Evaluation of Serum Superoxide Dismutase Activity, Malondialdehyde, and Zinc and Copper Levels in Patients With Keratoconus

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Purpose: The aim of this study was to evaluate the relationship between antioxidant superoxide dismutase (SOD) enzyme activity, malondialdehyde (MDA) as a lipid peroxidation marker, and some trace elements such as zinc (Zn) and copper (Cu) levels in patients with keratoconus.

Methods: A total of 58 patients with keratoconus and 53 control subjects with similar age and sex were evaluated in this study. The modified Krumeich keratoconus classification was used to divide the patients into 4 stages. Serum SOD activity, MDA, and zinc and copper levels were compared between the patient and control groups.

Results: The median serum SOD activity, MDA, and Zn and Cu levels were 27.2 (42.4–13.7) U/mL, 10.2 (11.9–8.5) nmol/mL, 87.9 (104.6–76.5) μmol/L, and 103.2 (117.9–90.3) μmol/L in the keratoconus group and 26.2 (32.5–14.4) U/mL, 8.8 (11.4–7.1) nmol/mL, 100.5 (121.1–81.8) μmol/L, and 98.4 (120.3–83.4) μmol/L in the control group, respectively. There was a statistically significant difference between the MDA and Zn levels of the keratoconus group and control subjects but not between the respective SOD activities or Cu levels ($P = 0.016$, $P = 0.031$, $P = 0.440$, and $P = 0.376$, respectively). We found no significant difference between the keratoconus group stages for serum SOD activity, serum MDA, and Zn and Cu levels ($P > 0.05$), and there was also no significant correlation between the keratoconus group stages and serum SOD activity, serum MDA, and Zn and Cu levels ($P > 0.05$).

Conclusions: There is imbalance in the systemic oxidant/antioxidant status where Zn deficiency also plays a role in patients with keratoconus.

Key Words: keratoconus, malondialdehyde, oxidative stress, superoxide dismutase, zinc

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Keratoconus is one of the most common indications for penetrating keratoplasty.[1,2] This progressive degenerative disease affects approximately 1/2000 persons, mostly young individuals, and can cause scarring, irregular astigmatism, and impaired visual acuity. The disorder is characterized by corneal thinning, leading to progressive protrusion.[3] Histopathological features include iron deposition in basal layers of the corneal epithelium, thinning of the epithelium, breaks in Bowman layer, compaction of stromal collagen lamellae, breaks in Descemet membrane, and endothelial cell loss.[3,4] The disorder is multifactorial with unclear etiology and both genetic and environmental factors playing a role.[3,5] It is frequently seen together with other disorders such as eye rubbing, atopy, vernal keratoconjunctivitis, Down syndrome, retinitis pigmentosa, Leber congenital amaurosis, Marfan syndrome, mitral valve prolapse, osteogenesis imperfecta, and Ehlers–Danlos syndrome.[3]

Oxidative stress has been shown to play a critical role in the pathogenesis of keratoconus.[6–8] Antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase, and reductase are normally able to prevent cell damage by reactive oxygen species (ROS) in the normal cornea. However, accumulation of ROS such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals in keratoconus can cause corneal damage without being prevented by antioxidant defense systems.[6,7] These corneas have lower levels of both enzymatic and nonenzymatic antioxidants such as SOD, class 3 aldehyde dehydrogenase, and glutathione while the catalase levels are increased.[7,8] Inducible nitric oxide synthase activity, nitrotyrosine, and malondialdehyde (MDA) levels are also increased.[6]

Lipid peroxidation develops in response to ROS accumulation and causes production of highly reactive electrophilic aldehyde species. MDA is an aldehyde used as a biomarker in lipid peroxidation.[9]

We are not aware of any studies on the relationship between keratoconus and MDA levels. The aim of this study was to evaluate the relationship between antioxidant SOD enzyme activity, MDA as a lipid peroxidation marker, and some trace elements such as zinc (Zn) and copper (Cu) levels in patients with keratoconus.

MATERIALS AND METHODS

A total of 111 subjects recruited from the ophthalmology clinics of Ahi Evran University Training and Research

Hospital and Ulucanlar Eye Training and Research Hospital were enrolled in this study. The sample size consisted of approximately 10% of patients with keratoconus who presented to our clinics every year. At first, clinical archives were reviewed and all cases who had no disorders other than keratoconus found. Random sampling was then performed. The patients were then invited to the clinics and informed about the study. Volunteering patients with keratoconus were then included in the study. The study population consisted of 58 patients with keratoconus and 53 control subjects. Age-matched healthy volunteers with no ocular diseases or refractive errors were selected randomly as the control group. Consent was obtained from the local ethics committee, and the study was conducted according to the Declaration of Helsinki. The participants provided written voluntary informed consent.

Full ophthalmic examination and corneal topography were performed in all participants, and keratoconus was diagnosed according to the Collaborative Longitudinal Evaluation of Keratoconus study criteria.[10] The Pentacam Scheimpflug imaging system was used for corneal topography and evaluated as discussed previously.[11] We used the modified Krumeich keratoconus classification to divide the patients into 4 stages, with the stage of the more advanced eye used for bilateral cases.[12] Patients with an ocular disorder such as glaucoma, corneal trauma, corneal scarring, uveitis, or severe dry eye, suffering from any systemic disorder, systemic inflammation or clinical condition, or other findings of zinc deficiency or currently using antiinflammatory or antioxidant/vitamin treatment were excluded from the study.

Blood Sample Collection

After drawing blood from the patients and control subjects, we quickly separated the serum from the cells by centrifugation for 10 minutes at 3000*g* and then kept the samples at −80°C until they were studied.

SOD Enzyme Activity

We measured T-SOD enzyme activity as previously described,[13] based on measuring the absorbance increase at 560 nm caused by NBT reduction to NBTH2. One unit of SOD activity was the amount of enzyme protein that led to 50% inhibition in the reduction rate of NBTH2. The results were expressed in units per milliliter.

Measurement of Serum MDA Levels

We performed TBARS assay using the thiobarbituric acid (TBA) method as described by Van Ye et al[14] in their 1993 article. We used the reaction with TBA at 90 to 100°C to determine the MDA level. The principle is that MDA or similar substances react with TBA and produce a pink pigment that has an absorption maximum at 532 nm. To ensure protein precipitation, the sample is mixed with cold 20% (wt/vol) trichloroacetic acid and the precipitate is then centrifuged to form a pellet. An aliquot of the supernatant is placed into an equal volume of 0.6% (wt/vol) TBA in a boiling water bath for 30 minutes. After cooling, sample and blank absorbance are read at 532 nm and the results expressed as nanomole per milliliter, based on a graph where 1,1,3,3-tetramethoxypropane has been used as the MDA standard.

Measurement of Serum Zinc and Copper Levels

The stored samples were allowed to equilibrate to room temperature before the assay and then diluted with deionized water (1:10). Zinc and copper levels were analyzed using the atomic absorption spectrophotometer (Shimadzu-AA7000F; Japan) at 214 and 324 nm, respectively. Rat serum–based standards with known Zn and Cu concentration were used for calibration. Quality assurance was with the certified reference serum as the quality control.

Statistical Analysis

We used SPSS software version 22.0 for data analysis. Serum SOD activity, MDA, and Zn and Cu levels in the keratoconus and control groups were compared with the Mann–Whitney *U* test. The Kruskal–Wallis test and Spearman correlation test were other statistical tests used. Post hoc statistical power analysis was calculated with GPower 3.0.10 software (Franz Faul, University of Kiel, Kiel, Germany). A P value less than 0.05 was the criterion for statistical significance.

RESULTS

Fifty-eight patients with keratoconus consisted of 35 women and 23 men, and 53 control subjects consisted of 34 women and 19 men with the mean age of 25.4 ± 7.3 and 25.3 ± 7.1 years, respectively and no significant difference between the groups in terms of sex or age ($P = 0.680$ and $P = 0.822$, respectively). The number of stage 1, 2, 3, and 4 keratoconus patients was 19, 19, 12, and 8, respectively. Post hoc power analysis results were 85%, 80%, 49%, and 42% for Zn, MDA, SOD, and Cu, respectively. A limitation of this study was the small sample size of SOD and Cu according to post hoc power analysis.

There was a statistically significant difference between the MDA and Zn levels of the keratoconus group and control subjects but not between the respective SOD activities or Cu levels ($P = 0.016$, $P = 0.031$, $P = 0.440$, and $P = 0.376$, respectively, and the results are shown in Table 1). We found

TABLE 1. Median SOD Activities, Levels of MDA, and Concentration of Serum Trace Elements in Patients With Keratoconus in Comparison With Control Subjects

Parameters	Keratoconus	Control	*P*
SOD, U/mL	27.2 (42.4–13.7)	26.2 (32.5–14.4)	0.440
MDA, nmol/mL	10.2 (11.9–8.5)	8.8 (11.4–7.1)	0.016*
Zn, μmol/L	87.9 (104.6–76.5)	100.5 (121.1–81.8)	0.031*
Cu, μmol/L	103.2 (117.9–90.3)	98.4 (120.3–83.4)	0.376

*Statistically significant.

no significant difference between the keratoconus group stages for serum SOD activity, serum MDA, and Zn and Cu levels ($P = 0.695$, $P = 0.548$, $P = 0.492$, and $P = 0.929$, respectively), and there was also no significant correlation between the keratoconus group stages and serum SOD activity, serum MDA, and Zn and Cu levels ($P = 0.695$, r = 0.053; $P = 0.979$, r = −0.004; $P = 0.895$, r = 0.018; and $P = 0.802$, r = 0.035, respectively).

DISCUSSION

ROS are produced endogenously as a result of normal cellular activity, mostly in the mitochondria. High ROS levels can cause cellular damage by reacting with DNA, lipids, and proteins. However, living systems have enzymatic and nonenzymatic cellular defense mechanisms that limit ROS levels and effects. These defense mechanisms are made up of antioxidant molecules such as vitamin C and vitamin E and antioxidant enzymes such as SOD, catalase, and glutathione peroxidase. Normally, ROS cannot overcome cellular defense mechanisms and reach levels that could cause cellular damage. However, imbalance between ROS production and antioxidant defense systems such as excessive production of ROS after exposure to ionizing or ultraviolet radiation can lead to increased levels of modified lipid and protein molecules and DNA, and also cellular damage related to modulation of gene and protein expression and alteration of DNA polymerase activity.[15]

Elevated levels of ROS can induce lipid peroxidation, leading to a variety of highly reactive electrophilic aldehyde species such as MDA, 4-hydroxy-trans-2-nonenal, and acrolein. These aldehydes in turn react with cellular proteins, DNA, and RNA in a concentration-dependent manner, activating signaling cascade molecules and transcription factors and causing inflammation.[16] They have been found to be associated with many inflammatory disorders induced by oxidative stress such as neurodegenerative diseases, metabolic syndrome, diabetes mellitus, and cancer and are also thought to play a role in age-related macular degeneration and diabetic retinopathy.[16–18] MDA has also been used as a biomarker of lipid peroxidation for many years.[9]

The cornea is constantly exposed to light and UV in a wide spectrum, making it particularly susceptible to oxidative stress. Buddi et al[6] have demonstrated increased MDA levels in keratoconus corneas and thought that this could play a role in keratoconus pathogenesis. Our study is the first to investigate the relationship between systemic MDA levels and keratoconus. Serum MDA levels were found to be high in these studies, demonstrating that systemic MDA levels are also high in these patients and indicating the presence of systemic oxidative stress in addition to localized elevation in keratoconus corneas. However, there are also studies reporting no difference in total serum oxidant levels in patients with keratoconus.[19–21]

SOD catalytically converts superoxide radical to oxygen and hydrogen peroxide. It therefore prevents ROS accumulation and is an important component of the antioxidant defense system. The cornea has a strong antioxidant system because of its constant UV exposure, and SOD is part of this system. Kenney et al[7] have found similar RNA levels and protein distribution for CuZnSOD for keratoconus and normal corneas. Another study has found low extracellular SOD levels but similar CuZnSOD and MnSOD levels in keratoconus corneas compared with normal corneas.[22] Many gene studies on the relationship between keratoconus and CuZnSOD have reported various results with no clear consensus.[23] We are aware of only one study on SOD levels in patients with keratoconus, and high plasma SOD levels have been reported.[24] However, we did not find a significant difference between the keratoconus and control groups regarding serum SOD levels in our study.

Zinc and Cu are essential elements that play a fundamental role in activation of a wide range of enzymes. Dudakova et al[25] have reported that Cu imbalance could affect keratoconus development. However, our study and a previous study have found no difference in systemic Cu levels in patients with keratoconus compared with the control group.[24] Zn deficiency is very common. Clinical manifestations include growth retardation, male hypogonadism in adolescents, diarrhea, alopecia, rough skin, impaired immunity, delayed wound healing, neurosensory disorder, and cognitive impairment.[26] Inadequate Zn consumption can also increase sensitivity to oxidative stress by causing changes in antioxidant defense systems. Antioxidant functions of Zn are performed through various mechanisms and one of these is being incorporated in the structure of CuZnSOD.[27] CuZnSOD is an important enzyme for oxidative stress. Many studies have emphasized that oxidative stress can develop due to decreased CuZnSOD following Zn deficiency. However, in addition to studies reporting decreased CuZnSOD activity with Zn deficiency, there are some that report no change.[28,29] Some studies also report increased CuZnSOD activity and increased antioxidant defense to compensate in cases of oxidative stress developing due to decreased antioxidant defense following Zn deficiency.[30,31] Another study has reported decreased Zn and increased SOD levels in the plasma of patients with keratoconus.[24] Comparison of CuZnSOD levels in keratoconus corneas with healthy corneas has revealed no significant difference.[7,22] In addition, Zn deficiency also causes increased MDA levels in the blood.[28] We found decreased Zn and increased MDA levels in the serum. This result, interpreted in light of previous studies, indicates that ROS can normally be easily eliminated with strong antioxidant mechanisms in the cornea but oxidative stress developing due to imbalance in the systemic oxidant/antioxidant status can contribute to keratoconus development. It is therefore possible that a Zn-rich diet may slow down progression of the disorder in patients with keratoconus. In this study, patients with keratoconus with any other findings of zinc deficiency were excluded. We therefore believe the relationship between keratoconus and clinical manifestations of Zn deficiency should be evaluated in future studies.

This study is the first to report systemic MDA levels in patients with keratoconus, and elevated serum MDA levels have been found. In conclusion, there is imbalance in the systemic oxidant/antioxidant status where Zn deficiency also plays a role in patients with keratoconus. This result needs to be verified with further studies and larger sample sizes.

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