Total Oxidant Status, Total Antioxidant Capacity and Oxidative Stress Index Levels in Patients With Mature and Immature Senile Cataract

Matür ve İmmatür Senil Kataraktlı Hastalarda Total Oksidatif Durum, Total Antioksidan Kapasite ve Oksidatif Stres İndeksi Düzeyleri

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ÖZ

Amaç: Matür ve immatür senil kataraktlı hastaların serum ve aköz humörlerinde total oksidatif durum (TOS), total antioksidan kapasite (TAC), oksidatif stres indeksi (OSI) değerlerini araştırmak.

Gereç ve Yöntemler: Katarakt cerrahisi yapılan 34 hasta çalışmaya dahil edildi. Hastalar matür (Grup 1, n=17) ve immatür (Grup 2, n=17) kataraktlı olmak üzere 2 gruba ayrıldı. Tüm hastalardan serum ve aköz humör örnekleri alındı ve spektrofotometrik olarak ölçüldü. Serum ve aköz TOS, TAC ve OSI değerleri 2 grup arasında karşılaştırıldı. İstatistiksel analiz için Kolmogorov-Smirnov testi ve Mann-Whitney U testi kullanıldı.

Bulgular: Ortalama TOS değeri grup 1 ve 2'de sırasıyla 508.7 ± 45.9 ve 245.6 ± 15.8 olup aradaki fark istatistiksel olarak anlamlıydı (p=0.0001). Ortalama TAC değeri Grup 1'de 2.28 ± 0.6 , Grup 2'de 3.83 ± 2.1 olup, istatistiksel anlamlılık saptandı (p=0,005). Ortalama OSI değeri ise Grup 1 ve 2'de sırasıyla 13.9 ± 4.1 ve 10.4 ± 1.4 bulundu ve Grup 1 de OSI değeri istatistiksel olarak daha yüksekti (p=0.005). Serum TOS, TAC ve OSI değerleri gruplar arasında istatistiksel olarak farklı değildi.

Sonuç: Matür kataraktlı hastaların aköz humörleri artmış oksidatif stres ve azalmış antioksidan kapasite ile karakterizedir. Artmış oksidatif stresin senil katarakt patogenez ve matürasyonunda rolü olduğunu düşünmekteyiz.

Anahtar Kelimeler: Aköz humor, katarakt, serum, oksidatif stres

ABSTRACT

Purpose: To investigate the total oxidant status (TOS), total antioxidant capacity (TAC) and oxidative stress index (OSI) of the aqueous humor and serum in patients with mature and immature senile cataract.

Materials and Methods: Cataract surgery patients (n=34) were enrolled in the study. We included 17 patients with mature cataracts (Group 1) and 17 patients with immature cataracts (Group 2). Samples of the aqueous humor and serum were taken from all patients and measured spectrophotometrically. Serum and aqueous TOS, TAC and OSI levels were compared in the two groups. The Kolmogorov-Smirnov test and Mann-Whitney U test were used for statistical analyses.

Results: The mean TOS level in Group 1 and 2 patients was 508.7 ± 45.9 and 245.6 ± 15.8, respectively, which is a statistically significant difference (p=0.0001). The mean TAC level in Group 1 and 2 was 2.28 ± 0.6 and 3.83 ± 2.1, respectively. This difference was statistically significant (p=0.005). The mean OSI in Group 1 and 2 was 13.9 ± 4.1 and 10.4 ± 1.4, respectively, with the mean OSI level statistically higher in Group 1 (p=0.005). The differences in mean TOS, TAC and OSI levels in the serum were not statistically significant.

Conclusion: The aqueous humor of mature cataract patients is characterised by increased oxidative stress and decreased antioxidant capacity. The increased oxidative stress may play a significant role in the pathogenesis and maturation of senile cataracts.

Keywords: Aqueous humor, cataract, serum, oxidative stress

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INTRODUCTION

Age-related cataract is one of the leading causes of visual impairment and reversible blindness among older people worldwide.[1] According to the statistics of the Ministry of Health, Labour and Welfare of Japan in 2010, nearly 40% of medical expenses in ophthalmology were related to the treatment of cataracts in patients over 65 years of age.[2] It is important to determine the causes while considering the prevention of cataracts.

The effects of oxidative stress have been studied since the 19th century when the concept of oxidation-reduction reactions with reactive oxygen species (ROS) was introduced. ROS—including such physiologic substances as superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen—contain highly reactive oxygen atoms.[3] The aqueous humor (AH) is known to contain several active oxidative agents such as H_2O_2 and superoxide anions.[4] Under physiological conditions, free radicals are actively neutralized by the antioxidant system that naturally occurs in the AH.[5,6] It is now well established that excess levels of ocular oxidants can cause damage to the lens and other tissues, which are implicated in normal aging and in the pathogenesis of several eye diseases such as pseudoexfoliation syndrome, diabetic retinopathy, glaucoma and senile cataract (SC).[7]

Oxidative stress can be defined as an increase in oxidants and/or a decrease in antioxidant capacity. For many decades researchers have studied many markers of oxidative stress and antioxidant defense, including measurement of enzymatic antioxidants (superoxide dismutase, catalase, glutathione peroxidase etc.) and nonenzymatic antioxidants (carotenoids, tocopherols, ascorbate, bioflavonoids, bilirubin, uric acid etc) and measurement of ROS production (superoxide anion, hydroxyl radical, hydrogen peroxide etc).[8] Concentrations of different oxidant-antioxidant markers can be measured in laboratories separately, but the measurements are time-consuming, labor-intensive and costly and require complicated techniques. Hence, TAC considers the cumulative effect of all antioxidants present in blood and body fluids. TOS also represents the cumulative action of oxidants and their synergistic interaction. Furthermore, redox balance between oxidation and antioxidation can be determined by the ratio percentage of the TOS to TAS, regarded as OSI.[9,10]

In the present study, we aimed to measure the levels of TOS, TAC and OSI in the aqueous humor and serum of patients with SC for exploring the role of oxidative stress in cataract formation. Additionally, we extended these investigations to determine if there was a correlation between TOS, TAC, and OSI levels with the cataract maturity.

MATERIALS AND METHODS

The eyes of 34 patients admitted for cataract surgery were included in this prospective, non-randomized study. This study was performed in accordance with the Declaration of Helsinki and informed consent was obtained from all patients. All patients underwent a complete ophthalmologic examination that included medical history, visual acuity, slit-lamp biomicroscopy with and without dilatation, applanation tonometry, and dilated funduscopy. The clinical type and maturity of the cataracts were determined by routine ophthalmic examination. The cataracts were graded according to the Emery and Little classification based on the degree of cataract hardness.[11] The immature cataract group (n=17) had 11 nuclear, 2 posterior subcapsular, 1 cortical and 3 mixed opacities cases; the remaining cases (n=17) included white mature cataract. Patients with pre-existing ocular disease or past ocular surgery including laser therapy were excluded from this study. Patients were excluded if they had systemic disease (diabetes mellitus, kidney disease, hypertension, etc) or were taking medications such as vitamin A, C, E and nonsteroidal anti-inflammatory drugs that may affect general oxidative stress.

Sample Collection

Samples of blood and AH collected from all participants on the day of surgery. Venous blood samples were placed on ice and centrifuged within 1 hour (3500 x g at 4șC for 10 min), and the supernatants were stored at -20 șC until analysed. At the beginning of the surgery, about 0,1- 0,2 ml of the AH was aspirated from the anterior chamber with a 26 gauge insulin syringe under an operating microscope with special care to avoid contamination. Samples were stored immediately at -80 șC until biochemical analysis. No complications occurred at any step of the AH sampling.

Biochemical Determinations of TAC, TOS and OSI

Measurement of Total Antioxidant Capacity: Serum TAC was evaluated using a novel automated method developed by Erel.[12] This method produces hydroxyl radicals, which are the most potent biological radical. In the assay, ferrous ion solution in reagent 1 is mixed with hydrogen peroxide present in reagent 2. Sequentially-produced radicals such as the brown dianisidinyl radical cation produced by the hydroxyl radical are also potent radicals. This method measures the antioxidative effect of the sample against potent free-radical reactions that are initiated by the hydroxyl radical. The assay has excellent precision values of more than 97%. The results are expressed in mmol Trolox equiv/L.

Measurement of Total Oxidant Status: Serum TOS was evaluated using a novel automated method developed by Erel.[13] Oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by the glycerol molecules that are abundant in the reaction medium. Ferric ions generate a coloured complex with xylenol orange in an acidic medium. Colour intensity, which can be measured spectrophotometrically (V-530; Jasco®, Tokyo, Japan), is related to the quantity of oxidant molecules present in the sample. The assay is calibrated with

hydrogen peroxide, and the results are expressed as micromolar hydrogen peroxide equivalents per litre (μmol H_2O_2 equiv/L).

Determination of Oxidative Stress Index: The OSI was defined as the ratio of TOS to TAC levels. For calculations, TAC units were changed to mmol/L, and the OSI was calculated according to the following formula.[12,13] OSI (arbitrary units)=TOS (μmol H_2O_2 equiv/L)/ TAC (mmol Trolox equiv/L).

Statistical Analysis

Statistical analysis was performed using SPSS for Windows version 20.0 (SPSS, Chicago, IL, USA). The Kolmogorov-Smirnov test was used to test the normality of data distribution. The data were expressed as arithmetic means ± standard deviations or median, minimum and maximum values. TOS, TAC and OSI levels in the aqueous humor and serum were compared statistically by using Mann-Whitney U test in the two groups taking into account the number of patients. A two-sided p value of <0.05 was considered statistically significant.

RESULTS

We studied 17 eyes with mature senile cataracts (Group 1) and 17 eyes with immature senile cataracts (Group 2) in patients undergoing phacoemulcification surgery. The mean ± SD age of the Group 1 (66.4 ± 8.3 years) and Group 2 (68.3 ± 8.7 years) did not differ statistically (Student's t-test p= 0.68). The demographic and clinical features of the Group 1 and 2 are presented in Table 1. Thirty-four AH and blood samples were obtained from 34 patients with either mature or immature SC.

A significant increase in TOS was found in the aqueous humor of Group 1 (508.7 ± 45.9 μmol H_2O_2 equiv/L) versus Group 2 (245.6 ± 15.8 μmol H_2O_2 equiv/L; p<0.001). The TAC levels in the aqueous humor were 2.28 ± 0.6 mmol Trolox equiv/L in Group 1 and 3.83 ± 2.1 mmol Trolox equiv/L in Group 2. The mean TAC levels between the groups were significantly different (p=0.005). The OSI (ratio of TOS/TAC) was 13.9 ± 4.1 and 10.4 ± 1.4 in Groups 1 and 2, respectively. This metric was significantly higher in Group 1 patients than Group 2 (p=0.005). These data are shown in Table 2.

Table 1. Demographics and clinical characteristic of the patient groups

Demographics and clinical characteristic	Group 1 (n=17)	Group 2 (n=17)	p
Age (years)	66.4 ± 8.3	68.3 ± 8.7	0.68
Female/male (n)	9/8	10/7	0.72

Group 1 includes patients with mature cataract; Group 2 includes patients with immature cataract. p<0.05

Table 2. Total oxidant status, total antioxidant capacity and oxidative stress index levels in the aqueos humor of the patients

AH measurements	Group 1 (n=17)	Group 2 (n=17)	p
TOS			
mean±SD	508.7 ± 45.9	245.6 ± 15.8	0.0001
median(min-max)	521.2 (226.2 -1041.9)	256.3 (141.2 – 341.9)	
TAC			
mean±SD	2.28 ± 0.6	3.83 ± 2.1	0.005
median(min-max)	3.77 (1.38 – 7.84)	2.32 (1.17 – 3.58)	
OSI			
mean±SD	13.9 ± 4.1	10.4 ± 1.4	0.005
median(min-max)	12.4 (9.65 – 25.5)	10.4 (8.49 – 14.7)	

TOS total oxidant status (μmol H_2O_2 equiv/L) , TAC total antioxidant capacity (mmol Trolox equiv/L), OSI oxidative stress index, AH aqueous humor, min: minimum, max: maximum. p<0.05

When blood samples were compared between the groups, the average TOS levels were 104.9±27.8 in Group 1 and 100.9±26.6 in Group 2. There was no significant difference between groups in terms of TOS levels (p=0.75). The mean TAC of the serum was 1.12 ± 0.1 and 1.19 ± 0.2 in Group 1 and 2, respectively. Although the mean TAC value in Group 1 was lower than Group 2, the difference was not statistically significant (p=0.29). Blood OSI levels were found to be 9.59 ± 2.8 in Group 1 and 8.92 ± 2.4 in Group 2. There was no significant difference found in OSI levels between the groups (p=0.55) (Table 3).

Table 3. Total oxidant status, total antioxidant capacity and oxidative stress index levels in the serum of the patients

Serum measurements	Group 1 (n=17)	Group 2 (n=17)	p
TOS			
mean±SD	104.9 ± 27.8	100.9 ± 26.6	0.75
median (min-max)	104.4 (41.6 – 146.2)	93.5 (59.6 – 151.6)	
TAC			
mean±SD	1.12 ± 0.1	1.19 ± 0.2	0.29
median (min-max)	1.11 (0.92 – 1.32)	1.15 (0.88 – 1.98)	
OSI			
mean±SD	9.59 ± 2.8	8.92 ± 2.4	0.55
median (min-max)	9.57 (3.59 – 14.5)	8.61 (5.52 – 15.1)	

TOS total oxidant status, TAC total antioxidant capacity, OSI oxidative stress index, min:minimum, max: maximum. p<0.05

DISCUSSION

Cataract formation represents a serious problem in the elderly with approximately 25% of the population aged >65 years and about 50% aged >80 years experiencing a serious loss of vision as a result of this condition.[14] Oxidative damage to the lens plays a significant role in the pathogenesis of many forms of cataracts. A significant proportion of lenses and aqueous humor taken from cataract patients have elevated H_2O_2 levels.[15] DNA damage in the lens epithelial cells and progression of nuclear cataracts were reported by ROS and ultraviolet light exposure in senile cataract patients.[16,17]

Multiple reports have found about oxidative stress in ocular disease in the literature, but these patients had generally diabet or glaucoma together with cataract. Latarya et al.[18] found that AH phophatase levels in patients with glaucoma were lower than cataract. Uzun et al reported that increased glutathione peroxidase and catalase activity and decreased TAC in the AH of patients with pseudoexfoliation.[19] Additionally, Beyazyıldız et al showed that increased TOS levels and decreased TAC levels in diabetic retinopathy patients.[20] In our study, our patients had only cataract and we found that increased TOS and OSI levels and decreased TAC levels in AH in progressed senile cataract. Cataract and other systemic or ocular diseases share oxidative stress components in their pathophysiology.

This study showed that cataract maturity has a significant impact on the levels of AH oxidants and antioxidants. There are several studies in the literature demonstrating that cataract formation increases AH oxidant status and decreases AH antioxidant capacity.[4,21-23] Miric et al.[7] measured SOD and catalase, which are antioxidant enzyme, in order to study the relationships between cataract maturity and AH enzymatic antioxidants. They found that SOD and catalase levels were significantly low in mature cataract. However, Sawada et al.[3] found that SOD activity and protein concentrations were higher in progressed nuclear cataract. They claimed that the different results of SOD activity were related with difference of biochemical techniques. Beyazyıldız et al.[24] found that there was no influence of cataract grades on the TOS, TAC and OSI of the patients with pseudoexfoliation. As a result of their study, the differences in the TAC and TOS were related to pseudoexfoliation rather than cataract. In the present study, AH TOS and OSI levels were higher and TAC levels were lower in the mature cataracts when compared to the immature cataracts (Figure 1). Although the parameters studied are different, the results of these studies and the present study are parallel, as all showed that increased oxidative stress markers and decreased antioxidants in senile cataracts.

In some studies, oxidative stress and decreased antioxidant defences were observed in the serum of patients with glaucoma or diabetes.[5,6,25,26] Kirboğa et al.[25] indicated that serum TOS levels in patients with diabetic retinopathy were significantly higher than those in the control group, while the total thiol and TAC levels were lower. But there was no study found in the literature assessing the TOS, TAC and OSI levels in the serum of patients with senile cataract. Our results reveal that the mean serum TOS and OSI levels in the mature cataract group were higher than those in the immature cataract group. The mean serum TAC levels were lower. There was no significant difference between the two groups regarding TOS, TAC and OSI levels in the serum. Our patients had no other ocular or systemic diseases. However, the serum parameters are not very specific to cataracts because there are active homeostatic mechanisms at hemato-camerular barrier. Oxidative stress in the AH might be much more important than systemic oxidative stress at predicting cataracts.

In conclusion, we report decreased TAC and increased TOS-OSI level in the AH from patients with mature senile cataracts. Although this study is limited by the small sample size, the results suggest that oxidative stress can be associated with maturation of senile cataracts. Further work is needed to elucidate whether there exists an association between oxidative stress and maturation of senile cataract.

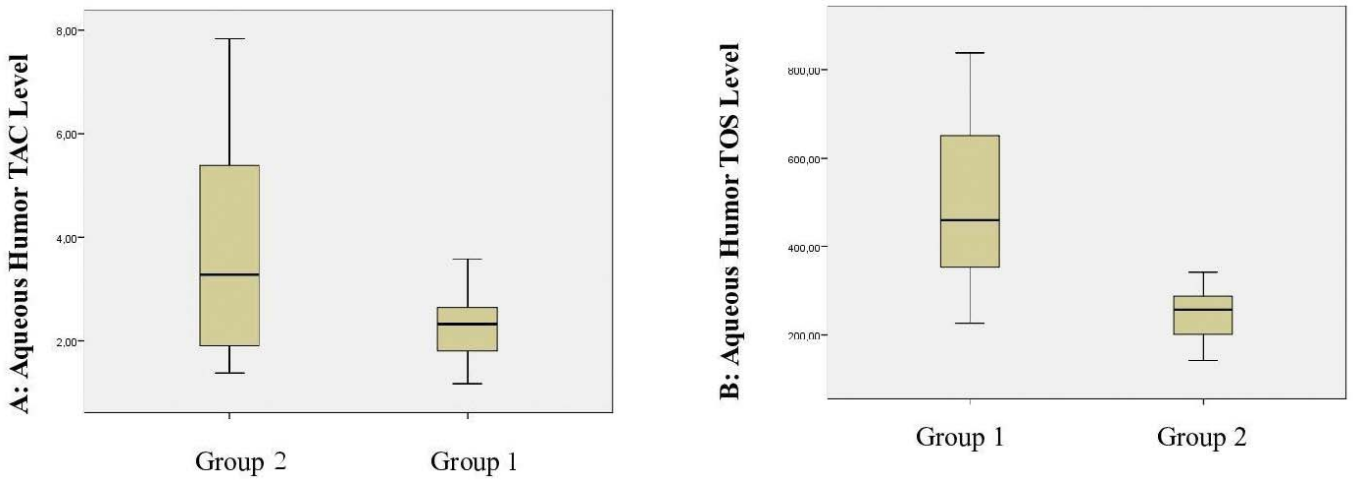

Figure 1: *TAC and TOS levels in AH of groups. A: TAC levels B: TOS levels*

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