

## KATARAKLI LENSLEERDE GLUTATYON, GLUTATYON S TRANSFERAZ VE GLUTATYON REDÜKTAZ DÜZEYLERİ

### GLUTATHIONE, GLUTATHIONE REDUCTASE AND GLUTATHIONE S-TRANSFERASE LEVELS IN HUMAN CATARACTOUS LENSES

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#### SUMMARY

Glutathione is enzymatically synthesized in the lens and generally thought to play a role as a scavenger for radicals produced by oxidative stress via an oxidation-reduction system. The role of sulphur-containing compounds is thought to be associated with maintenance of the transparency of the lens. Glutathione and its related enzymes were measured for cataractous human lens nucleus. Glutathione decreased progressively with development of cataract. The level of glutathione in the nucleus was analyzed in terms of the patient age groups and in terms of lens pigmentation. Results are expressed as mean  $\pm$  standart deviation. We have found that glutathione and its related enzymes in the nuclei of senil cataractous patients seemed to have no correlation with age groups. The glutathione of brown-colored lenses was  $0.39 \pm 0.28$   $\mu\text{mol/g}$  lens, The glutathione S-transferase was  $81.75 \pm 66.07$   $\text{nmol/g}$  lens, glutathione reductase was  $97.25 \pm 36.0$   $\text{nmol/g}$  lens wet weight, the glutathione of yellow-colored lenses was  $0.71 \pm 0.66$ , the glutathione S-transferase was  $96.93 \pm 38.30$   $\text{nmol/g}$  lens, glutathione reductase was  $126.7 \pm 80.0$   $\text{nmol/g}$  lens wet weight. The difference between two group means aren't statistically significant ( $p > 0.05$ ).

**Key words:** cataract, glutathione, sulphur -containing compounds in lens

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### ÖZET

Glutasyon (GSH) lenslerde enzimatik olarak sentez edilir ve genellikle oksidasyon-reduksiyon sistemlerinde bulunan oksidatif stress tarafından oluşturulan serbest radikallerin tutulmasında rol oynar.Sülfür içeren bileşiklerin rolünün lensin şeffaflığının sürdürülmesi ile ilgili olduğu düşünülmektedir.Kataraktlı lens nükleusunda glutasyon ve ilgili enzimler ölçülmüştür.Kataraktın gelişmesiyle glutasyon progressif olarak azalmaktadır Nükleustaki glutasyon düzeyleri hastaların yaş grupları ve lens pigmentasyonuna göre sınıflandırıldı. Sonuçlar  $\pm$  standart sapma olarak verildi Elde edilen sonuçlara göre glutasyon ve ilgili enzimlerin sonuçlarının senil kataraktlı hastaların nükleusunda yaş grupları ile bir korelasyon göstermediği belirlendi.Lenslerin renklerine göre yapılan sınıflamanın sonuçlarına göre kahverengi lenslerin glutasyon düzeyleri  $0.39 \pm 0.28 \mu\text{mol/g lens}$ , glutasyon S-transferaz düzeyleri  $81.75 \pm 66.07 \text{ nmol/g lens}$ , glutasyon redüktaz düzeyleri  $97.25 \pm 36.0 \text{ nmol/ g lens}$  olarak bulundu. Sarı renkli nükleusların glutasyon, glutasyon S-transferaz ve glutasyon redüktaz düzeyleri ise  $0.71 \pm 0.66, 96.93 \pm 38.30, 126.7 \pm 80.0$  olarak bulundu.

Lenslerin renklerine göre yapılan sınıflamada ise kahverengi ve sarı renkli nükleusların GSH ve ilgili enzim düzeyleri arasında istatistiksel olarak bir fark bulunmadı ( $p > 0.05$ ).

### INTRODUCTION

Two thirds of the normal lens is composed of water and the remainder of structural protein.The beta and gamma crystallins of the lens contain considerable amount of sulfur,which is present as sulfhydryl groups easily accessible for reaction with oxidizing agents (1).The lens cortex has a high concentration of another sulfhydryl-containing

substance, glutathione. This tripeptide is vital to the normal metabolism of the lens. It is regarded as one of the primary defenses against oxidation damage. Loss of glutathione has been reported during aging of the lens and during cortical cataract formation. For these reasons glutathione has been extensively studied (2,3).

Reduced glutathione (GSH) has an important role in the maintenance of lens transparency by protecting protein sulfhydryl groups by oxidation (4).

The amount of GSH in the human lens has been shown to decrease with aging and during the formation of cataracts, even in the early stages (5, 6).

In this study we examine GSH, GSH S transferase (GST), GSH reductase (GR) levels in senile cataractous lenses.

## MATERIALS AND METHODS

### Lens Sample

Cataractous lenses were obtained from senile cataractous lenses removed from patients ages between 43 and 92 years (women  $69.56 \pm 10.93$ , men,  $69.75 \pm 8.75$ ) during planned extracapsular cataract extraction (PEKKE) performed at the Department of Ophthalmology Istanbul University Cerrahpaşa Medical Faculty. The lenses were stored at  $-70^{\circ}\text{C}$  upto assay time.

The nuclei of these lenses were used for assaying glutathione or enzyme activities.

### Enzyme Assay

After determining the wet weight with a sensitive mechanic scale, the samples were homogenised with 0.15 % KCL solution at  $4^{\circ}\text{C}$  in a glass homogenizer and centrifuged for 1.5 hours at  $10,000 \times g$ . Before centrifugation 0.5 ml homogenate separated for glutathione assay.

After centrifugation an aliquot of the supernatant was used as the enzyme source.

Glutathione reductase was determined at  $25^{\circ}\text{C}$  according to method of Beutler (7).

The activity of the glutathione reductase was expressed in units/ g of lens nucleus. The unit was defined as the amount of enzyme that catalyzed the oxidation of 1nmol of NADPH / min.

The glutathione - S- transferase was measured at 25 °C as described by Habig at all (8) Activity of the enzyme was expressed as nmol /gram of lens.

#### Glutathione Assay

10% w /v lens homogenate was used for glutathione assay This assay was depend on conjugation of glutathione with 1- chloro 2,4 dinitrobenzene (9), product was measured spectrophotometrically (Jasco model 7800 UV/ vis spectrophotometry ) These results were expressed as micromol /gram of lens.

#### Materials

NADPH, GSSG, GSH, were obtained from Sigma KCl, Na<sub>2</sub>HPO<sub>4</sub>, Tris HCL, Etilendiamintetraaceticacid, 5,5 dithiobis(2-nitrobenzoic acid), 1 chloro 2,4 dinitrobenzene were purchased from Merck.

### RESULTS

The glutathione content ,glutathione S-transferase and glutathione reductase activities were determined at the nuclei of 29 senile cataractous lenses and results were analyzed in terms of lens pigmentation and patient ages.Average values were calculated.The results were shown in table I and II. When groups are compared to lens pigmentation(table I),results were statistically not significant for GSH,S-transferase, and Glutathione reductase The groups are compared according to lens coloring The results were not statistically significant for brown and yellow colored lenses.

The groups are compared according to patients age.The results were not statistically significant for all GSH and linked enzymes in all groups.

### DISCUSSION

The lens of the eye contains free aminoacids and their derivates in high concentration reduced glutathione(GSH) and taurine being major constituents.The role of sulphur containing compounds is thought to be associated with transparency of the lens (10,11).

The content of glutathione in the lens nucleus was found to be moderately lowered in mature cataract

Table I. Glutathione ,Glutathione S-transferase, Glutathione reductase in lens nuclei of human senile cataracts Cataracts were classified according to lens pigmentation

	GSH	GST	GR
Brown	0.39 ± 0.28	81.75 ± 66.07	97.25 ± 36.0
Yellow	0.71 ± 0.66	96.93 ± 38.30	126.7 ± 80.0

Table II. Glutathione, Glutathione S-transferase , Glutathione reductase in lens nuclei of human senile cataracts. Results were classified in terms of patient age groups

	GSH	GST	GR
40-50	0.28	142.3	138.2
51-60	0.43 ± 0.42	85.10 ± 44.62	148.16 ± 32.79
61-70	0.87 ± 0.89	84.20 ± 58.71	92.15 ± 27.33
71-80	0.54 ± 0.43	76.21 ± 42.44	83.48 ± 44.31
90	1.27	70.09	88.50

In the present study the content of glutathione in the lens nucleus was not found statistically different in brown colored lenses.

Xie et al. found a significant decrease in glutathione in the brown cataracts (12) But Rao,Sadasivudu and Cotlier reported a sharp decrease in GSH at the stage of yellow coloring (11)

Glutathione reductase in human cataractous lenses has been studied by several investigators(13,14,15).

The activity of glutathione reductase was determined for nuclei of 7 brown colored ,22 yellow colored cataractous lenses(Table I) When compared with yellow colored lenses GR activity,was not statistically significant ( $p>0.05$ ).but Xie et al. showed that glutathione reductase activity of brown cataracts significantly lowered from yellow colored lenses

The glutathione S -transferase activity measured for nuclei of 29 cataractous lenses its activities in brown colored lenses were not found statistically significant in the yellow colored lenses

Glutathione S transferase are thought to play a physiological role in initiating the detoxication of potencial alkylating agents. According to age classification there was one patient at 40-50 years age, 3 patients at 51-60 years age, 13 patients 61-70 years age, 10 patients 71-80 years age, one patient 81-90 years age and one patient above 90 years age. We can not compare all groups with each other because of small number of patients at 40-50, 51-60, 81-90 and >90 groups. We can only compare 61-70 and 71-80 age groups. The GSH, GST and GR values between these two groups were statistically not significant ( $p > 0.05$ ).

Rathbun, Bovis and Holleschau were found decreased glutathione peroxidase, glutathione reductase and glutathione S-transferase activities in the rhesus monkey as function aging (16).

In the future we can plan to make this study by extending the age groups.

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