·Brief Report·

Decreased paraoxonase1 activity and increased malondialdehyde and oxidative DNA damage levels in primary open angle glaucoma

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Abstract

• **To investigate the malondialdehyde (MDA) levels, paraoxonase1 (PON1) activity and 8 –hydroxy 2 –deoxyguanosine (8–OHdG) levels in the primary open angle glaucoma (POAG) patient. Blood samples from 52 healthy individuals and 53 patients with POAG were analyzed for MDA and 8 –OHdG by high –performance liquid chromatography (HPLC) and PON1 by spectrophotometry. The data obtained were analyzed statistically. MDA levels were 10.46±8.4 and 4.70±1.79 μmol; PON1 levels were 121± 39.55 and 161.62±60.22 U/mL; and 8–OHdG values were 1.32 ±0.53/10^6 dG and 0.47 ±0.27/10^6 dG in the POAG patients and the control group, respectively. The difference was significant in MDA levels, 8–OHdG levels and PON1 activity in POAG patients in comparison with controls (P<0.001). We concluded that the observed increase in MDA and 8–OHdG levels may be correlated with decreased PON1 activity. Oxidative stress plays an important role in glaucoma development.**

• **KEYWORDS:** glaucoma; paraoxonase; 8-hydroxy 2-deoxyguanosine
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INTRODUCTION

Glaucoma is an optic neuropathy and lead to blindness worldwide. It is a multifactorial disease but the pathogenesis of glaucoma is still unclear despite intensive clinical and basic researches. Many researches indicate that oxidative stress has an important role in the primary open angle glaucoma (POAG) pathogenesis. The breakdown of balance between the generation and clearance system of reactive oxygenspecies (ROS) can cause the damage of membranes and proteins, lipids and nucleic acids[1].

ROS-mediated oxidation of lipids in cell membrane leads to formation of the lipid peroxidation products. These products derived from polyunsaturated fatty acid (PUFA) are unstable and form a complex series of compounds. Lipid peroxidation is an indicator of cellular oxidative stress. Malondialdehyde (MDA) is widely accepted as a marker of peroxidative cell membranes damage that is induced by chemical and/or physical oxidative stress. Elevated level of MDA is associated with a variety of diseases [2]. ROS leads to breaks or base modifications in DNA, including the 8-hydroxy 2-deoxyguanosine (8-OHdG). Therefore, 8-OHdG can be used as a biomarker of oxidative DNA damage sensitively. Many studies have showed increased levels of 8-OHdG, in patients with diabetes mellitus and diseases with inflammation and ageing[3].

Paraoxonase1 (PON1) is an enzyme with antioxidant properties, synthesized in the liver, and secreted in the plasma. It has been extensively investigated in several disorders, including age-related pathologies such as senil macular degeneration, coronary artery disease, atherosclerosis, type-2 diabetes, and Alzheimer's disease. PON1 is a free-radical scavenging system for human body. It prevents oxidation of low-density lipoprotein *via* metabolizing the lipid peroxides[4].

The aim of this study is to evaluate the role of oxidative stress in the pathogenesis of POAG by measuring MDA levels, PON1 activity, and 8-OHdG levels.

SUBJECTS AND METHODS

Medical Ethics Committee of Ataturk University Medical Faculty approved the study. The study involved 53 patients with POAG and 52 healthy control individuals. We described POAG patients who have intraocular pressure (IOP) higher than 21 mm Hg, cup/disk (C/D) rate 0.3 and retinal nerve fiber layer (RNFL) defects in OCT and visual field defects. All of our patients are Caucasian. The inclusion criteria were

based on 40 to 80 year-old patients having POAG. Patients under 40 years old, using cigarettes or vitamins, having thyroid function disorders, liver or renal dysfunction, anemia, coronary heart disease, hypertension, neoplastic disease, history of cerebrovascular accident, anterior optic neuropathy, retinal vascular disease or diabetes mellitus were excluded from the study. All patients underwent a detailed ophthalmologic evaluation. Patients were informed about the study. Venous peripheral blood samples (10 mL) were collected from the patients.

MDA concentrations of all groups in blood plasma sample were evaluated by high-performance liquid chromatography (HPLC) with fluorescent detection (HPLC-FLD)[5]. Serum PON1 activity was also measured [5]. 8-OHdG and dG levels were measured by using HPLC with electrochemical (HPLC-ECD) and variable wave length (HPLC-UV) detectorsystems in the hydrolyzed DNA samples [6]. The independent sample t-test was used to calculate the statistical significance. A P-value of <0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Demographic and biochemical datas are shown in Tables 1 and 2. There were statistically significant differences between the two groups in MDA, PON1 and 8-OHdG levels (P<0.001). Statistical analysis revealed that plasma MDA levels negatively correlated with plasma PON1 activity (r= -0.283, P<0.001) and positively correlated with 8-OHdG levels (r=-0.283, P<0.001) in the patient group.

Many risk factors including positive family history of glaucoma, increasing age, and elevated IOP have been identified. Although the pathogenesis of glaucoma is still unclear, oxidative stress appears to have a role in glaucomatous optic nerve damage[7].

In vitro models and clinical studies have shown that ROS-mediated oxidative mechanisms play a role in the etiology of glaucoma. ROS-mediated oxidative stress is in correlation with increased levels of lipid peroxidation in glaucoma patients. MDA level, showing the oxidative damage in cells and tissues, is the indicator of lipid peroxides[8]. In this study, similar to the results of earlier reports, we found higher MDA concentrations in the glaucoma patients.

The 8-OHdG, a DNA product of oxidative damage, can serve as a sensitive biomarker for many disorders [9]. In this study, we detected an overall elevation of 8-OHdG-induced ROS-mediated oxidative damage in patients with glaucoma. Recent studies demonstrated that oxidative DNA damage induced the increase of ROS formation and/or decrease in antioxidant status in the sera and aqueous humor of glaucoma patients [10]. Furthermore, in a recent study Charissou *et al*[11] demonstrated that oxidative damage leading to lipid peroxidation may be accompanied by an increase in oxidative DNA damage.

Table 1 Clinical and demographic characteristics of all groups $\bar{x} \pm s$

Parameters	Patient group (n=53)	Control group (n=52)	P
Age (a)	58.81±12.23	61.53±10.95	>0.05
Male (%)	58	54	>0.05

Table 2 The data on oxidative status assessment between patient group and control group $\bar{x} \pm s$

Parameters	Patient group (n=53)	Control group (n=52)	P
8-OHdG/10^6 dG	1.32±0.53	0.47±0.27	<0.001
MDA (μmol)	10.46±8.4	4.70±1.79	<0.001
PON (U/mL)	121±39.55	161.62±60.22	<0.001

MDA: Malondialdehyde; PON: Paraoxonase. P<0.001 was considered statistically significant between patient group and control group.

Inagaki *et al* [12] conducted a study to determine whether genetic polymorphisms affecting high-density lipoprotein (HDL)-associated antioxidant enzymes were associated with POAG. They concluded that PON1 gene polymorphisms may influence the features of Japanese patients with POAG [12]. Although the reduction mechanism of serum PON1 activity is not fully understood in glaucoma patients, it may be attributable to greater inactivation of PON1 by increased generation of ROS[13].

Previous studies showed that PON1 neutralizes the harmful effects of lipid peroxides by decreasing covalent linkages between lipid peroxidation products[14].

Similarly, Baskol *et al* [15] determined a significant negative correlation between MDA and PON1 activities. According to their results, they concluded that increased generation of ROS causes inactivation of PON1 and increased lipid peroxidation product such as MDA levels. Likewise, glaucoma patients exhibited lower PON1 activity than the control group in our study. We determined a significant negative correlation between MDA levels and PON1 activities. This first report in the POAG patients about corelation PON1, 8-OHdG and MDA level.

In summary, our findings support that oxidative stress has a significant role in glaucoma pathogenesis and indicates that anti-oxidative therapy may have a role in glaucoma treatment.

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