

# Serum malondialdehyde, monocyte chemoattractant protein-1, and vitamin C levels in wet type age-related macular degeneration patients

Ramazan Kürşad Zor<sup>ID</sup>, Serpil Erşan, Erkut Küçük<sup>ID</sup>, Gamze Yıldırım<sup>ID</sup> and İsmail Sarı

## Abstract

**Purpose:** The purpose of this study was to investigate the serum levels of malondialdehyde (MDA) which is a marker of oxidative stress, monocyte chemoattractant protein-1 (MCP-1) which has an important role in inflammation, and vitamin C which has antioxidant properties in patients with wet age-related macular degeneration (wAMD).

**Methods:** Thirty patients with wAMD were included in the study and serum levels of MDA, MCP-1, and vitamin C were compared with healthy participants ( $n = 30$ ). Serum vitamin C and MDA levels were measured using a spectrophotometric method. Serum MCP-1 levels were determined by the ELISA method.

**Results:** MCP-1 and MDA levels were higher in patients with wAMD compared with the control group ( $p < 0.05$ ). Serum vitamin C levels were lower in patients with wAMD compared with the control group ( $p < 0.05$ ).

**Conclusions:** The increase in the MCP-1 levels in patients with wAMD may be associated with increased inflammation in wAMD. Decreased serum vitamin C and elevated MDA levels in patients with wAMD suggest increased oxidative stress in wAMD patients. These results indicate that the increased oxidative stress and inflammation can play a role in the pathogenesis of wAMD.

**Keywords:** age-related macular degeneration, choroidal neovascularization, inflammation, malondialdehyde, monocyte chemoattractant protein-1, oxidative stress

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## Introduction

Age-related macular degeneration (AMD) is a progressive disease caused by abnormalities in the photoreceptor / retinal pigment epithelium / Bruch's membrane / choroid complex.<sup>1</sup> It is the single largest cause of irreversible visual loss in the developed world. It is divided into two as wet type (wAMD) and dry type (dAMD). dAMD is characterized by atrophy of the retinal pigment epithelium (RPE), while wAMD is associated with choroidal neovascularization (CNV) and overexpression of vascular endothelial growth factor (VEGF).<sup>2</sup> AMD is thought to be the result of a complex multifactorial interaction between

metabolic, functional, genetic, and environmental factors. Although aging is the most important risk factor, factors such as nutrition, light, and smoking have been shown to contribute to the pathogenesis of AMD by inducing oxidative stress.<sup>3</sup> The oxidative stress burden of the retina is higher than other tissues due to sunlight exposure and high oxygen concentration.<sup>4</sup> Depending on lifestyle choices as cigarette smoking and other contributing factors such as genetics, this oxidative stress may increase uncontrollably, which results in a high concentration of free oxygen radicals in the environment.<sup>4</sup> Increased oxidative stress causes increased levels of oxidized lipids,

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Correspondence to:  
**Ramazan Kürşad Zor**  
Department of  
Ophthalmology, School  
of Medicine, Niğde Ömer  
Halisdemir University, Bor  
Yolu, Niğde 51100, Turkey.  
[kursadzor@hotmail.com](mailto:kursadzor@hotmail.com)

**Serpil Erşan**  
**İsmail Sarı**  
Department of  
Biochemistry, School of  
Medicine, Niğde Ömer  
Halisdemir University,  
Niğde, Turkey

**Erkut Küçük**  
Department of  
Ophthalmology, School  
of Medicine, Niğde Ömer  
Halisdemir University,  
Niğde, Turkey

**Gamze Yıldırım**  
Department of  
Ophthalmology, Niğde  
Ömer Halisdemir  
Education and Research  
Hospital, Niğde, Turkey



especially from polyunsaturated fatty acids such as phosphatidylcholine on the cell membranes.<sup>4</sup> The peroxidation of the lipids which were required for cellular and metabolic functions in the retina causes the formation of different cleavage products such as malondialdehyde (MDA) and malondialdehyde-acetaldehyde (MAA).<sup>5</sup> Such molecules are very reactive and can interact with various matrix proteins, cellular proteins, and cellular membranes. As a result of this interaction, antigenic molecules are formed and may result in inflammation associated with the wrong targeting of healthy molecules or cells by the immune system.<sup>4</sup> Malondialdehyde (MDA) is a highly reactive three-carbon dialdehyde produced as a product of polyunsaturated fatty acid (PUFA) peroxidation by free radicals and is an important indicator of lipid peroxidation.<sup>6</sup> Little is known about the direct relationship between MDA and AMD. Many of the lipid oxidation products have been shown to be proinflammatory and cytotoxic to photoreceptors and RPE.<sup>7</sup> Oxidative stress and the resulting free oxygen radicals can cause DNA damage in addition to changing the lipid structure. It has been demonstrated that DNA damage can cause inflammatory response in RPE cells in AMD patients.<sup>8</sup> Oxidative stress-mediated inflammatory process has been reported to trigger degenerative and inflammatory cascades in the retina.<sup>9</sup> Inflammatory cells also cause a series of reactions that result in excessive oxidative stress at the inflammation site.<sup>10</sup> Although the exact mechanism cannot be elucidated, inflammation plays a critical role in the development of neovascularization in AMD.<sup>11</sup> Ambati and colleagues<sup>12</sup> demonstrated that the pro-inflammatory cytokine and chemokine panel including interleukin (IL)-1, IL-6, IL-8, tumor necrosis factor (TNF), interferon gamma (IFN $\gamma$ ), monocyte chemoattractant protein-1 (MCP-1) accelerates AMD progression. MCP-1 / CCL2 is a member of a C-C chemokine family and plays an important role in the development of inflammation with its activating and chemotactic effects on monocytes / macrophages.<sup>13</sup> MCP-1, which plays an important role in the mechanism of inflammation, may also contribute the pathogenesis of retinal diseases as well as many other diseases. It is thought that it may be important in the pathogenesis of wAMD due to its angiogenic and inflammatory effects and may be a new target molecule in the treatment.

While reactive oxygen radicals and oxidative stress in the tissues increase in parallel with

aging, antioxidant capacity decreases.<sup>9</sup> The nutritional imbalance can make this situation even worse in terms of antioxidant capacity. In the Age-Related Eye Disease Study (AREDS), the effect of high doses of vitamin C, vitamin E, beta carotene, and zinc on AMD were evaluated and it was concluded that these antioxidant agents decrease the odds of progression to advanced AMD.<sup>14</sup>

Although an important relationship between antioxidants and AMD has been reported, this issue is still controversial in the literature.<sup>15,16</sup>

In this study, we aimed to investigate the serum levels of MDA which is a marker of oxidative stress, MCP-1 which has an important role in inflammation, and vitamin C which has antioxidant properties in patients with wAMD and compare the results with healthy participants.

### Materials and methods

The study was approved by the Ethics Review Board of Niğde Ömer Halisdemir University (Protocol No: 2019/15). Detailed information about the procedure was given to all patients and healthy participants, and written consent was received from each patient and participant before taking blood samples. The study was conducted in accordance with the principles of the Declaration of Helsinki. Approximately 5 mL of venous blood was collected from 30 patients with wAMD and 30 control. Then, these blood samples were centrifuged at 1600  $\times$ g for 10 minutes and serum samples were obtained. Sera were stored at  $-80^{\circ}\text{C}$  until analysis day.

All patients included in the study were older than 50 years of age and axial length was between 22.0 mm and 25.0 mm. Retinal angiomatous proliferation, maculopathy with myopic CNV or CNV due to angioid streaks; patients with glaucoma, uveitis, smoking history, and systemic diseases were excluded from the study. Patients with wAMD in one or both eyes were accepted but patients with wAMD in one eye and geographic atrophy above 175  $\mu\text{m}$  in the other eye were excluded. The diagnosis of wAMD was made by clinical examination, fundus photograph, optical coherence tomography, and fundus fluorescein angiography. Control blood was obtained from 30 age- and gender-matched healthy participants who did not have systemic or ocular disease.

**Table 1.** Demographic data of patients and the control group.

	Patient group (N = 30)	Control group (N = 30)	p
Gender (female/male)	12/18	17/13	0.200
Age (year ± SD)	71.2 ± 9.8	66.1 ± 9.7	0.055
SD, standard deviation.			

### Measurement of serum MDA levels

Malondialdehyde levels (MDA), an end-product of lipid peroxidation, were measured according to the thiobarbituric acid reactive substances (TBARS) method in serum samples from patients and controls. TBARS is a well-established assay for screening and monitoring lipid peroxidation. The standard absorption curve for MDA quantification was prepared using 1,1,3,3-tetraethoxypropane and the values were expressed as mmol/L.<sup>17</sup>

### Serum MCP-1 levels measurement

Human MCP-1 levels were measured with ELISA kit (Elabscience Biotechnology, Wuhan, China) according to instructions of the manufacturers.

### Measurement of serum vitamin C levels

Ascorbic acid is oxidized by copper. Dehydroascorbic acid and diketogulonic acid are formed. These products are treated with 2,4-dinitrophenylhydrazine to form the derivative bis-2,4 dinitrophenylhydrazone. This compound, in strong sulfuric acid, undergoes a rearrangement to form a product with an absorption band that is measured at 520 nm. The reaction is run in the presence of thiourea to provide a mildly reducing medium, which helps to prevent interference from non-ascorbic acid chromogens.

### Statistical analysis

The Statistical Package for the Social Sciences (SPSS) version 16 (SPSS, Chicago, IL) was used for the statistical analysis. Parametric data were evaluated by the independent sample *t*-test and categorical data by the chi-square test. Nonparametric data were evaluated by Mann–Whitney *U* test. A *p* value ≤ 0.05 was considered as significant.

## Results

There were 12 female and 18 male patients in the wAMD group while 17 female and 13 male

patients in the control group. The mean age of the patient group was 71.2 ± 9.8 years, and the control group was 66.1 ± 9.7 years. There was no significant difference between the groups in terms of age (*p* = 0.055) and gender (*p* = 0.200) (Table 1).

Table 2 summarizes the statistical comparison of MCP1, vitamin C, and MDA levels determined in the serum samples of patient and control group. MCP1 median levels were 127.90 pg / mL in the patient group and 84.55 pg / mL in control group. The median MDA levels were 2.14 mmol / mL in the patient group and 0.79 mmol / mL in the control group. Both MDA and MCP1 levels were significantly higher in the patient group than in the control group. When the vitamin C levels of the patient and control groups were compared, it was found to be significantly lower in the patient group compared with the control group (Table 2).

Table 3 shows the comparison of parameters measured in the patient groups by the age groups. When the patients were divided into two age groups (46–70 and 71–87), no significant difference between the age groups in the levels of MDA, MCP-1, and vitamin C was found (*p* > 0.05).

## Discussion

Lipids are carried from the choroid to the RPE. Likewise, they are removed from the RPE by joining the circulation passing through the brunch membrane. These lipids secreted from RPE are called “lipid-like particles” and they accumulate in the brunch membrane and play a role in the age-related thickening of the Bruch’s membrane.<sup>18</sup> This accumulation may increase over time, causing the formation of drusen. As it is known, the progression of AMD is related to the number and size of drusen. The pathophysiology of AMD is not fully understood; however, findings from ongoing studies broaden our knowledge of the disease and its underlying mechanism. The

**Table 2.** Serum MCP-1, MDA, and vitamin C levels in patients and control participants.

	<b>Patient Median (Min-Max); N = 30</b>	<b>Control Median (Min-Max); N = 30</b>	<b>p value</b>
Vitamin C (mg/dL)	0.86 (0.71–1.25)	1.12 (0.73–1.92)	0.0001*
MDA (mmol/mL)	2.14 (0.57–5.01)	0.79 (0.50–2.79)	0.0001*
MCP-1 (pg/mL)	127.90 (62.10–492.50)	84.55 (57.30–106.2)	0.0001*

MCP-1, monocyte chemoattractant protein-1; MDA, malondialdehyde.  
Data are expressed as the median (min-max).  
\* $p < 0.05$ .

**Table 3.** Serum levels of MCP-1, MDA, and vitamin C levels in the patient group according to age subgroups.

	<b>Age groups (years)</b>		<b>p value</b>
	<b>46–70 age group Median (Min-Max) N = 16</b>	<b>71–87 age group Median (Min-Max) N = 14</b>	
Vitamin C (mg/dL)	0.87 (0.75–1.25)	0.86 (0.71–1.25)	0.3520
MDA (mmol/mL)	2.14 (0.57–4.43)	2.14 (0.57–5.01)	0.9070
MCP-1 (pg/mL)	121.55 (62.10–171.00)	127.90 (62.10–492.50)	0.2840

MCP-1, monocyte chemoattractant protein-1; MDA, malondialdehyde.  
Data are expressed as the median (min-max).

pathogenesis of AMD is thought to be the result of a complex multiple interactions between metabolic, functional, genetic, and environmental factors. It has been frequently reported that aging, oxidative stress, inflammation, and their relationship play a role in the pathogenesis of AMD. The oxidative stress burden of the retina is higher than other tissues due to sunlight exposure and high oxygen concentration.<sup>4</sup> Increased oxidative stress causes an increase in the level of oxidized lipids in the retina. The peroxidation of the lipids which required for cellular and metabolic functions also in the retina causes the formation of different cleavage products such as MDA and MAA.<sup>5</sup> MDA is a biological marker of oxidative stress and it is the main and most frequently studied product of polyunsaturated fatty acid peroxidation. This aldehyde is a highly toxic molecule and should be considered more than a lipid peroxidation marker. Its interaction with DNA and proteins is potentially mutagenic and atherogenic.<sup>19</sup> Because of these properties, the relationship

between serum MDA levels and AMD is of interest. In a study by Shen and colleagues,<sup>20</sup> mean serum MDA levels were found to be significantly higher in AMD patients compared with the control group, but also higher in late AMD patients compared with early AMD patients. Totan and colleagues<sup>21</sup> found that serum MDA levels were higher in AMD patients compared with healthy controls. In another study, it was shown that increased MDA levels induce cytotoxicity and VEGF expression in RPE cells in vitro.<sup>22</sup> A recent study in mice showed that MDA is not only an indicator of AMD, but also directly contributes to the pathogenesis of AMD, and that MDA causes autophagy dysfunction in cultured RPE cells. In the same study, it was found that MDA causes increase in VEGF levels and progression of CNV in eyes with wAMD.<sup>23</sup> In another study by Matsuura and colleagues,<sup>2</sup> it was reported that MDA levels were significantly correlated with CNV area in patients with wAMD. Our finding of elevated serum MDA levels in patients with

wAMD is consistent with the role of oxidative stress in wAMD, but also reinforces the notion that MDA levels can be used as a marker of oxidative stress.

Many lipid oxidation products have been shown to be proinflammatory and cytotoxic to photoreceptors and RPE.<sup>7</sup> Molecules formed by oxidative damage may exhibit antigenic properties and eventually cause the inflammation associated with the wrong targeting of healthy molecules or cells by the immune system.<sup>4</sup> Free oxygen radicals formed as a result of oxidative stress can also cause DNA damage in addition to changing lipid structure. DNA damage causing an inflammatory response in the RPE layer in senile macular degeneration (SMD) patients has been demonstrated.<sup>8</sup> Inflammatory cells activated in the inflammation site release many enzymes (such as neutral proteases, elastase, collagenase, acid hydrolases, phosphatases, and lipases), reactive species (superoxide, hydrogen peroxide, hydroxyl radical, hypochlorous acid), and chemical mediators (eicosanoids, complement components, cytokines, chemokines, nitric oxide) and thus cause tissue damage and oxidative stress.<sup>10</sup> After all, we can say that inflammation and oxidative stress are interrelated and they are closely linked pathophysiological processes. If oxidative stress occurs as the primary abnormality in an organ, inflammation develops as a result and further augments oxidative stress. Conversely, if inflammation is the primary event, oxidative stress will develop as a result, which will further increase inflammation.<sup>24</sup>

So far, many studies have demonstrated the importance of inflammation in AMD. Histological examination of patients with AMD showed macrophages and associated giant cells in areas with RPE atrophy, Bruch's membrane destruction, and choroidal neovascularization.<sup>25</sup> It is known that macrophages are a rich source of angiogenic cytokines and are associated with choroidal, tumoral, and inflammatory angiogenesis.<sup>26</sup> Ambati and colleagues<sup>12</sup> also demonstrated that the proinflammatory cytokine and chemokine panel including IL-1, IL-6, IL-8, TNF, IFN $\gamma$ , and MCP-1 accelerate SMD progression. MCP-1 is a protein that specifically attracts blood monocytes and tissue macrophages to its source through its interaction with the cell surface receptor CCR2.<sup>27</sup> MCP-1 is produced structurally by retinal microvascular endothelial cells and increased by proinflammatory molecules.<sup>28</sup> Du and colleagues showed that oxidative damage increased

MCP-1 secretion in retinal cells. They concluded that increased MCP-1 caused by oxidative damage promotes the AMD process by attracting macrophages to the retina and inducing them to release proinflammatory factors.<sup>29</sup> In support of this study, in a study conducted by Jonas and colleagues, it was found that intraocular MCP-1 concentration, subfoveal neovascular membrane type, and macular edema were significantly correlated in wAMD patients.<sup>30</sup> In another study, peripheral blood mononuclear cells from wAMD patients were reported to produce higher levels of MCP-1 and CXCL8 (IL-8), and it was concluded that high levels of MCP-1 could contribute to uncontrolled retinal inflammation and CNV formation in the macula.<sup>11</sup> Also, Pober and colleagues reported that circulating immune cells can also be recruited to participate in inflammation.<sup>31</sup> When the studies on the relation between AMD and MCP-1 in the literature are examined, it is suggested that both MCP-1 secreted from the retinal epithelial cells and high serum MCP-1 levels cause recruitment of macrophages to the macular region, and MCP-1 induced macrophages may have an important role in the formation of wAMD. In our study, serum MCP-1 levels of wAMD patients were found to be significantly higher than the control group in accordance with the findings of previous studies. These data and previous studies support the opinion that MCP-1 is involved in the pathogenesis of wAMD and that high serum MCP-1 levels may pose a risk for CNV. Accordingly, in the future, a potential anti-MCP1 treatment may lead to a reduction in angiogenic factors suggesting a new treatment possibility in wAMD. Indeed, Ehling and colleagues reported that inflammation-related angiogenesis in progressive liver fibrosis was promoted by CCL2-dependent monocytes during the progression of fibrosis and that the inhibition of monocyte infiltration by targeting CCL2 prevented this angiogenesis.<sup>32</sup> In addition to wAMD, MCP-1 is a potential point of intervention for the treatment of various diseases including multiple sclerosis, rheumatoid arthritis, atherosclerosis, and insulin-resistant diabetes.<sup>33</sup>

With aging, decreased blood flow together with RPE and Bruch membrane changes and thickening cause uncontrolled accumulation of cellular debris, making the eye more sensitive to proinflammatory processes.<sup>8</sup> During aging, oxidative damage increases and antioxidant capacity decreases simultaneously, thereby reducing the natural repair capacity of RPE cells.<sup>9</sup> Oxidative

damage which increases with aging is associated with diseases such as Parkinson, Alzheimer's, atherosclerosis, and cancer, as well as AMD.<sup>4</sup> As it is a tissue with a high concentration of oxygen and polyunsaturated fatty acids, the retina is highly sensitive to oxidative stress. Therefore, dietary intake of antioxidant vitamins and minerals and their serum concentration are suggested to be an important factor in the prevention of macular degeneration.<sup>14</sup> For example, vitamin C is a water-soluble antioxidant that has a role in the capture of free radicals and helps to renew other antioxidants such as vitamin E.<sup>34</sup> When the literature is reviewed, there are conflicting results showing that antioxidant treatment has or does not have efficacy in AMD.<sup>14,15,16,35</sup> In a study conducted in 2017, wet and dry AMD patients received a preparation consisting of vitamin C (408 mg), vitamin E (241 mg), zinc (30 mg), and lutein (9 mg) once a day for 3 months and a decrease from the initial serum MDA levels have been reported. However, it is found that this decrease was significant in the wAMD patient group, but not statistically significant in the dAMD patient group.<sup>2</sup> In our study, serum MDA levels were significantly higher in wAMD patients compared with the control group, while serum vitamin C levels were significantly lower in wAMD patients. The lower antioxidant vitamin C levels and higher MDA levels in wAMD patients in our study are consistent with the studies suggesting the role of increased oxidative stress in wAMD. The inverse relation between MDA and vitamin C found in our study supports the notion of vitamin C in antioxidant dietary supplement preparations that may help to reduce systemic oxidative stress. In light of the available information, it is obvious that a higher number of homogenized and reliable studies are needed for determining the effectiveness of antioxidant dietary supplements.

The most important limitation of our study was that we did not evaluate the levels of MDA, MCP-1, and vitamin C in dAMD patients. In addition, it was not investigated whether the patients received vitamin C treatment before the study. Another limitation of our study is that we did not make the differential diagnosis of polypoidal choroidal vasculopathy as we did not perform indocyanine green angiography.

In conclusion, serum MDA and MCP-1 levels were found to be significantly higher and vitamin C levels were significantly lower in the wAMD

patient group compared with the control group, indicating that the increased oxidative stress and inflammation can play a role in the pathogenesis of wAMD. High levels of MDA and MCP-1 may be significant risk factors for wAMD, and they may be novel molecules that may be targeted for treatment. The inverse relationship between vitamin C and MDA suggests that antioxidant effect of vitamin C can also be used in the treatment.

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### Conflict of interest statement

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### Ethical approval

Ethics committee approval in accordance with the Helsinki Declaration: Clinical Study Registration number: 2019/ 15 Niğde Ömer Halisdemir University Ethics committee.

### Informed consent

All participants were informed verbally and in writing about the study. Informed consent form was signed by participants included in the study.

### ORCID iDs

Ramazan Kürşad Zor  <https://orcid.org/0000-0002-3233-7906>

Erkut Küçük  <https://orcid.org/0000-0002-1474-9237>

Gamze Yıldırım  <https://orcid.org/0000-0003-3058-6308>


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