CLINICAL SCIENCE

The role of oxidative stress and antioxidants in the pathogenesis of age-related macular degeneration

Zuhal Yildirim,¹ Nil Irem Ucgun,¹ Filiz Yildirim¹

¹Etimesgut Public Health Laboratory, Ankara, Turkey. ²Second Ophthalmology Clinic, Ankara Numune Education and Research Hospital, Ankara, Turkey. ³Duatpepe Government Hospital, Clinic of Internal Medicine, Polatlı, Ankara, Turkey.

OBJECTIVE: To investigate the role of oxidant/antioxidant status and protein oxidation in the development of age-related macular degeneration.

METHOD: The activities of serum superoxide dismutase and glutathione peroxidase and the levels of serum malondialdehyde, advanced oxidation protein products, glutathione and vitamin C were measured in 25 patients with age-related macular degeneration and 25 control subjects without age-related macular degeneration.

RESULT: The malondialdehyde and advanced oxidation protein product levels in the serum were significantly higher in the age-related macular degeneration patient group than in the control group (p<0.05). The superoxide dismutase activity in the serum was significantly lower in the age-related macular degeneration patient group than in the control group (p<0.05). The levels of vitamin C and glutathione and the activity of glutathione peroxidase in the serum were unchanged between groups (p>0.05).

CONCLUSION: The results of the present study suggest that decreased effectiveness of the antioxidant defense system and increased oxidative stress may play a role in the pathogenesis of age-related macular degeneration.

KEYWORDS: Oxidative stress; Antioxidants; Protein oxidation; AMD.

INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of irreversible visual impairment and blindness among people aged 60 years and older.¹ Although the disease presents a serious social and economic problem, its pathogenesis and etiology are still unclear.²⁻⁴ Based on the disease’s clinical and pathological features, there are two subtypes of late AMD: atrophic (dry) and neovascular (wet), both of which can lead to significant visual loss.⁵

Over the past decade, the body of literature regarding the modifiable factors associated with AMD has grown considerably and includes cigarette smoking, age,⁶ nutritional factors,⁶⁻⁸ obesity⁹ and insufficient antioxidants in the diet.¹⁰

The eye is an exceptional organ because of its continuous exposure to environmental chemicals, radiation, and atmospheric oxygen.¹¹ These oxidative stresses have been implicated in the possible pathophysiology of various ocular diseases, such as AMD, cataracts, glaucoma, uveitis, and pseudoexfoliation syndrome. Reactive oxygen species (ROS) are involved in this process. Several ocular degenerative disorders have been studied, and the presence of oxidative stress has been demonstrated through markers of lipid peroxidation, the activity of antioxidant enzymes, and the levels of low-molecular-weight antioxidants.¹²

Non-enzymatic lipid peroxidation is an example of a free radical-associated process through which oxidative stress promotes cellular damage. Serum malondialdehyde (MDA) is the end product of the primary reactions that lead to the significant oxidation of such polyunsaturated fatty acids in cellular membranes and, thus, serves as a reliable marker of oxidative stress.¹³ Protein oxidation is currently considered to be an important factor in a variety of diseases, such as Alzheimer’s and Parkinson’s diseases, cancer, hypertension, cardiovascular disease, diabetes, ischemia-reperfusion injury and aging.¹⁴⁻¹⁵

Advanced oxidation protein products (AOPP) are described as dityrosines that contain cross-linked protein products. Importantly, this definition excludes protein aggregates that form as a result of disulfide links following low-level oxidative stress. Therefore, the presence of AOPP may be a better and more accurate marker of oxidative stress than lipid peroxidation products.¹⁶

Endogenous antioxidants, including such non-enzymatic scavengers as glutathione (GSH) and such antioxidant enzymes as superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT), are the first lines of
defense against oxidative stress and act by scavenging potentially damaging free radical moieties.\textsuperscript{17}

Ascorbate (vitamin C) is the most effective aqueous-phase antioxidant found in human blood. Increasing evidence suggests that, within the aqueous phase, vitamin C plays a vital role in the antioxidant defense mechanism of the eye, protecting ocular tissues against photooxidative damage by acting as a free radical scavenger.\textsuperscript{18}

Determining the level of oxidative stress encountered by the human eye has not yet been attempted in clinical diagnoses and/or treatment.\textsuperscript{19} The aim of the present study was to investigate the role of antioxidants and protein and lipid peroxidation in the development of AMD. Therefore, we measured the activities of the SOD and GPx enzymes and the serum levels of MDA, AOPP, GSH and vitamin C in patients with AMD and in control subjects not exhibiting AMD.

**MATERIALS AND METHODS**

This study included 15 men and 10 women (mean age ±SD; 65.16 ± 13.96 years) with AMD and choroidal neovascular (CNV) membrane, which is secondary to AMD. The control group included 15 men and 10 women (mean age ±SD; 65.72 ± 12.62 years). No statistically significant differences between the groups were observed in terms of age and sex. Patients with other ophthalmic conditions (e.g., glaucoma, uveitis, psoedexfoliation syndrome, other progressive retinal diseases) and systemic diseases (e.g., diabetes, arthritis, coronary arterial disease, peripheral vascular disease) were excluded.

All subjects, both in the control group and the patient group, completed a questionnaire confirming the following information: age, gender, non-smoker status, non-consumption of supplements, such as vitamins and/or antioxidants. All patients with AMD were first tested to ensure that they did not ingest antioxidant vitamins and minerals. After obtaining blood samples from the patients, we initiated anti-vascular endothelial growth factor therapy.

All patients underwent a comprehensive ophthalmic examination. CNV was diagnosed by slit-lamp biomicroscopy of the fundi, color fundus photographs, fundus fluorescein angiographies, and optical coherence tomography of the fundi, color fundus photographs, fundus.

**RESULTS**

DISCUSSION

AMD is a complex, multifactorial disease of aging for which several theories of pathogenesis have been proposed,
including oxidative damage\textsuperscript{25} and ocular perfusion abnormalities.\textsuperscript{26}

Oxidative stress may cause injury to the retinal pigment epithelium (RPE), the Bruch’s membrane, and the choroid, which are layers in the eye involved in the pathophysiology of AMD.\textsuperscript{27-31}

Liang et al.\textsuperscript{32} demonstrated that human RPE cells exposed to oxidative stress or rod outer segments exhibited damage primarily to mitochondrial (mt) DNA and that damaged mtDNA was not efficiently repaired.

The retina is particularly susceptible to oxidative stress due to its high concentration of oxygen, its high proportion of polyunsaturated fatty acids, and its exposure to visible light.\textsuperscript{33} Prior reports have suggested that the retina is susceptible to lipid peroxidation\textsuperscript{34,35} and that this susceptibility also increases with aging in the macular region.\textsuperscript{35}

Previous studies demonstrated that the plasma MDA levels were higher in an AMD patient group than in a control group.\textsuperscript{36,37} In the present study, we found that the serum MDA levels were significantly higher in the AMD patient group than in the control group, in agreement with a previous study.\textsuperscript{38}

Protein oxidation is also a useful marker for the evaluation of oxidative stress in vivo. Many different types of protein oxidative modifications can be induced by free radicals. A prior study demonstrated that the levels of protein carbonyl groups in the serum were higher in the AMD patient group than in the control group.\textsuperscript{38}

AOPP measurements reflect the generation of free radicals and the degree of protein oxidation.\textsuperscript{39,40} In the present study, we found that the levels of AOPP in the serum were significantly higher in the AMD patient group than in the control group. Therefore, this study demonstrated that protein oxidation, a useful oxidative stress marker, is upregulated in AMD, suggesting increased oxidative stress.

Antioxidant enzymes are a primary defense system that protects biological macromolecules from oxidative damage. SOD, CAT and GPx are antioxidant enzymes that form part of the complex system that protects the retina from oxidative damage, and all three of these enzymes are found in the photoreceptors and the RPE.\textsuperscript{41} SOD is a key antioxidant enzyme involved in the metabolism of oxygen free radicals.\textsuperscript{41} A previous report suggested that the activities of SOD in plasma and erythrocytes were lower in the AMD patient group than in the control group.\textsuperscript{42} In the present study, we found that the activities of SOD in the serum were significantly lower in the AMD patient group than in the control group.

| Table 1 - Oxidation markers are down-regulated in AMD patients and Control Subjects (mean ± SD). |
|---------------------------------|-----------------|-----------------|
| AMD group (n = 25) | Control group (n = 25) |
| SOD (U/mL) | 4.99 ± 3.09* | 9.91 ± 4.42 |
| GPx (U/mL) | 12.97 ± 11.23 | 15.79 ± 11.20 |
| MDA (nmole/L) | 6.39 ± 1.80* | 4.90 ± 1.97 |
| AOPP (umole/L) | 251.89 ± 69.58* | 181.43 ± 43.11 |
| GSH (nmole/mL) | 310.05 ± 102.51 | 331.03 ± 129.45 |
| Vitamin C (umole/L) | 2.54 ± 1.50 | 3.14 ± 1.54 |

\*p < 0.05, as compared with the control group.

In conclusion, increased oxidative stress, which causes oxidative damage to lipids and proteins and decreases antioxidant capacity, may lead to irreversible damage in the form of AMD. Further studies that analyze samples obtained both from the serum and the aqueous humor are required to confirm our findings.

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