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Lens Capsular Glutathione Level and Glutathione Peroxidase Activity Among Diabetic Patients +

Sinan Emre*, Burhan Ateş**, Ahmet Taşar*, Selim Doğanay*,

*Department of Ophthalmology, Faculty of Medicine, Inonu University,
**Department of Chemistry, Faculty of Arts and Sciences, Inonu University, Malatya, Turkey

Purpose: To evaluate the level of glutathione (GSH) and gluthathione peroxidase (GSH-Px) activity in the anterior lens capsule of patients with cataract in diabetic patients with meticulous control and in patients with senile cataract.
Method: The anterior lens capsules in 18 eyes with diabetes under tight glycemic control with cataract (Group A) and in 26 eyes with senile cataract (Group B) were collected in phacoemulsification surgery. The specimens were thawed, centrifuged and the supernatants of the homogenate obtained from the samples were analyzed for the GSH level and GSH-Px enzyme activity.
Results: Group A composed of 10 female and 8 male patients and mean age of patients was 60.3 ± 8.3 years. Group B composed of 11 female and 15 male patients and mean age of the patients was 63.9 ± 12.2 years. Mean of the measurements for GSH level and GSH-Px activity at anterior capsule were 82.4±16.5 vs. 97.5±12.5 nmol/mg and 27.0±9.7 vs. 36.9±9.3 μmol/mg protein, in group A and B, respectively. The differences between groups were without any statistical significance for both parameters($p>0.05$).
Conclusion: The detorious effect of diabetes on anti-oxidant mechanisms is a well known subject. Our results indicate that approximate level of GSH and GSH-Px activity between diabetics with tight glucose control group and age-matched control group may be the result of meticulous control of hyperglycemia.

Key Words: Cataract, Diabetes mellitus, Glutathione (GSH), Glutathione peroxidase (GSH-Px), Meticulous controlled diabetes

Diyabet Hastalarının Lens Ön Kapsüllerinde Glutatyon Seviyesi ve Glutatyon Peroksidaz Aktivitesi

Amaç: Bu çalışmada yaşa bağlı kataraktı bulunan hastalar ile sıkı kontrol altındaki diabet hastalarının lens ön kapsüllerinde ölçülen glutatyon (GSH) seviyesi ve glutatyon peroksidaz (GSH-Px) aktiviteleri değerlendirilmiştir.
Yöntem: Çalışmaya tip II diabeti ve kataraktı bulunan hastalar ile yaş uyumlu olacak şekilde yaşa bağlı kataraktı bulunan hastalar dahil edildiler. Çalışma kapsamında sıkı diabet kontrolünde olan 18 hasta ile (Grup A), yaşa bağlı kataraktı bulunan 26 hastanın (Grup B) fakoemulsifikasyon yöntemi ile yapılan katarakt operasyonlarında alınan lens ön kapsülleri dahil edilmiştir. Örnekler çözünüp, santrifüje edilmelerinden sonra numunelerden GSH seviyeleri ve GSH-Px enzim aktiviteleri ölçülmüştür.
Sonuçlar: Grup A ortalama yaşları 60.3 ± 8.3 yıl olan, 10 bayan ve 8 erkek hastadan oluşmaktaydı. Grup B ise ortalama yaşları 63.9 ± 12.2 yıl olan, 11 bayan ve 15 erkek hastadan oluşmaktaydı. Grup A ve B için lens ön kapsülünde ölçülen ortalama GSH seviyeleri sırasıyla 82.4±16.5 ve 97.5±12.5 nmol/mg iken ortalama GSH-Px aktivite düzeyleri sırasıyla 27.0±9.7 ve 36.9±9.3 μmol/mg protein idi. Her iki parametre içinde gruplar arasındaki fark istatistiksel olarak anlamlı düzeyde tespit edilmedi ($p>0.05$).
Yorum: Diabetin anti-oksidan mekanizmalar üzerindeki zararlı etkisi iyi bilinmektedir. Bizim sonuçlarımızda GSH seviyesinin ve GSH-Px aktivitelerinin sıkı kontrollü diabet grubunda ve yaşa bağlı kataraktı grubu arasında yakın değerlerde çıkmış olması, diabetin sıkı kontrolünün diabetin lens üzerindeki etkilerini hafifletmek bakımından önemli olduğunu düşündürtmektedir.

Anahtar Kelimeler : Katarakt, Diabetes mellitus, Glutation (GSH), Glutation peroksidaz (GSH- Px), Sıkı kontrollü diabet

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Hyperglycemia secondary to uncontrolled glucose regulation is the cause of diabetes and diabetic complications.[1,2] Hyperglycemia of a short period time cause tissue damage by mechanisms involving repeated acute changes in cellular metabolism. On the other hand, despite the restoration of euglycemia, exposure of long-lived molecules to high glucose causes cumulative changes.[2]

Increased intracellular glucose levels leads to increased enzymatic conversion of glucose to the polyalcohol sorbitol, associated with decrease of NADPH and glutathione.[2,3] The loss of antioxidant reducing equivalents increases sensitivity to oxidative stress associated with intracellular reactive oxygen species (ROS). Hyperglycemia induces the production of superoxide which inhibits glucose-6-phosphate dehydrogenase (G6PD). This enzyme is the rate-limiting enzyme of the pentose phosphate pathway and this pathway is important for maintaining reducing equivalents to the antioxidant defense system. The product, NADPH, is the cell's principal reductant and is required for providing reducing equivalents to the glutathione based system.[2]

ROS due to light catalysed reactions causes oxidative damage in the transparent ocular media, aqueous humor and lens, and has been thought to be a major factor in the development of age-onset cataracts. It is clearly documented that both visible and UV radiations to which the lens is constantly exposed cause the production of ROS. ROS are capable of causing oxidative modification of cellular macromolecules such as lens crystallins, glutathione, cytoskeletal elements, and membrane thiols and they lead opacification of lens and cataract formation.[3]

Glutathione (GSH) is the predominant low-molecular-weight thiol in animal cells. Most of the cellular GSH is present in the cytosol. Cellular GSH concentrations are reduced markedly in response to protein malnutrition, oxidative stres and many pathological conditions. GSH is the major redox that determines the antioxidative capacity of cells but its value can be affected by other redox couples. GSH is the major substrate of enzyme glutathione peroxidase (GSH-Px). Major part of the glutathione is in the GSH form, minor part is in oxidised glutathione (GSSG) form and at normal conditions GSH/GSSG ratio is quite high. GSH/GSSG is the major redox couple that determines the antioxidative capacity of cells.[4]

GSH-Px, at the presence of high amount of H_2O_2 catalysis the oxidation of GSH to GSSG and meanwhile detoxifies the H_2O_2 by converting into water and molecular oxygen. The oxidized glutathione GSSG is reduced back to GSH by a NADPH-dependent reductase, glutathione reductase.[4]

The purpose of this study was to determine and compare the level of GSH and activity of GSH-Px in diabetic patients with meticulous glucose control and nondiabetic senile cataractous lenses.

MATERIALS AND METHODS

Clinical specimens

Eighteen patients with type 2 diabetes under meticulous control with cataract (10 female, 8 male, mean age was 60.3 ± 8.3) and 26 patients with senile cataract (11 female, 15 male, mean age was 63.9 ± 9.8) were included in this study. The mean duration of diabetes was 10.7 years. Participants in the control group had no clinical evidence of any disease state which might effect the antioxidant capacity. All diabetics were examined for microvascular complications; such as serum creatinine, microalbuminuria, creatinine clearence measurements for diabetic nephropathy; funduscopic examination for screening of diabetic retinopathy and for macrovascular complications. HbA1c levels were < % 7.0 as the recommendation of American Diabetes Association.[5] All patients with type 2 diabetics were treated with standart insulin with meticulous control.

To be included in the study, informed consent was obtained from all participants. The study was approved by the ethical committee of Inonu University School of Medicine and carried out in accordance with the Declaration of Helsinki.

Uncomplicated phacoemulsification of cataract and hydrophobic acrylic foldable posterior chamber intraocular lens implantation was performed on all of the cataractous lenses by two surgeons. After a temporal or superior clear corneal tunnel incision was made, a dispersive viscoelastic material (sodium hyaluronate 3%–chondroitin sulfate 4%,Viscoat, Alcon) was administered into the anterior chamber and the lens capsule was removed by continuous curvilinear capsulorhexis of 5.5 mm diameter with the help of a rhexis forceps. The collected capsules were transmitted in an ice container and frozen at -80 ºC till biochemical analysis.

Tissue for enzyme activity studies was homogenized (PCV Kinematica Status Homogenizator, Littau-Luzern, Switzerland) in 200 μm ice-cold phosphate-buffered saline (pH 7.4). The homogenate was sonified (Bronson sonifier 450, Danburg, CT, USA) with an ultrasonifier by six cycles (20-s sonications and 40-s pause on ice). The homogenate was centrifuged (10 000 g, 5 min) and cell-free supernatant was subjected to enzyme assay immediately.

Determination of GSH level and GSH-Px activity

Total GSH assay the formation of 5-thio-2-nitrobenzoate (TNB) was followed spectrophotometrically at 412 nm.[6] The amount of GSH in the extract was determined as nmol/mg protein utilizing a commercial GSH as the standard. Enzyme assay activity of GSH-Px was determined spectrophotometrically. The activity of GSH-Px was determined in a coupled assay with glutathione reductase by measuring the rate of NADPH oxidation at 340 nm using H_2O_2 as the substrate.[7] Specific activity is given as the amount of NADPH (μmol) disappeared per min per mg protein.

The protein content of samples was determined using the colorimetric method of Lowry et al. [8] using bovine serum albumin as the standard. All analyses were performed in duplicates.

Statistical analysis

Statistical analysis were performed with SPSS for Windows version 12.0 program (SPPS Inc., Chicago, IL). All data were reported as means ± standart deviation (SD). Mann-Whitney U test was used for comparison of variables in groups. A value of $p< 0.05$ was considered statistically significant.

RESULTS

The study population characteristics are shown in Table 1. Lens capsular GSH and GSH-Px activity levels of the patients with diabetic and senile cataract are presented in Table 2. The difference for capsular glutathione (GSH) levels in diabetic and senile cataracts were statistically unsignificant (respectively 82.4±16.5 vs. 97.5±12.5 nmol/mg protein, $p>0.05$). Also capsular GSH-Px activities were statistically unsignificant in diabetic patients compared with the senile cataractous patients (respectively 27.0± 9.7 vs. 36.9±9.3 μmol/mg protein,($p>0.05$).

Table 1. Demographic features of patients.

Group	Eyes	Mean Age (years) ± SD	Gender (F/M)
A (DM)	18	60.3 ± 8.3	10 / 8
B (Control)	26	63.9 ± 12.2	11 / 15

DM: Diabetes mellitus, F: Female, M: Male

Table 2: GSH level and GSH-Px activity readings according to study groups and their statistical analysis.

Group	Mean ± SD	
	GSH (nmol / mg protein)	GSH-Px (μmol / mg protein)
A (DM)	82.47 ±16.52	27.0±9.79
B (Control)	97.54 ± 12.56	36.99±9.31
p	0.161	0.101

DM: Diabetes mellitus, GSH: Glutathione, GSH-Px: Glutathione peroxidase
$p< 0.05$ was needed for statistical significance

DISCUSSION

The human lens consists of three metabolically different zones: the epithelium, the cortex and lens nucleus or core. Lens capsule is the external envelope which covers the cortex and nucleus and it is rich of epithelial cells under the collagenous surface. These cells are the metabolically active cells and some of these cells elongate to form lens fiber cells. In these cells, the major gene products of the lens, the crystallins are produced.[9]

Oxidative stress is thought to play a major role in the initiation and progression of diabetic complications.[1] The glycometabolic imbalance is also an important cataractogenic factor in diabetics. The hazardous effects of the ROS are neutralized in the lens by antioxidants such as ascorbic acid, vitamin E, the glutathione system, superoxide dismutase and catalase.[10] While antioxidant activities of enzymes such as superoxide dismutase, catalase and GSH-Px decrease in blood and different tissues of patients with diabetes mellitus, the levels of the ROS such as superoxide anion radicals increase.[10,11] These alterations suggest that free oxygen radicals and

antioxidant mechanisms might play an important role in the pathogenesis of the diabetic cataract.

During the development of senile cataract the enzymatic (superoxide dismutase, GSH-Px) and nonenzymatic (ascorbate, cysteine, GSH) antioxidant system activities are reduced in the lens with aging. In various studies which investigated the role of oxidant stress in the development of cataract, lens lipid peroxides are reported to be increased.[10,12] On the other hand, an insufficiency in enzymatic and nonenzymatic antioxidant systems with aging is also reported.[10]

Different researchers reported that lens lipid peroxides are increased and antioxidant enzyme activities are decreased inside cataractous lenses.[10,13] Authors stated that lens glucose, glycated protein and lipid peroxides were higher in diabetic cataractous patients when compared with senile cataract. In these studies, the decrease in SOD and catalase activities were more pronounced in diabetic cataractous lenses than in senile lenses. Inactivation of these enzymes may result in an elevation of the H_2O_2 and O_2^- levels in the lens, and this may be responsible for the oxidative modification of lens protein. These results further support the idea that inactivation of antioxidative enzymes may be important for the development of diabetic complications.[12,15]

GSH participates in various cellular reactions but primarily scavenges free radicals. GSH is also important for proliferation of cells, protein synthesis, cell-cycle regulation, immune system homeostasis, utilization of lipid, glucose and aminoacid as well as various metabolic pathways.[5] Human crystalline lens has unique properties, like being completely transparent in spite of the high protein content, and the high thiol content. It has been assumed that high concentration of reduced GSH keeps the thiol groups in the reduced form and prevent the disulfide bonds formation which lead cross-linking and aggregation.[14] Low GSH levels have been reported in human lens with age,[15] and in human cataract lenses,[16] in diabetic cataract lenses,[17] and finally in experimentally induced cataract models.[18,19]

The loss of GSH occurs in nearly all experimental cataracts. It is interesting to note that large decreases in GSH occur in the nuclear region while cortical GSH remains normal. This result strongly suggests that the postulated role of extra-lenticular oxidants such as hydrogen peroxide in cortical cataract development is not important. There also appears to be a threshold of GSH concentration to maintain its antioxidant effectiveness.[9] Decreased GSH synthesis may be the one cause of low GSH level, the activity of GSH synthesis enzymes γ-glutamylcysteine synthetase (GCS) and glutathione synthetase (GS) has been found lower in old clear human lenses. Age-related deficiency in γ –cystathionase activity which is essential for cysteine synthesis from methionine, and failure to regenerate GSH from GSSG via glutathione reductase (GR) and NADPH may be other causes of lowered GSH levels.[14]

There is increasing evidence that products of the Maillard reaction may play an important role, not only in diabetic complications but also of widespread age related pathologies. Advanced glycation end products (AGEs) accumulate in the intracellular and/or extracellular environment of ocular structures, contributing to the development of diseases such as diabetic retinopathy, cataract formation and atherosclerosis.[20] The experiments demonstrate that glycation with different sugars alter the GR activity in time-dependent manner and enzyme loses almost half of the activity after 5 days of incubation with low glucose concentration (5 mM), and almost 80% of initial activity after 15 days.[21] In both the aged and diabetic lenses, advanced glycation end products (AGEs) formation of various molecules have been shown to be markedly elevated, indicating the involvement of the later Maillard compounds in cataract formation. Glycation of lens crystallins induces significant age-related alterations, leading to aggregation and to cataract formation.[20] GSH can prevent glycation and by different study groups, participation of GSH in Maillard reactions as an anti-glycation agent has been described and they showed that the presence of GSH in *in vitro* glycation mixtures significantly prevents the formation of sugar mediated protein–protein crosslinking.[22,23]

A comparative study on blood and lens antioxidant status in normal, senile, and diabetic cataract patients found G6PD and GR activities in blood were lower in both pathological groups than in the control and lower in diabetic than in senile cataracts, whereas in lens both enzymes show higher activity in diabetes than in senile cataract.[17] According to Ganea and Harding the presence of the molecular chaperone *a*-crystallin in the lens could contribute to a partial protection of enzymes against inactivation.[14]

Except the enzymes directly involved in maintaining the normal redox status of the cell, G6PD is a potent agent against reactive oxygen species. It catalyzes the

former reaction of the pentose phosphate pathway and converts glucose-6- phosphate to 6-phosphoglucono-δ lactone by reducing $NADP^+$ to NADPH. After a serial reactions, the cellular requirement level of NADPH for biosynthetic processes and protection against oxidative damage in the lens is sufficiently maintained. In case of any oxidative stress, the pentose phosphate pathway is capable to increase the NADPH supply which is needed by the GSH protective system. Lens becomes more vulnerable to oxidative injury, in the presence of pentose phosphate pathway deficiency.[14]

Similar to our results there are also reports[24] of increased intensity of the oxidative stress in local compartment of diabetic patients, compared to other age-related cataract patients without any statistical significance. Lack of statistical significance in our study may be related to characteristics of the group, diabetics were under meticulous insulin treatment and free of any diabetics complication. Also, although the difference was not statistically significant, in our study group the patients with age-related cataract was composed of older patients compared to diabetic group. Higher mean age of these patients may be reason of close values from these two different groups.

In conclusion, the critical role of oxidative stres in age and diabetes-related cataract has been documented tremendously. Despite the reports of significant differences of GSH and GSH-Px between diabetes and age-related cataracts, the difference was not significant in our patients with age-related cataracts and meticulous controlled diabetics. In our opinion, it is necessary to plan a multicentered, standardized study to investigate effects of tight glycemic control on the anti-oxidant enzyme profile of patients with diabetes mellitus.

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Corresponding Address:

Dr. Sinan EMRE
Inonu University Medical Faculty,
Turgut Ozal Medical Center, Research Hospital,
Ophthalmology Department
TR-44280 Malatya, Turkey
Tel : 422 341 06 60
Fax : 422 341 0728
E-Mail: emre@inonu.edu.tr & mdsinanemre@yahoo.com