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Original Article

Oxidative Stress Levels in Aqueous Humor from High Myopic Patients

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Purpose: To compare oxidative stress status in the aqueous humor of highly myopic eyes and control eyes.

Methods: Aqueous humor samples were collected from 15 highly myopic eyes (high myopia group) and 23 cataractous eyes (control group) during cataract surgery. Central corneal thickness, corneal endothelial cell density, hexagonality of corneal endothelial cells, and cell area of corneal endothelial cells were measured using specular microscopy. Axial length was measured using ultrasound biometry. 8-Hydroxydeoxyguanosine (8-OHdG) and malondialdehyde levels were measured using enzyme-linked immunosorbent assay.

Results: 8-OHdG level was lower in the aqueous humor of myopic patients than in that of control group ($p = 0.014$) and was positively correlated with central corneal thickness and negatively correlated with axial length (r = 0.511, $p = 0.02$; r = -0.382, $p < 0.001$). There was no correlation between 8-OHdG level and corneal endothelial cell density, hexagonality, or cell area. Malondialdehyde level did not show any correlation with any parameters evaluated.

Conclusions: 8-OHdG might be a sensitive biomarker for evaluating oxidative stress status in the eye. Oxidative stress level was lower in the aqueous humor of highly myopic eyes compared to that in control eyes, which indicates lower metabolic activity in these eyes.

Key Words: 8-Hydroxydeoxyguanosine, Axial eye length, Corneal pachymetry, High myopia, Malondialdehyde

Oxidative stress is involved in the pathogenesis of many eye diseases, including dry eye syndrome, cataract, and age-related macular degeneration [1-3]. Oxidative stress impairs cellular function by damaging lipids, proteins, RNA, and DNA, in addition to causing inflammation and cell death [4,5]. 8-Hydroxydeoxyguanosine (8-OHdG), an oxidized DNA nucleoside, results from the oxidation of guanine and is excreted in bodily fluids [6,7]. 8-OHdG is one of the most widely used biomarkers of cellular oxidative stress [6]. Malondialdehyde (MDA) is an endogenous genotoxic product of enzymatic and oxygen radical-induced lipid peroxidation [8].

Aqueous humor is continually produced by the ciliary body and is in direct contact with the anterior surface of the lens, iris, and corneal endothelial cells, before draining out of the eye via the trabecular meshwork [8,9]. Oxidative stress in aqueous humor reflects the balance among the oxidative states of the lens, corneal endothelium, and retina. Corneal endothelial cells are in direct contact with the aqueous humor [9] and can therefore secret their metabolic

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products directly into the aqueous humor, whereas metabolic products from lens epithelial cells and the retina must diffuse through the anterior lens capsule and anterior hyaloids membrane to reach the aqueous humor [10].

High myopia has been reported to be associated with various complications and is defined as a long axial length (AXL) [11,12]. Retinal degeneration is one of the major complications leading to severe vision loss [11,12]. Many causes of high myopic retinal degeneration, including oxidative stress and retinal ischemia, have been suggested [11,13]. An association between AXL and cytokine levels in aqueous humor has also been reported [14-16]. However, to the best of our knowledge, oxidative stress level in the aqueous humor of patients with high myopia has not yet been evaluated.

In this study, we investigated the oxidative stress status of aqueous humor in patients with high myopia and corresponding controls.

Materials and Methods

This comparative control study investigated aqueous humor levels of 8-OHdG and MDA in 15 highly myopic eyes and 23 control eyes. Highly myopic eyes were defined as those with myopia of -6 diopters (D) or more or AXL 26 mm or longer. In the controls, aqueous humor samples were collected from senile cataract patients free from other ocular or systemic diseases. The study protocol complied with the provisions of the Declaration of Helsinki and was reviewed and approved by the institutional review board/ethics committee of Hallym University Medical Center, Seoul, Korea. Patients were enrolled from the Ophthalmic Centers in Hallym University Kangnam Sacred Heart Hospital between July and December 2010.

All patients underwent a complete ophthalmic examination, including refraction, measurements of AXL and best-corrected visual acuity, indirect stereoscopic ophthalmoscopy, fluorescein angiography, and color fundus photography. Central corneal thickness (CCT), corneal endothelial cell density, hexagonality of corneal endothelial cells, and cell area of corneal endothelial cells were measured using specular microscopy. AXL was measured using ultrasound biometry (CineScan; Quantel Medical, Bozeman, MT, USA). The Lens Opacities Classification System II, which is based on photographic standards, was used to assess cataracts [17,18]. Slit-lamp microscopic examinations (BM-900; Haag-Streit, Koeniz, Switzerland) were performed by board-certified ophthalmologists or ophthalmologists in training.

Sample collection

In all patients, anterior chamber paracentesis was performed before incision during cataract surgery, and no steroids were administered before surgery. Undiluted aqueous humor samples were collected in sterile tubes and stored at -80°C until analysis.

Measurement of 8-OHdG and malondialdehyde using enzyme-linked immunosorbent assay

The aqueous humor levels of 8-OHdG and MDA were measured using the commercially available competitive 8-OHdG ELISA kit (Cayman Chemical, Ann Arbor, MI, USA) and MDA sandwich ELISA kit (Cell Biolabs Inc., San Diego, CA, USA), according to the respective manufacturer's instructions. Briefly, for the 8-OHdG assay, aqueous humor samples were incubated with 8-OHdG-acetylcholinesterase conjugate and an 8-OHdG monoclonal antibody in an enzyme immunoassay plate that had been pre-coated with goat anti-mouse antibodies. The enzyme immunoassay plate was incubated, washed, and developed with Ellman's reagent, and absorbance was evaluated at 410 nm. For the MDA assay, aqueous humor samples were added to the microtiter plate precoated with an anti-MDA antibody. After incubation at 37°C for 1 hour and extensive washing, the plate was incubated for 1 hour with 100 μL of a biotinylated mouse anti-MDA antibody, followed by incubation for 1 hour with 100 μL of streptavidin-peroxidase conjugate. Then, TMB/E (3,3′,5,5′-tetramethylbenzidine) was added to each well and incubated for 5 to 10 minutes, after which the absorbance of plate was measured at 450 nm in a multi-plate reader (Spectramax Plus 384 plate reader; Molecular Devices, Sunnyvale, CA, USA). Serial dilutions of recombinant human 8-OHdG and MDA served as standards.

Statistics

Experimental data are expressed as mean ± standard deviation. The results were analyzed using the Mann-Whit-

ney *U*-test and Spearman's correlation test. All statistical analyses were performed using SPSS ver. 14.0 (SPSS Inc., Chicago, IL, USA). A *p*-value less than 0.05 was considered significant.

Results

Fifteen highly myopic eyes of 10 patients (high myopia group) and 23 cataract eyes of 23 patients (control group) were included in this study (Table 1). The mean age was 61.3 ± 10.3 years in the highly myopic group and 66.1 ± 12.1 years in the control group. The male to female ratio was 5 : 10 in the highly myopic group and 6 : 17 in the control group. The mean refractive error (spherical equivalents) was -10.27 ± 3.78 D in the highly myopic group and +0.61 ± 1.66 D in the control group. Nucleosclerosis grade was not different between the control group (2.27 ± 0.70) and the highly myopic group (2.27 ± 0.68; $p = 0.777$, Mann-Whitney *U*-test). Intraocular pressure was also not different between the control group (13.78 ± 2.70 mmHg) and the highly myopic group (15.00 ± 2.98 mmHg; $p = 0.314$, Mann-Whitney *U*-test).

Oxidative stress marker levels in aqueous humor and axial length

The levels of 8-OHdG and MDA in aqueous humor are shown in Fig. 1A-1D. 8-OHdG levels were lower in the high myopia group compared to the control group ($p = 0.014$, Mann-Whitney *U*-test) (Table 2). MDA level was not significantly different between the control and high myopia groups. The level of 8-OHdG was dependent on AXL (r = -0.382, $p < 0.001$, Spearman's correlation coefficient). MDA level did not correlate with AXL.

Correlations between 8-OHdG and ocular parameters

The level of 8-OHdG correlated positively with CCT (r = 0.511, $p = 0.02$, Spearman's correlation coefficient) (Fig. 2A). There was no correlation between 8-OHdG level and corneal endothelial cell density, hexagonality, or cell area (Fig. 2B-2D).

Correlations between malondialdehyde and ocular parameters

MDA level did not show any correlation with CCT, AXL, corneal endothelial cell density, hexagonality, or cell area (Fig. 3A-3D).

Discussion

Oxidative stress has been reported to be associated with various ocular diseases [11-16,19,20]. 8-OHdG has been used as a biomarker for oxidative DNA damage [20]. In this study, 8-OHdG level in aqueous humor was positively associated with CCT and negatively associated with AXL, whereas it was not associated with the measured parameters of corneal endothelial cells. These results suggest that 8-OHdG level in aqueous humor reflects the

Table 1. Demographic data

	Control	High myopia group	*p*-value
Number	23	15	
Age (yr)	66. 1 ± 12.1	61.3 ± 10.3	0.213
Male : female	6 : 17	5 : 10	
Spherical equivalent (diopter)	+0.61 ± 1.66	-10.27 ± 3.78	<0.001*
Axial length (mm)	23.1 ± 0.6	28.2 ± 2.2	<0.001*
Central corneal thickness (μm)	525.8 ± 24.4	497.6 ± 24.8	0.002*
Endothelial cell count (/mm^2)	2,442.1 ± 292.3	2,572.3 ± 359.9	0.119
Hexagonality (%)	52.4 ± 10.7	59.3 ± 18.9	0.551
Cell area (μm^2)	415.4 ± 51.9	397.5 ± 68.0	0.112

Values are presented as number or mean ± standard deviation.
*Statistically significant by Mann-Whitney *U*-test.

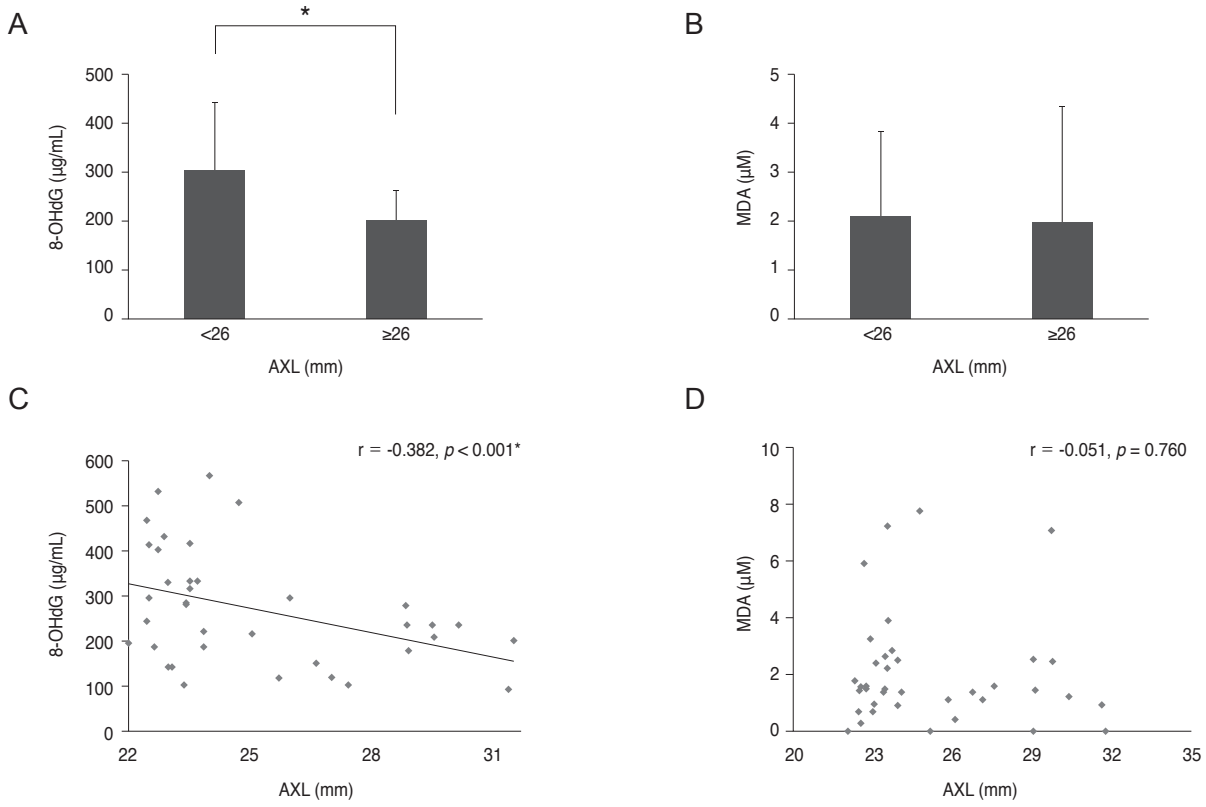

Fig. 1. Oxidative stress marker levels in aqueous humor and axial length (AXL). (A) 8-Hydroxydeoxyguanosine (8-OHdG) level in aqueous humor was lower in the high myopia group compared to the control group (p = 0.014, Mann-Whitney U-test). (B) Malondialdehyde (MDA) levels were not different between control and high myopia groups. (C) 8-OHdG level decreased depending on AXL (r = -0.382, p < 0.001, Spearman's correlation coefficient). (D) MDA level did not correlate with AXL. *Statistically significant.

Table 2. Oxidative stress marker levels in aqueous humor of control and high myopia groups

	Control (n = 23)	High myopia group (n = 15)	p-value
8-OHdG level (μg/mL)	311.6 ± 127.7	212.5 ± 103.2	0.014*
MDA level (μM)	2.1 ± 1.7	1.9 ± 2.4	0.224

Values are presented as mean ± standard deviation.
8-OHdG = 8-hydroxydeoxyguanosine; MDA = malondialdehyde.
*Statistically significant by Mann-Whitney U-test.

oxidative stress status of the whole eye, not only the corneal endothelium. Differences in CCT between myopia and emmetropia remain controversial [21]. It has been reported that CCT does not correlate with degree of myopia [22]. Conversely, negative correlation between refraction degree and CCT has been reported [23]. In this study, CCT was thinner in patients with high myopia than it was in the controls.

High myopia is a degenerative disease [14]. A variety of cytokines present in aqueous humor have been reported to be related to high myopia [14-16]. Elevated levels of matrix metalloproteinase-2 (MMP-2), tissue inhibitor of MMP-1 (TIMP-1), TIMP-2, and TIMP-3 have been found in eyes with elongated axes [14]. Concentration of transforming growth factor-β2 has been shown to positively correlate with AXL [16]. Conversely, concentration of vascular endothelial growth factor in aqueous humor has been negatively correlated with AXL [15]. High myopia results in retinal pigment epithelium degeneration [12]. It has been suggested that the metabolic activity in high myopia deceases due to retinal pigment epithelium degeneration [24-26]. Ultraviolet light-blocking glasses worn to correct the myopia could reduce the oxidative stress level in the aqueous humor.

Free radical production leading to oxidative stress is an initiating factor in the development of maturity-onset cataract [27,28]. Elevated level of MDA has been found in the cataract lenses and vitreous of myopic patients compared with non-myopic patients with cataract [29]. αA-crystallin,

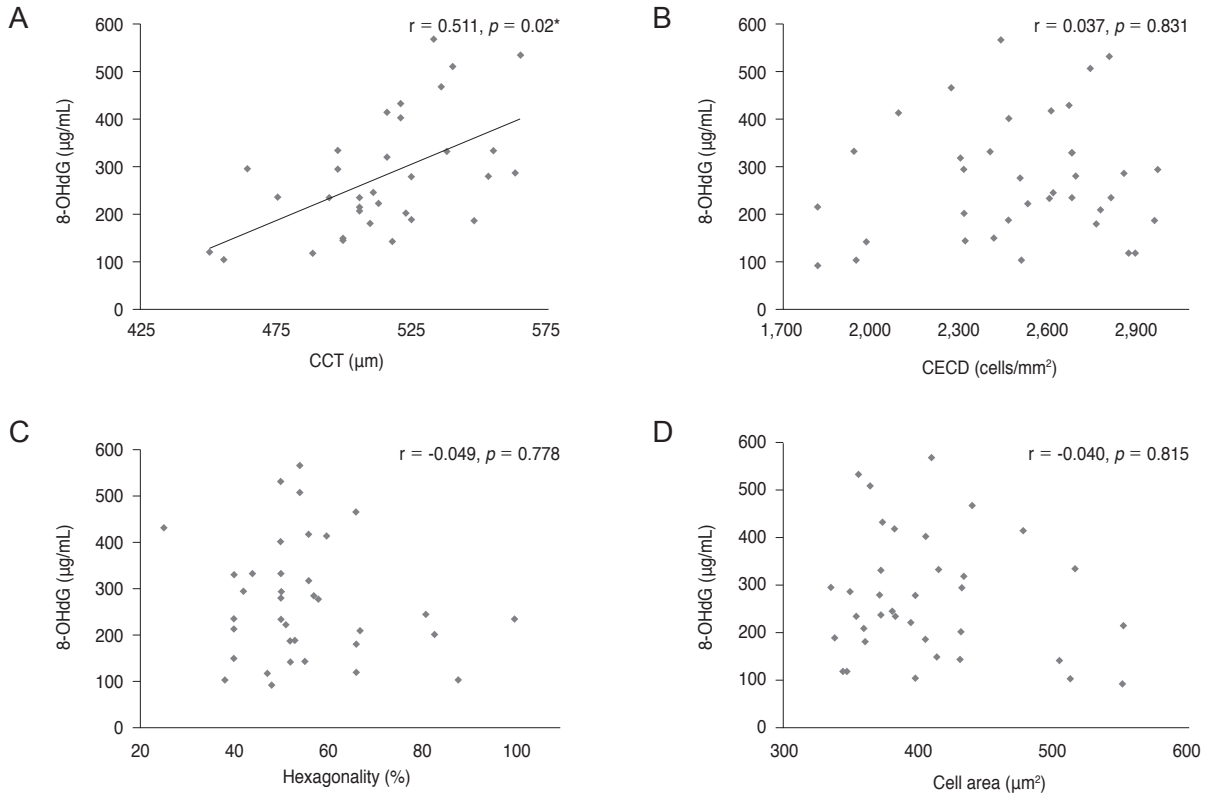

Fig. 2. Correlation between 8-hydroxydeoxyguanosine (8-OHdG) and corneal endothelial cells. (A) 8-OHdG level was positively correlated with central corneal thickness (CCT) (r = 0.511, $p = 0.02$, Spearman's correlation coefficient). (B) 8-OHdG level did not correlate with corneal endothelial cell density (CECD). (C) 8-OHdG level did not correlate with cell hexagonality. (D) 8-OHdG level did not correlate with cell area. *Statistically significant by Spearman's correlation coefficient test.

the best-characterized structural protein of the human lens, acts as a chaperone under conditions of oxidative stress, thereby maintaining lens transparency [30]. High myopia has been reported as a risk factor for dark nuclear cataract, and CpG islands in the crystallin alpha A promoter are hyper-methylated in lens epithelial cells of patients with high myopic dark nuclear cataract [31]. However, the role of oxidative stress in high myopia is not well understood. In contrast to other studies [32], this study found that 8-OHdG level was lower in the highly myopic group compared to the control group and was negatively correlated with AXL. Reduced metabolism in degenerative high myopia [33] might lead to a decrease in oxidative stress level. Aqueous humor level of 8-OHdG was found to be higher in patients with exudative age-related macular degeneration, and the level correlated with macular lesion area [19]. One serious complication of high myopia causing vision loss is choroidal neovascularization [34]. Oxidative stress can be localized around choroidal neovascularization areas in high myopia patients. Furthermore, high myopia has been suggested as a risk factor for glaucoma [35]. Aqueous humor level of 8-OHdG increases and total antioxidant status decreases in the serum and aqueous humor of glaucoma patients [20]. Glaucoma associated with high myopia might be different from that with normal AXL. Age is an important factor for oxidative stress in eyes [2]. Cataract is a common cause of increased oxidative stress in aqueous humor [2]. However, in this study, cataract status was not different between the control and high myopia groups. One limitation of this study is that the comparison of 8-OHdG level in the aqueous humor between the high myopia group and non-cataract emmetropia group was not performed according to ethnics. However, further study is necessary to compare 8-OHdG level in the aqueous humor between age-matched high myopia and non-cataract emmetropia groups in order to support our conclusions.

In contrast, there were no significant differences in MDA level between the two groups. The production of MDA results from oxidative damage of lipids by free radi-

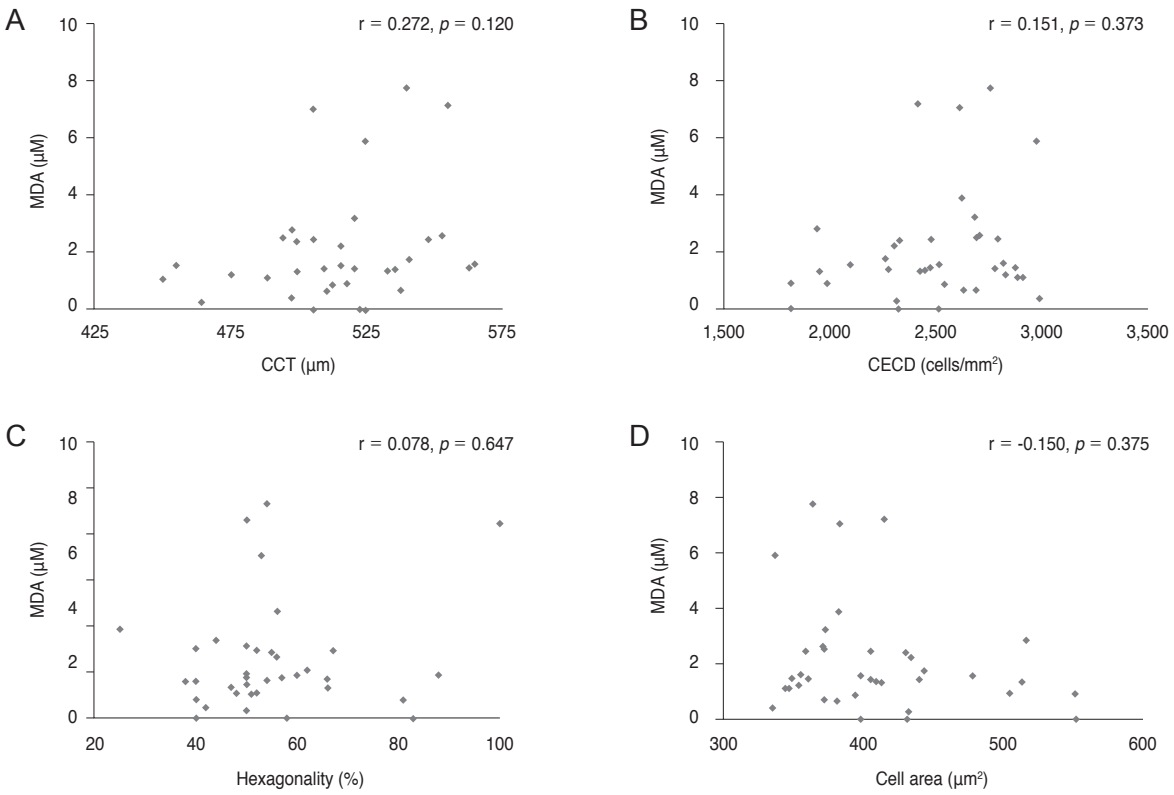

Fig. 3. Correlation between malondialdehyde (MDA) and corneal endothelial cells. (A) MDA level did not correlate with central corneal thickness (CCT). (B) MDA level did not correlate with corneal endothelial cell density (CECD). (C) MDA level did not correlate with cell hexagonality. (D) MDA level did not correlate with cell area.

cals [36]; therefore, MDA level is frequently used as an indicator of oxidative damage to lipids resulting from free radicals [37]. There might be a potential reason for the differences between the 8-OHdG and MDA results. The molecular weight of 8-OHdG (283 g/mol) is higher than that of MDA (72 g/mol); thus, it is possible that 8-OHdG is not able to be washed out into the trabecular meshwork but is retained in the anterior chamber. A previous study showed that 50% of 0.18-μm particles were caught in the trabecular meshwork and in the juxtacanalicular tissue [38]. In general, molecules having a molecular weight less than 500 g/mol are able to permeate the anterior chamber [39]. With increasing age, increased cross-linking might occur, leading to accumulation of extra-cellular material in the juxtacanalicular tissue, increased outflow resistance, and development of glaucoma [40]. Another reason for 8-OhdG accumulation is that is a very stable product of oxidative DNA damage following enzymatic cleavage after reactive oxygen species-induced 8-hydroxylation of guanine base in mitochondrial and nuclear DNA [41]. The half-life of MDA is very short in the human body [42]. MDA has a room-temperature half-life in plasma of approximately 2 hours.

In this study, 8-OHdG level was evaluated only in aqueous humor. However, 8-OHdG level in the vitreous might be more reflective of the oxidative stress level in the posterior segment, although a correlation between aqueous humor and vitreous levels has been reported [19]. Further study is necessary to compare 8-OHdG levels in vitreous of patients with high myopia.

In conclusion, 8-OHdG, a biomarker of oxidative stress, was found at lower levels in myopic patients compared to the control group. 8-OHdG level positively correlated with CCT and negatively correlated with AXL. There was no correlation between MDA level and CCT or AXL. 8-OHdG could be a sensitive marker for the evaluation of oxidative stress status of the eye. Oxidative stress level was lower in eyes with high myopia, which indicates lower metabolic activity in highly myopic eyes.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgements

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