

## SUPEROXIDE DISMUTASE ACTIVITY IN CLEAR AND CATARACTOUS HUMAN LENSES

Dr. Mehmet BALCI\*, Dr. Nazmi ZENGİN\*\*, Dr. Ömer AKYOL\*\*\*, Dr. İlker DURAK\*\*\*

\* Ministry of Health, State Hospital, Eye Clinic, Soma, Manisa,

\*\* Selçuk University, Faculty of Medicine, Dept. of Ophthalmology, Konya

\*\*\* Ankara University, Faculty of Medicine, Dept. of Biochemistry, Ankara

### SUMMARY

*Superoxide dismutase (SOD) is one of the important enzymes that protects the ocular lens from oxidative damage. In this study, SOD activities in twenty immature, and twelve mature cataractous lenses and five clear lenses were compared. The mean SOD activity in the clear lens group was measured to be  $0.487 \pm 0.153$  U/mg protein whilst it was  $0.408 \pm 0.114$  U/mg, and  $0.229 \pm 0.103$  U/mg protein in the mature and immature cataractous groups, respectively. These results indicated significantly lowered SOD activities in mature cataractous lenses as compared to those of the clear ones ( $p < 0.05$ ). Although our results revealed a gradual decrease in activity with maturation of cataract, the difference observed between immature and mature cataractous lenses was not statistically significant ( $p > 0.05$ ). Meaningfully decreased SOD activities were found in the nuclei of all lens groups when compared to those of the cortex ( $p < 0.05$ ). Results of the present study support the hypothesis that oxidative stress might play a role in senile cataractogenesis.*

*Key Words : Lens, cataract, superoxide dismutase, oxidative stress, free radicals.*

### ÖZET

#### **Şeffaf ve Kataraktlı İnsan Lenslerinde Süperoksit Dismutaz Aktivitesi**

*Superoksit dismutaz (SOD) lensi oksidatif stresten koruyan önemli enzimlerden birisidir. Bu çalışmada 20 immatür, 12 matür kataraktlı ve 5 şeffaf lensteki SOD düzeyleri karşılaştırıldı. Şeffaf lens grubunda SOD aktivitesi  $0.487 \pm 0.153$  U/mg protein iken immatür katarakt grubunda  $0.408 \pm 0.114$  U/mg, matür katarakt grubunda  $0.229 \pm 0.103$  U/mg protein olarak bulundu. Bu sonuçlar matür kataraktlı grupta şeffaf lens grubuna oranla SOD aktivitesinin anlamlı derecede azaldığını göstermektedir ( $p < 0.05$ ). Kortekstekilye karşılaştırıldığında her üç grup lenste de nükleer SOD aktivitesi anlamlı derecede düşüktü ( $p < 0.05$ ). Çalışmamızın sonuçları oksidatif stresin senil katarakt gelişiminde rol oynayabileceği hipotezini desteklemektedir.*

*Anahtar Kelimeler : Lens, katarakt, superoksit dismutaz, oksidatif stres, serbest radikaller*

### INTRODUCTION

Intermediate products of oxygen such as singlet oxygen, superoxide and hydroxyl radicals, and hydrogen peroxide are called free radicals. These intermediates are highly toxic, and can react with vital macromolecules such as enzymes, proteins, lipids,

and nucleic acids to bring about severe structural and functional damage to the cell (1). Tissues are equipped with enzymatic (eg superoxide dismutase (SOD), catalase, glutathione peroxidase, etc.), and non-enzymatic (eg ascorbate, vitamin E, metal-binding proteins, etc.) antioxidant systems to de-

toxify the free radicals. When the tissue suffers from either an increased production of free radicals or a decreased dismutation capacity, or a combination of both, pathologic conditions such as carcinogenesis, atherosclerosis, stroke, and cataractogenesis, etc. may ensue (2).

Cataract is one of the major causes of preventable blindness throughout the world (3). Although several mechanisms have been proposed, its pathogenesis has remained obscure. Results of recent research suggested that the oxidative damage might play a role in cataract formation (4). However, the relative contribution of the oxidant and antioxidant factors to this process is yet to be determined.

In the present study, SOD activities of clear, and cataractous lenses at different stages of maturation were investigated in an effort to elucidate its possible involvement in cataractogenesis.

## MATERIALS AND METHODS

Twenty immature (4 subcapsular, 10 cortical and 6 nuclear) and twelve mature cataractous lenses were obtained from patients (18 men and 14 women, age  $64 \pm 4$  year) who underwent intracapsular cataract extraction for senile cataract. Clear lenses were

obtained from age - and sex - matched fresh cadavers, maximum six hours postmortem. The lenses were stored at  $-20\text{ C}$ , and assayed within one week of collection.

The lenses were thawed, and capsula and cortex were removed from nucleus by using a Graefe knife; so that two samples were prepared from each lens: 1) capsula and cortex, and 2) nucleus.

Wet weights of samples were determined by sensitive mechanic scale (Gebr, Bosch S200, Germany). The samples were then homogenized with distilled water by using a homogenizer (B. Braun Melsungen AG, Type 853 202, W Germany). The suspension was added same volume of ethanol: chloroform (5:3, v/v), and centrifuged at  $15000 \times g$  for 15 minutes. Aliquots removed from the resulting supernatant were assayed for SOD activity.

Protein was determined according to Lowry et al. (5). The nitroblue tetrazolium (NBT) method of Sun et al. (6) was used to determine SOD activity. Amount of protein required to inhibit NBT reduction by 50 % was defined as one unit (U) of SOD activity.

Results were expressed as mean  $\pm$  standart deviation (SD), and Student's t test was used for statistical analyses of significance.

Table 1. SOD activities in clear and cataractous human lenses

Lens type	Enzyme activity (U/mg protein)		
	Total	Cortical	Nuclear
Clear (n=5)	$0.487 \pm 0.153$	$0.642 \pm 0.205$	$0.332 \pm 0.201$
Immature cataractous (n=20)	$0.408 \pm 0.214$	$0.505 \pm 0.239$	$0.311 \pm 0.189$
Subcapsular (n=4)	$0.418 \pm 0.226$	$0.479 \pm 0.206$	$0.329 \pm 0.213$
Cortical (n=10)	$0.409 \pm 0.219$	$0.521 \pm 0.276$	$0.297 \pm 0.179$
Nuclear (n=6)	$0.397 \pm 0.197$	$0.514 \pm 0.245$	$0.305 \pm 0.182$
Mature cataractous (n=12)	$0.229 \pm 0.103$	$0.356 \pm 0.141$	$0.102 \pm 0.065$

## RESULTS

The mean SOD activity in the clear lens group was measured to be  $0.487 \pm 0.153$  U/mg protein whilst it was  $0.408 \pm 0.214$  U/mg protein, and  $0.229 \pm 0.103$  U/mg protein in the immature and mature cataractous lens groups, respectively. No difference of mean SOD activity was noted between subtypes of immature lenses ( $p > 0.05$ ). However, our results indicated significantly lower SOD activity in mature cataractous lenses as compared to that of the clear lenses ( $p < 0.05$ ).

Although the results presented in Table 1 revealed a gradual decrease in activity with maturation of cataract, the difference between immature and mature cataractous lenses was not statistically significant ( $p > 0.05$ ). The differences between mean SOD activities of subtypes of immature cataractous lenses were also found to be statistically insignificant ( $p > 0.05$ ). When comparing the activity in the nucleus with that of the cortex, significantly decreased activities were found in all the groups ( $p < 0.05$ ).

## DISCUSSION

The ocular lens is continuously exposed to ultraviolet light. This may initiate photochemical reactions which ultimately lead to intraocular generation of free radicals (7). It is well known that the lens contains a very effective antioxidant system (8). Since the lens has a limited protein turnover capacity, especially in the nucleus, enzymes of this system play an important role in preventing the accumulation of modified proteins. However, preventive capacity of the antioxidant system decreases with aging (9), and thereby the lens becomes more susceptible to oxidative damage. Thus, the oxidation of protein sulfhydryl groups results in intra and intermolecular disulfide bond formation, and increased cross-linking leading to high molecular weight protein aggregate formation, and cataractogenesis. The modification of lipids in the lens membrane can also be an additional cataractogenic mechanism (10).

In the present study, SOD activities of cataractous lenses were found to be significantly lower

than those of the clear ones. There has been much controversy in the literature concerning the mechanism of decreased SOD level in cataractous lenses. Fecondo and Augusteyn (11) proposed the possibility of increased reactive species of  $O_2$  causing inhibition of antioxidant enzymes. In contrary, Bhuyan et al. (12) suggested that impaired enzymatic defences lead to the accumulation of reactive species of  $O_2$ . Taken in conjunction with our previous findings (13) which indicated an increased xanthine oxidase activity in cataractous lenses, the results of the present study favours for Fecondo and Augusteyn's opinion.

In all lenses, SOD activity in the nucleus was lower than those measured in the cortex. Since nucleus has older and relatively inactive cells as compared with cortex, this may reflect a general aging phenomenon which has been observed with several enzymes and metabolites in various animal lenses (11). Increased SOD consumption due to limited protein turnover in the nucleus can be an additional factor responsible for the reduced SOD activity.

As one can see from the magnitude of the standard deviations in relation to the mean SOD activities, a wide range of enzyme activities were obtained in the same type of lenses. This may reflect the inadequacies of the classification system used (immature vs mature), but, more likely, they are attributable to individual differences of the antioxidant status in the lenses studied.

It is difficult to compare the SOD activities obtained in this study with those from other laboratories (11,15,16) because of the differences in assay methods. However, our ratio of SOD activity in normal to cataractous in lens types are comparable to that obtained by Bhuyan et al. (12).

Results of the present study support the hypothesis that oxidative stress might play a role in senile cataractogenesis. Our results also suggest antioxidant capacity of the lens decreases as the opacity increases. However, it is not clear for the time being whether the oxidative stress is the single initiating factor or not.

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