Investigation of oxidative and antioxidative status in patients with diabetic cataracts

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Aim: To investigate the oxidative and antioxidative status in diabetic patients with cataracts and nondiabetic patients with cataracts as compared to controls.

Materials and methods: There were 149 subjects included in the present study. Blood was collected from type 2 diabetic patients suffering from cataracts (diabetic cataract; n = 42), senile nondiabetic patients suffering from cataracts (nondiabetic cataracts; n = 60), and healthy subjects of a control group (n = 47). Serum levels of total oxidant status (TOS), total antioxidant status (TAS), total thiol levels (TTLs), and paraoxonase 1 (PON1) were investigated in the samples.

Results: TTLs were significantly reduced in diabetic cataract patients (444.8 µmol/L) compared with both nondiabetic cataract patients (525.1 µmol/L) and the control group (569.9 µmol/L). Plasma PON1 levels also were significantly reduced in diabetic cataract patients (241.9 U/L) relative to the control group (178.3 U/L). There were no statistically significant differences between TAS and TOS levels among groups.

Conclusion: The present study demonstrates a significant reduction in serum TTLs and serum PON1 activities in diabetic cataract patients compared with controls. These findings represent a failure in the antioxidant system, which may be related to the cataractogenic process.

Key words: Oxidative status, antioxidative status, diabetic cataract

1. Introduction

The process of cataractogenesis is complex and multifactorial and is induced by number of risk factors (1–3). Among these, diabetes has been implicated as a major risk factor for the development of cataracts (4,5). Many clinical, epidemiological, and preclinical studies evaluating the relationship between diabetes and cataractogenesis have shown that cataract formation occurs more frequently, at earlier ages, and progresses much faster in diabetic individuals compared with nondiabetics (6,7) Although the cataractogenic effect of diabetes has been demonstrated, the exact mechanism is still unclear (8–10). Multiple pathogenic mechanisms, such as increased oxygen free radical formation, abnormal glycosylation of lens proteins, advanced glycation process, and increased tissue sorbitol concentration, have been proposed (11–13).

Oxidative stress induced by excessive reactive oxygen species (ROS) has a detrimental role in all diabetic complications, including diabetic cataracts (14). Oxidative stress status is defined as the production of larger amount of ROS than can be scavenged or inactivated by available antioxidant systems. In addition to damaging vital biological molecules, such as DNA, cellular proteins, and lipids, and leading to serious pathological results (15), it has been considered for a long time that excessive amounts of ROS have an important role in cataractogenesis (16).

Among various different parameters that have been used to measure oxidative stress status, total oxidant status (TOS) and total antioxidant status (TAS) are widely used and safe markers for the evaluation of oxidative stress levels (17,18) .

Antioxidants are molecules that protect cellular components against free radical-induced oxidative damage. There are many antioxidant systems in the human body that deactivate the ROS. The effect of reduced levels of antioxidant molecules on pathological processes in many diseases has been extensively investigated (19). It has been demonstrated that the concentration of antioxidant molecules in serum and lenses is significantly reduced in

cataract patients with diabetes compared with nondiabetic cataract patients and controls (20,21).

Thiols are one of the most important members of the antioxidant defense system (22,23).Significantly reduced thiol levels have been reported in patients with exudative age-related macular degeneration (ARMD) (24). To the best of our knowledge, the plasma total thiol levels (TTLs) in diabetic cataracts have never been investigated.

Paraoxonase is another important member of the antioxidant defense system (25,26). Expression of paraoxonase 1 (PON1) in the human diabetic cataractous lens has been reported (27). Moreover, lower PON1 levels in the serum of diabetic patients suffering from cataracts were also reported (28). Based on these results it was proposed that, within the lens, antioxidant properties of PON1 may provide protection against oxidative stress. The number of studies evaluating the effect of PON1 activities in diabetic cataracts is very limited (27,28).

The aim of this study was to investigate the levels of TOS, TAS, TTL, and PON1 in diabetic patients with diabetic cataracts and nondiabetic cataract patients compared to a control group.

2. Materials and methods

2.1. Subjects and sample collection
Our study population consisted of diabetic cataract patients (n = 42), nondiabetic cataract patients (n = 60), and healthy subjects as a control group (n = 47).

The study was conducted in the Department of Ophthalmology, Ankara Atatürk Research and Training Hospital. The study protocol was approved by the Ethics Committee of the Atatürk Research and Training Hospital and was conducted according to the Declaration of Helsinki and Good Clinical Practice Guidelines. The participants were informed about the nature of the study and informed consent was obtained. All subjects were over 55 years of age. Cataract types in the eyes of diabetic and nondiabetic patients were nuclear. Subjects who had histories of hypertension, cardiovascular disease, renal dysfunction, hepatic disease, hypo- or hyperthyroidism, and traumatic or toxic cataract were excluded from the study. None of the subjects were taking vitamins or antioxidant supplements or were smokers.

2.2. Samples
Blood samples were drawn into plain vacutainers from the antecubital veins of healthy controls and patients. The blood was allowed to clot for 30 min and serum specimens were centrifuged for 10 min at 4000 rpm, transferred to Eppendorf tubes, and stored at −80 °C until analysis.

2.3. Measurement of biochemical parameters
Serum hemoglobin (Hb), hemoglobin A1C (HbA1c), apolipoprotein A (ApoA), and fibrinogen were measured in the samples. Biochemical analyses were performed with a routine chemistry laboratory (Cobas P8000 and C6000, Roche Hitachi, Mannheim, Germany).

2.4. Measurement of paraoxonase activities
Paraoxonase activities were measured using paraoxon substrate. The rate of paraoxon hydrolysis (diethyl-p-nitrophenylphosphate) was measured by monitoring the increase of absorbance at 412 nm at 37 °C on an autoanalyzer (Advia 2400, Siemens, Japan). The amount of generated p-nitrophenol was calculated from the molar absorption coefficient at pH 8, which was 17,000 M^{-1} cm^{-1} (29). Paraoxonase activity was expressed as U/L.

2.5. Measurement of total oxidant status
The TOS levels of the sera were determined using a novel automated measurement method (17). Oxidants present in the sample oxidize ferrous o-dianisidine complexes into ferric ions. The oxidation reaction was enhanced by glycerol molecules that are abundantly present in the reaction medium. The ferric ions form a colored complex with xylenol orange as an acidic medium. Therefore, the color intensity, measured spectrophotometrically, was related to the total number of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 equiv/L).

2.6. Measurement of total antioxidant status
The TAS of sera was measured using an automated colorimetric measurement method for TAS developed by Erel (18). In this method, the hydroxyl radical, the most potent biological radical, is produced by the Fenton reaction and it reacts with the colorless substrate O-dianisidine to produce the dianisyl radical, which is bright yellowish-brown in color. Upon the addition of a plasma sample, the oxidative reactions initiated by the hydroxyl radicals present in the reaction are suppressed by the antioxidant components of the plasma, preventing the color change and thereby providing an effective measurement of TAS. The assay results were expressed as mmol Trolox equiv/L.

2.7. Measurement of total thiol status
TTL serum concentrations or sulfhydryl groups (-SH) were measured by the methods originally described by Elmman (30) and modified by Hu (31). Here, thiols interact with 5,5'-dithiobis-(2-nitrobenzoic acid), forming a highly colored anion with a maximum peak at 412 nm. Here, this method was adapted to an automated biochemistry analyzer for the first time (Advia 2400, Siemens, Japan).

2.8. Statistics
Data analysis was performed by using SPSS 11.5 for Windows (SPSS Inc., Chicago, IL, USA). Data were

shown as mean ± SD or mean (95% confidence interval [CI]). Whether the mean differences among groups were statistically significant or not was evaluated by analysis of covariance (ANCOVA) after adjustment for age. According to the ANCOVA, while the clinical parameters were assumed as dependent variables, study groups and age were assumed as a fixed factor and a covariate factor, respectively. The Bonferroni adjusted multiple comparison test was applied for determining which group differed from which others. A P-value of less than 0.05 was considered statistically significant.

3. Results

Patients' biochemical parameters examined during the research are shown in Table 1. Overall, the level of fibrinogen and ApoA were significantly reduced in diabetic cataract patients compared to the control group. HbA1c levels were significantly higher in the diabetic cataract group compared to both nondiabetic cataract patients and the control group. Similar results were also observed for Hb levels.

TTL, PON, TAS, and TOS levels are presented in Table 2. Overall, TTLs were significantly reduced in diabetic cataract patients (P < 0.001) compared with both nondiabetic cataract patients and the control group. There were also statistically significant differences between thiol levels in nondiabetic cataract patients and the control group (P < 0.05) (Figure 1).

Plasma PON1 activities were significantly reduced in diabetic cataract patients relative to the control group (P < 0.05). Although PON1 activities in these subjects were also reduced compared to nondiabetic cataract patients, the difference was not clinically significant. Similar results were also observed between nondiabetic subjects with cataracts and the control group (Figure 2). There were no statistically significant differences between TAS (P = 0.126) and TOS (P = 0.254) levels among groups.

Table 1. Biochemical parameters of study groups (mean ± SD).

Variables	Controls (n = 47)	Nondiabetic cataracts (n = 60)	Diabetic cataracts (n = 42)	P-value
Fibrinogen (mg/dL)	295.8 (255.8–335.8)	355.8 (322.5–389.2)	407.8 (370.1–445.5)	<0.001
ApoA (mg/dL)	147.4 (137.1–157.6)	136.3 (128.0–144.6)	123.6 (114.1–133.1)	0.005
HbA1c (%)	5.67 (5.33–6.04)	5.85 (5.56–6.14)	7.33 (7.01–7.66)	<0.001
Folic acid (ng/mL)	9.6 (8.5–10.6)	7.8 (6.9–8.7)	9.4 (8.4–10.4)	0.018
Hb (g/dL)	13.9 (13.4–14.4)	13.8 (13.4–14.2)	12.8 (12.4–13.3)	<0.001

ApoA: apolipoprotein A, HbA1c: hemoglobin A1c, HB: hemoglobin.

Table 2. Enzyme levels of study groups (mean ± SD).

Variables	Controls (n = 47)	Nondiabetic cataracts (n = 60)	Diabetic cataracts (n = 42)	P-value
TTL (µmol/L)	569.9 (544.6–595.3)	525.1 (504.3–545.8)	444.8 (421.0–468.6)	<0.001
PON (U/L)	241.9 (209.8–274.1)	202.5 (176.2–228.8)	178.3 (148.1–208.5)	0.024
TAS (mmol Trolox equiv/L)	2.81 (2.70–2.92)	2.69 (2.61–2.78)	2.82 (2.72–2.93)	0.126
TOS (µmol H2O2 equiv/L)	14.6 (12.9–16.4)	15.1 (13.7–16.6)	13.3 (11.7–15.0)	0.254

TTL: total serum thiol levels, PON: serum paraoxonase activity, TOS: total serum oxidant status, TAS: total serum antioxidant status.
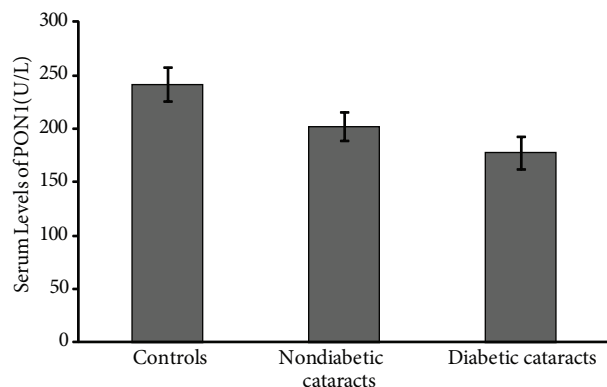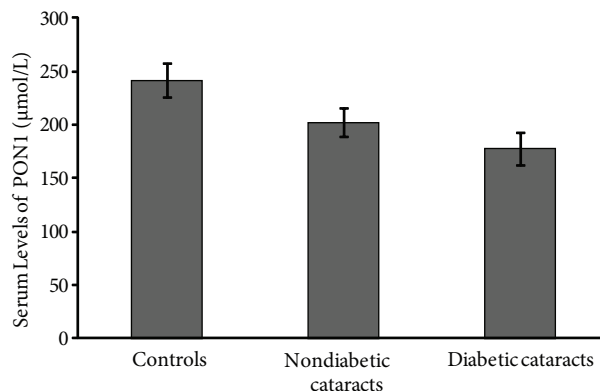
Figure 1. TTLs of study groups.

Figure 2. PON1 activities of study groups.

4. Discussion

The results of the present study showed that serum TTL and PON1 levels were significantly lower in patients with diabetic cataracts compared with the control group. Statistically significant differences were also observed between TTLs of diabetic cataract patients and nondiabetic cataract patients. There were no significant differences in TAS and TOS levels in any of the groups.

Plasma TTLs indicate the total amount of thiol-carrying molecules in the plasma. Thiols are sulfhydryl groups (-SH) containing derivatives that have important biological functions (32). They are important in protein structure and function, in regulation of enzymatic activity and in transcriptional activity, and are also one of the most efficient members of the antioxidant defense system (22,23). Various characteristic features of thiols contribute to their powerful antioxidant properties, one of which is their ability to neutralize ROS.

ROS are essential molecules for the regulation of vital cellular functions; however, overproduction of them may damage vital biological molecules including proteins, lipids, and nucleic acids, leading to various pathological conditions (15). In lens tissue, excessive amounts of ROS lead to biochemical changes such as degradation, crosslinking, and aggregation of lens proteins; these may have an important role in cataractogenesis (16).

The exact source of ROS in lens tissue is not clearly defined but hyperglycemia in diabetes is one of the major contributors. Hyperglycemia in diabetes increases generation of ROS and directly induces oxidative stress. Increased production of ROS in diabetes has been implicated in the pathogenesis of diabetic complications (33). In lens tissue, excessive ROS initiates lipid peroxidation and, together with glycation of proteins, induces biochemical reactions that lead to lens fiber damage and light scattering (4,13).

The rates of production and removal of ROS are strictly regulated to maintain the physiological levels. To protect this sensitive balance, several enzymatic and nonenzymatic antioxidant mechanisms in the body work in order to deactivate the excessive ROS efficiently (19). Several antioxidant molecules being taken through diet also play a role in the deactivation process and provide protection against ROS-induced oxidative injury (34–37).

In various pathological conditions, such as stroke, coronary artery diseases, and liver failure, decreased level of thiols have been observed (38–41).

Low thiol levels were also observed in wet-type ARMD patients compared with the control group (24). To the best of our knowledge, plasma TTL levels have not been studied previously in cataract patients.

Paraoxonase is another important antioxidant enzyme that protects low-density lipoprotein from oxidation and has a role in detoxification of organophosphates. Significantly reduced PON1 activities have been reported in hyperthyroidism (42), diabetes (43), cardiovascular diseases (44), chronic renal failure (45), and age-related macular degeneration (46).

Hashim et al. evaluated the serum PON1 activities in diabetic and nondiabetic patients with cataracts and observed significantly reduced PON1 levels in both groups compared with the control group (27). Hashim et al. also demonstrated the expression of PON1 at the genetic and proteomic levels in human lens tissue and proposed that PON1 has an antioxidant role against oxidative stress (28). The results of the present study support the findings of Hashim et al.'s first study. We also observed significantly reduced PON1 activities in diabetic cataract patients compared with controls. PON1 activities in nondiabetic cataract patients were also reduced, but the difference was not statistically significant. The decrease in PON1

activities may be due to several factors. First, high glucose concentrations may be one of factors, because it was shown that diabetic patients have lower PON1 levels. In addition, PON1 activity is also lower in healthy subjects with elevated fasting glucose levels and in patients with metabolic syndromes (47,48). Second, reduction in PON1 activity may also develop as a result of increased ROS concentration (49). Jaouad et al. demonstrated that PON1 activity is significantly reduced after exposure to ROS (50), and so the lower PON1 levels that we observed may reflect high ROS concentrations. Moreover, we propose that reduced thiol levels are another factor that contributes to the decrease in PON1 activity. PON1 has a cysteine thiol group in its active site, which is necessary for PON1 activity (32). At low thiol levels, the availability of the thiol group for PON1 activity is decreased and this causes a decrease in PON1 activity (32). Whatever the cause, it is clear that in diabetic cataract patients PON1 activity was significantly reduced. Together with the reduced thiol levels, the results of the present study demonstrated that serum levels of 2 important antioxidant molecules are significantly decreased in diabetic patients with cataracts.

ROS are very unstable molecules and they have a very short half-life (6–10 s or less), and so their plasma or tissue concentration cannot be directly measured; instead, oxidative stress status is measured.

There are many studies in the literature that investigate the oxidative stress parameters in diabetic cataract patients (21,22). In our study, although TOS levels were similar in cataract patients and controls, TTLs were significantly lower in cataract patients. As mentioned above, thiols are a primary component of the physiological antioxidant defense system, and PON1 is another important member of this system; we suggest that there was a state of oxidative stress and ROS had increased. In response to excess ROS, thiols, as a primary antioxidant, were consumed and PON1 activity was also decreased. We propose that oxidative stress was compensated for and TOS was maintained at a normal level.

In conclusion, the levels of 2 important antioxidant molecules were significantly reduced in diabetic cataract patients compared with controls. This finding represents a decrease in the antioxidant defense system. Although no differences were found between levels of TOS and TAS, the decrease in antioxidant status may reflect that the increased ROS concentration induced oxidative stress.

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