SERUM MDA AND AOPP LEVELS IN AGE RELATED MACULAR DEGENERATION
Yaşa bağlı makula dejenerasyonunda serum MDA ve AOPP düzeyleri

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Abstract

Purpose: *Our aim was to reveal advanced oxidation protein products (AOPP), novel marker of oxidative stress as markers of protein oxidation, as well as malondialdehyde (MDA) levels as a marker of lipid peroxidation in age-related macular degeneration (AMD).*

Material and Methods: *Serum MDA and AOPP levels were measured in 27 patients with AMD and 10 healthy subjects. Serum MDA levels and oxidative stress biomarker, AOPP levels, were measured spectrophotometrically.*

Results: *When compared with healthy controls, MDA levels were found to be significantly higher in AMD patients ($p<0.001$); serum AOPP levels of patients with AMD did not show a statistically significant difference ($p>0.05$).*

Conclusion: *Increased MDA levels could indicate that lipid peroxidation might be implicated in the pathophysiology of AMD.*

Key Words: *Malondialdehyde, protein oxidation, age related maculopathy*

Özet

Amaç: *Bu çalışmada yaşa bağlı makula dejenerasyonu (AMD) hastalarında protein oksidasyonunun yeni bir belirleyicisi olan ileri okside protein ürünleri (AOPP) düzeyi ile lipid peroksidasyon göstergesi olan malondialdehit (MDA) düzeylerinin ölçülmesi amaçlandı.*

Materyal metod: *Serum MDA ve AOPP düzeyleri 27 AMD'li hasta ve 10 sağlıklı bireyde ölçüldü. Serum MDA ve AOPP düzeylerinin ölçümünde spektrofotometrik yöntem kullanıldı.*

Bulgular*: MDA düzeyleri, hasta grubunda anlamlı derecede yüksek bulundu ($p<0.001$); AOPP düzeyleri açısından hasta ve kontrol grupları arasında istatistiksel olarak anlamlı fark bulunmadı ($p>0.05$).*

Sonuç: *AMD hastalarında tespit edilen yüksek MDA düzeyleri, bu hastalığın patogenezinde lipid peroksidasyonunun rol oynayabileceğini göstermektedir.*

Anahtar Kelimeler: *Malondialdehit, protein oksidasyonu, yaşa bağımlı makülopati*

Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly population in the western world. At present, the exact cause of AMD remains unknown. Several risk factors have been elucidated, including smoking (1), atherosclerosis (2), increased plasma fibrinogen (3), and low levels of antioxidant vitamins (4) and antioxidant enzymes (5). An improved understanding of the aetiology and pathogenesis of AMD at the cellular and biochemical level is crucial to developing improved treatment and hopefully preventing this disease.

Free radicals are produced continuously, mainly by mitochondrial electron transport, enzymes such as xanthine oxidase and aldehyde oxidase, inflammation, xenobiotic metabolism and hyperoxia (6). The free radical theory of aging proposes that reactive oxygen species (ROS) cause oxidative damage over the life of the subject. It is the cumulative and potentially increasing amount of accumulated damage that accounts for the dysfunctions and pathologies seen in normal aging

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Geliş tarihi 18 Kasım 2003

(7). Several authors have suggested that the free radical hypothesis of aging applies to AMD in particular, because the central area of the neurosensory retina and the underlying retinal pigment epithelium may be susceptible to the cumulative toxic effects of continued photic damage (8, 9). It has been suggested that circulating ROS could damage the choriocapillaris and lead to AMD (10). During aging, the balance between the generation of ROS and ROS clearence can be disturbed resulting in oxidative damage to macromolecules such as lipids and proteins (11).

ROS-mediated oxidation of cell membrane lipids leads to the formation of lipid peroxidation products such as MDA (12). Oxidative modification of proteins in vivo may affect a variety of cellular functions involving proteins: receptor, signal transduction mechanism, transport system, and enzymes (13). The oxidative damage to proteins is reflected by increased levels of advanced oxidation protein products (AOPP), novel markers of oxidative stres (14).

The etiopathogenesis of AMD has not yet been clarified. To our knowledge, serum AOPP levels have not been investigated in AMD. Our aim was to reveal advanced oxidation protein products (AOPP), novel marker of oxidative stres, as markers of protein oxidation, as well as MDA levels as a marker of lipid peroxidation in AMD.

2. MATERIALS AND METHODS

2.1. Subjects
In this study we measured serum MDA and AOPP levels in 27 patients with AMD. Results are compared with age and sex matched 10 healthy controls.

There were 27 patients, ages ranging between 53 to 82 years, (median 68). A group of 10 healthy controls, ages ranging between 48 to 95 years (median 55), were also included in the study. None of the patients or none of those in the control group had any systemic disease or received any mineral or vitamin drugs.

Methods

All reagents were purchased from Sigma and Merck. Blood samples were obtained after an overnight fast and serum immediately seperated. Serum samples were stored at –70 ^{0}C until analysis.

2.2. Measurement of serum MDA concentration

Serum MDA levels were measured by a method described elsewhere (15). The principle of the method was based on the spectrophotometric measurement of the color occurred during the reaction to thiobarbituric acid with MDA. Concentration of thiobarbituric acid reactive substances (TBARS) was calculated by the absorbsance coefficient of malondialdehyde-thiobarbituric acid complex and expressed in nmol/ mL. As a standard MDA bis (dimethyl acethal)-TBA (thiobarbituricacid) complex was used.

2.3. Measurement of serum AOPP levels

AOPP were determinated in the serum spectrophotometrically and calibrated with chloramine-T solutions that, in the presence of potassium iodide, absorb at 340 nm (14). AOPP concentrations were expressed as micromoles of per liter of chloramine-T equivalents per liter of plasma (mmol/L).

2.4. Statistical analyses

Statistical evaluation was carried out with the SPSS® 10.0 (Statistical Packages for Social Sciences; SPSS Inc, Chicago, Illinois, USA). Data obtained from the study groups were compared by the non-parametric Mann Whitney U test; p value less than 0.05 was considered to be statistically significant. The values were presented as medians with their range in table I.

RESULTS
There was no statistically significant difference of age and sex distribution between the patients and control groups ($p>0.05$).

Serum MDA and AOPP levels are detected as 2,68 (1,06-6,15 nmol/mL) (Median (range)) and 210,26 (87,35-468,45 mmol/L) (Median (range)) respectively in the patient group. Serum MDA and AOPP levels are detected as 1.06 (0,62-1,31 nmol/ mL) (Median (range) and 159,77 (92,51-261,23 mmol/L) (Median (range) respectively in the control group.

Serum MDA and AOPP levels are shown on Table 1. When compared to healthy controls, MDA levels were found to be significantly higher in AMD patients ($p<0.001$); but serum AOPP levels of patients with AMD were not statistically different from the controls ($p =0.353$).

Table I. *Serum MDA and AOPP levels of patients with AMD and Controls*

	n	MDA (nmol/mL) Median (range)	AOPP (mmol/L) Median (range)
AMD	*27*	*2,68 (1,06-6,15)*	*210,26 (87,35-468,45)*
Control	*10*	*1,06 (0,62-1,31)*	*159,77 (92,51-261,23)*
p		*p<0.001*	*p =0.353*

DISCUSSION

ROS are molecules which contain an unpaired electron making them higly reactive. ROS are produced in all tissues during aerobic metabolism and they can also be formed by photochemical reactions (11). ROS overwhelms the protective systems and results in cell damage and lipid peroxidation, which play a crucial and perhaps causative role in the pathogenesis of a number of acute and chronic diseases, and also in the pathogenesis of AMD (10, 16).

The eye is at high risk to be damaged by oxidative mechanisms. One major reason is the exposure to light throughout life. It has been suggested that the biochemical composition of posterior segment structures (unsaturated fatty acids) is an important factor making the eye more susceptible to damage than other organs. High polyunsaturated fatty acid content of photoreceptor membranes particularly expose the retina to increased risk of lipid peroxidation by ROS (8, 17).

ROS can attack double bonds in polyunsaturated fatty acids, and thus induce lipid peroxidation; this in turn results in more oxidative damage (18). ROS-mediated oxidation of cell membrane lipids leads to the formation of lipid peroxidation products, such as MDA (12).

In our study, we found high MDA levels in AMD patients compared to controls and this is in accordance with some other studies (19, 20).

The significantly higher concentration of lipid peroxidation product, MDA, in patients with AMD indicates an important pathogenic role of oxido-reduction disturbance.

The vulnerability of proteins to ROS is now well documented (21). Oxidation of amino acid residues such as tyrosine, leading to the formation of dityrosine, protein aggregation, cross-linking, and fragmentation, is an example of ROS-mediated protein damage in vitro. In contrast, evidence for the presence of such oxidatively damaged proteins in vivo and their possible clinical significance was still lacking until recently (22, 23).

To our knowledge, this is the first study for a novel oxidative stress marker of protein, referred to as advanced oxidation protein products (AOPP) in AMD although we could not find a statistically significant difference between the patient and the control group.

This may be due to a relatively small sample size in our study. We are planning to continue our study in larger patient and control groups.

Our study demonstrated that increased MDA levels could indicate an implication of lipid peroxidation in the pathophysiology of AMD.

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