of two groups were similar. The mean follow-up period of cases in the study group was 6.23 ± 1.5 years. Demographic features of the two groups are summarized in Table 1.

Erythrocyte MPO, CAT activities, and plasma MDA levels are shown in Table 2. The mean activity ($\pm$SEM) of MPO was 0.53 ± 0.05 U/l in the study group and 0.42 ± 0.042 U/l in the controls. There was no significant difference in the MPO activity between the study group and the controls ($P = 0.329$).

Erythrocyte CAT activity was found to be 16346.5 ± 993.9 U/l in POAG patients and 16061.3 ± 1126.6 U/l in control cases. No statistically significant difference was found between the study group and the control group ($P = 0.919$).

Plasma MDA levels were significantly higher in the study group (6.88 ± 0.96 nmol/ml) than the controls (2.94 ± 0.26 nmol/ml) ($P = 0.0001$).

Table 1 Demographic features of the study and control groups

	Study group n = *40*	*Control group* n = *60*
Age (years)	57.3 ± 1.9	57.4 ± 1.0
Sex (m/f)	15/25	30/30
Duration of the disease	6.23 ± 1.5	—

Table 2 Statistical analysis results of the study and control groups

Variables	*Study group* (n = *40*) *Mean* $\pm$ *SE*	*Control group* (n = *60*) *Mean* $\pm$ *SE*	P
MPO activity	0.53 ± 0.05	0.42 ± 0.042	0.329
CAT activity	16346.5 ± 993.9	16061.3 ± 1126.6	0.919
MDA level	6.88 ± 0.96	2.94 ± 0.26	0.0001

Discussion

The prevalence of POAG is strongly age related.[9–12] Several studies have found a statistically significant increase in intraocular pressure with age.[13–15]

There is a general consensus that cumulative oxidative damage is responsible for aging.[16] There is an age-related rise in systemic oxidant load, and age-related morbidity is associated with low antioxidant defences.[17,18] Therefore, oxidative damage may play an important role in the pathogenesis of age-related diseases.[19]

Oxidative damage is a form of tissue injury that is initiated by reactive oxygen species known as free radicals. These reactive molecules can be formed during the course of normal aerobic metabolism or as a result of a particular insult such as light exposure. Complex defence mechanisms against such damage exist in tissues exposed to oxidative stress. However, when defences are inadequate, damage can occur. The final consequence of oxidative damage includes loss of normal structural and functional integrity of cells.[20] Within the eye, these damaging reactions have been proposed to be involved in the pathogenesis of age-related diseases.[21,22]

Oxidative damage has been hypothesized to play a role in the pathogenesis of glaucoma. As there are high aqueous concentrations of hydrogen peroxide and photochemical reactions in the anterior segment arising from aerobic metabolisms, the trabecular meshwork is exposed to high levels of oxidative stress.[16] Aqueous humour is known to contain several active oxidative agents such as hydrogen peroxide and superoxide anion.[23,24] It has been suggested that chronic oxidative insult induced by such agents can compromise trabecular meshwork function[25,26] and subsequently play a role in the pathogenesis POAG. De La Paz and Epstein[27] have demonstrated a decline in the specific activity of human trabecular superoxide dismutase, but not CAT, with increasing age, thus supporting the view that oxidative stress may be aetiologically involved in POAG.

In this study in which we investigated the systemic antioxidant enzyme activity in POAG patients, we found no evidence of an association between POAG and systemic enzyme activities of MPO, CAT ($P = 0.329$ for MPO, $P = 0.919$ for CAT). However, a statistically significant relationship was found between the presence of POAG and plasma MDA levels ($P = 0.0001$). The plasma MDA level, a by-product of lipid peroxidation, is a reliable and commonly used biomarker of the overall lipid peroxidation. Our finding of increased plasma MDA levels in POAG patients is not only consistent with the role of oxidative stress in POAG but also supports the idea that plasma MDA levels may be used as a marker of oxidative stress on a group basis.

Clearly, additional research is needed for further evaluation of the role of oxidative stress in POAG. We conclude from these results that systemic antioxidant enzyme activities evaluated in the red blood cells of individuals with POAG are not useful correlates of disease. There is no evidence for an underlying systemic oxidative stress, measured by antioxidant enzyme activities in red blood cells of patients, in POAG.

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