

Comparison of macular pigment optical density in patients with dry and wet age-related macular degeneration

Ayhan Ozyurt, Nilufer Kocak, Pınar Akan¹, Ozlem Gursoy Calan¹, Taylan Ozturk, Mahmut Kaya, Eyup Karahan², Suleyman Kaynak

Aim: The aim of the study was to evaluate the macular pigment optical density (MPOD) levels in patients with wet age-related macular degeneration (AMD), dry AMD, and also in healthy controls. **Settings and Design:** This study was conducted at Department of Ophthalmology, and the study design was a prospective study. **Patients and Methods:** Forty-eight patients with wet AMD, 51 patients with dry AMD, and 50 controls were included in the study. All patients were naive to both previous lutein or zeaxanthin administration and any previous intravitreal injections. Fundus reflectance (VISUCAM 500, reflectance of a single 460 nm wavelength) was used to measure the MPOD levels. Three groups were compared regarding age, gender, serum lutein, and zeaxanthin concentrations as well as MPOD levels. **Results:** Serum lutein and zeaxanthin levels were significantly higher in control group when compared with wet AMD (Group 1) and dry AMD (Group 2) ($P = 0.001$ and $P < 0.001$, respectively). Mean MPOD was found to be similar in all of the three study subgroups ($P = 0.630$). However, maximum MPOD was significantly higher in control group when compared with Group 1 and 2 ($P = 0.003$). There was no correlation between serum lutein or zeaxanthin concentrations and mean MPOD levels ($P = 0.815$, $r = 0.014$ and $P = 0.461$, $r = 0.043$, respectively), but there was a weak correlation between serum zeaxanthin concentration and maximum MPOD level ($P = 0.042$, $r = 0.124$). Maximum MPOD level was found to be correlated with the level of AMD (Group 1, 2, and 3; $r = 0.184$, $P = 0.041$). **Conclusion:** Maximum MPOD level was found to be lower in patients with AMD when compared with control cases. Mean MPOD and maximum MPOD levels were similar in wet and dry AMD Groups. These results can be applied clinically keeping in mind that MPOD measurements with one wavelength reflectometry may not be completely reliable.

Key words: Age-related macular degeneration, macular pigment optical density, serum lutein, serum zeaxanthin

Age-related macular degeneration (AMD) is the advanced form of age-related maculopathy (ARM) and is the leading cause of blindness in the elderly.^[1] There is a general consensus that cumulative oxidative damage is responsible for aging and may, therefore, play an important role in the pathogenesis of AMD. Oxidative stress, which refers to cellular damage caused by reactive oxygen intermediates (ROI), has been implicated in many disease processes, especially age-related disorders. ROIs include free radicals, hydrogen peroxide, and singlet oxygen, and they are often the byproducts of oxygen metabolism.^[2,3] Oxidative stress is a major risk factor for the pathological development of retinal diseases and vision impairment.

The macular pigment (MP) consists of xanthophyll, which is formed from the yellow carotenoid lutein, zeaxanthin, and meso-zeaxanthin. Located in the Henle fibers and in the inner plexiform layer, the highest MP density is found in the fovea.^[4,5] These pigments play an important role in protecting the retina against oxidative stress through different mechanisms.^[6]

Departments of Ophthalmology and ¹Biochemistry, School of Medicine, Dokuz Eylul University, ²Department of Ophthalmology, Karatas Hospital, Izmir, Turkey

Correspondence to: Dr. Mahmut Kaya, Mithatpasa Cad. 2, Karatas, No: 338 D: 12, Konak, Izmir 35220, Turkey. E-mail: mahmutkaya78@yahoo.com

Manuscript received: 04.05.16; Revision accepted: 22.05.17

Access this article online

Website:

www.ijo.in

DOI:

10.4103/ijo.IJO_365_16

Quick Response Code:



Various epidemiological studies have shown a varying association of AMD and MP although some authors reported no significant effect of MP levels on the development of AMD.^[7-10] Several studies have found no protective effect of *in vivo* measured MP optical density (MPOD) on different stages of ARM.^[11-15] Berendschot *et al.*^[16] did not find differences in MPOD between normal eyes and those with different stages of ARM. Data from the Carotenoids in Age-Related Eye Disease Study, the Blue Mountain Eye Study, and the Age-related Eye Disease Study (AREDS) provide evidence that low dietary intake of lutein and zeaxanthin is associated with an increased risk of AMD. Although no consensus has still been ensured on this issue, decreased MPOD levels are thought to be associated with the increased risk for the development of AMD.^[7,10,13,15-17] More recently, AREDS-2 research group revealed that addition of more lutein and zeaxanthin concentrations to the AREDS formulation was not found to be related to decreased risk of AMD progression.^[18] Furthermore, the dry and wet subtypes

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Cite this article as: Ozyurt A, Kocak N, Akan P, Calan OG, Ozturk T, Kaya M, *et al.* Comparison of macular pigment optical density in patients with dry and wet age-related macular degeneration. Indian J Ophthalmol 2017;65:477-81.

of AMD may have different etiologies and risk factors, and the need to examine MP in wet AMD separately has been emphasized.^[19]

A variety of techniques can be used to estimate the MPs; heterochromatic flicker photometry, fundus reflectometry, fundus autofluorescence, and resonance Raman spectroscopy.^[5] In our study, MPOD was measured by means of fundus reflectometry using the one-wavelength reflection method (VISUCAM500; Carl Zeiss Meditec AG, Jena, Germany). Hammer *et al.*^[14] found a good correlation between this OD measurement of the MP using one-wavelength reflectometry and the single OD measurement with autofluorescence. The aim of this study was to compare the MPOD levels in patients with wet and dry AMD as well as in healthy control cases.

Patients and Methods

Forty-eight patients with wet AMD, 51 patients with dry AMD, and 50 controls were included in the study. The Institutional Review Board approved the study, and fully informed consent was obtained from each participant (approval date and number: August 10, 2012, and 753GOA). The protocol followed the tenets of the Declaration of Helsinki. All study participants underwent a complete ophthalmic examination including the best-corrected visual acuity (BCVA) assessment, slit-lamp biomicroscopy, and fundus examination.

The wet AMD group (Group 1) consisted of 48 patients with exudative AMD in at least one eye. In patients with the diagnosis of bilateral exudative AMD, the eye with the better BCVA was selected for the study. The right eye was selected in case of the presence of equal BCVA in both eyes. The diagnosis of AMD was set and confirmed subjectively through clinical examination and objectively through spectral domain optical coherence tomography, color fundus photography, and fluorescein angiography. Group 2 consisted of 51 patients with bilateral involvement of early and intermediate (Stages 2 and 3 according to the AREDS I) subtype of dry AMD. When both eyes were eligible for enrollment, the right eye was preferred for the study. Group 3 included fifty elderly phakic patients without AMD or other retinal pathology in whom MPOD assessment was managed to be performed clearly. Healthy controls had no signs of AMD in either of the two eyes, and the right eye was preferred for the study in the participants whose both eyes were eligible for enrollment. All patients were naive to previous lutein or zeaxanthin administration and to any previous intravitreal injections. The patients having any ocular pathology other than AMD and those who underwent vitreoretinal surgery and retinal laser photocoagulation were excluded from the study.

Macular pigment optical density measurements

The optional MP density module for VISUCAM 500 was used reflectance of a single 460 nm wavelength based on a single blue-reflection fundus image to determine MPOD and its spatial distribution. A shading correction is used that approximates the reflectance of the fundus in the absence of MP. It is based on a three-dimensional parabolic function automatically fitted to fundus reflectance at peripheral locations. The participant was positioned in front of the fundus camera and instructed to look at a target inside. The fundus was illuminated by a monochromatic blue light. Four MPOD parameters were automatically calculated: maximum OD (MPOD measured at the peak), mean OD (mean MPOD within the measurement area), area (area where MP could be detected), and volume (sum of all ODs, as recommended by the manufacturer). Mean MPOD and maximum MPOD measurements of each eye were noted and statistically analyzed. BCVA was converted to the logarithm of the minimum angle of resolution (logMAR) and averaged.

Statistical analysis

Statistical analyses were performed using SPSS Windows version 15.0 (SPSS Science, Chicago, IL, USA). The normality of distribution was checked for all factors by Kolmogorov–Smirnov analysis. The data in the study followed normal distribution, and hence the parametric tests were used to determine significance. Univariate comparisons between Groups 1, 2, and 3 were made using Chi-squared test of independence for the categorical variable such as sex, whereas one-way ANOVA was used to compare average ages, serum lutein concentration, serum zeaxanthin concentration, and MPOD levels between the study groups. The relation of age with serum lutein concentration, serum zeaxanthin concentration, and MPOD levels was analyzed with linear regression analysis. The relation of MPOD measurements with age, gender, degree of AMD serum lutein, and zeaxanthin concentrations was evaluated with correlation analysis. $P < 0.05$ was considered statistically significant.

Results

Three study groups were not different with regard to age and gender ($P = 0.066$ and $P = 0.161$, respectively). The baseline characteristics of the study population are shown in Table 1. Age was not found to be correlated with serum lutein concentration, serum zeaxanthin concentration, mean MPOD level, and maximum MPOD level ($P = 0.773$, $P = 0.344$, $P = 0.543$, and $P = 0.541$, respectively). No statistical correlation was found between such parameters and gender in our study

Table 1: Demographics of study participants

	Group 1 Wet AMD (n=48)	Group 2 Dry AMD (n=51)	Group 3 Control (n=50)	P
Age	69.3±11.3	71.5±6.8	69.2±9.1	0.066*
Gender (female/male)	27/21	28/23	28/22	0.161**
Serum lutein concentration	0.327±0.111	0.328±0.181	0.336±0.032	0.021*
Serum zeaxanthin concentration	0.487±0.022	0.494±0.031	0.581±0.044	0.0001*
Mean MPOD level	0.157±0.689	0.148±0.035	0.155±0.737	0.630*
Maximum MPOD level	0.364±0.067	0.373±0.079	0.399±0.068	0.004*

There was a significant effect of maximum MPOD level on AMD $P < 0.05$ level for the three groups (Tukey *post hoc* test; $F(2,296)=5.71$, $P=0.004$). *ANOVA,

**Chi-square test. MPOD: Macular pigment optical density, AMD: Age-related macular degeneration

population ($P = 0.251$, $P = 0.152$, $P = 0.848$, and $P = 0.167$, respectively).

Serum lutein and zeaxanthin concentrations were significantly higher in control group when compared with Group 1 and 2 ($P=0.021$ and $P<0.0001$, respectively). However, the serum lutein and zeaxanthin concentrations did not differ significantly between the Group 1 and Group 2 patients ($P>0.05$ for each). Mean MPOD level was found to be similar in three groups ($P = 0.630$); however, maximum MPOD level was significantly higher in control group [Fig. 1] when compared with Group 1 and 2 [Fig. 2] ($P = 0.003$). Correlation analysis revealed that mean MPOD level had no relation with age, gender, serum lutein and zeaxanthin concentrations, or the level of the AMD. Moreover, there is our study that did not find any relationship between maximum MPOD level and serum levels of lutein and/or zeaxanthin. The logMAR level of BCVA had a low negative correlation with maximum MPOD (Spearman's $r = -0.23$; $P < 0.0001$), but no significant correlation was found with the mean MPOD. A significant correlation between BCVA and serum zeaxanthin was found ($r = -0.37$, $P < 0.0001$), but no significant correlation was found with serum lutein.

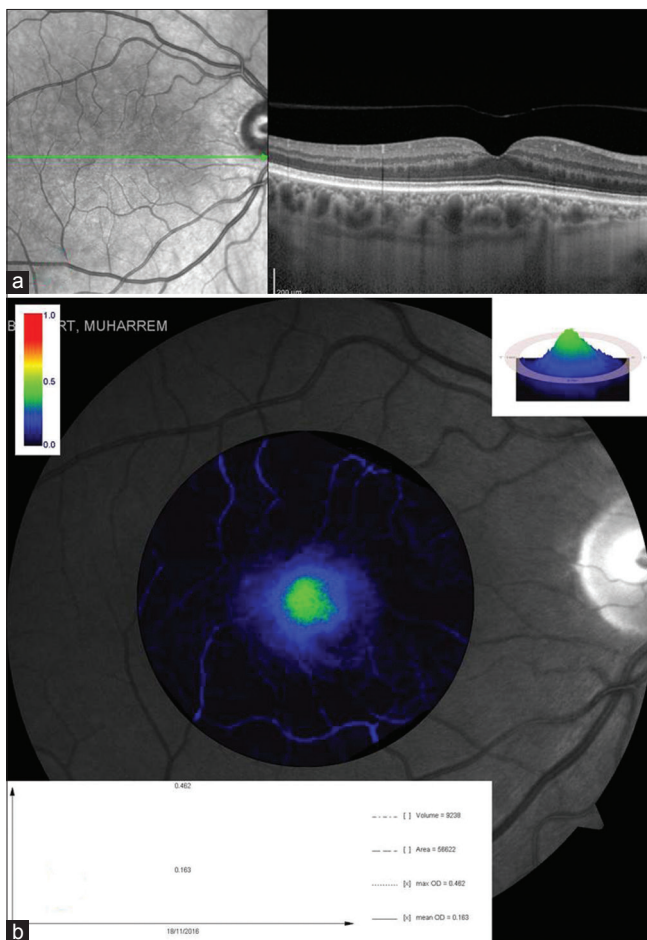


Figure 1: Representative spectral domain-optical coherence tomography image from a healthy participant (a). Measurement of macular pigment optical density. Macular pigment optical density is stated as maximum optical density and mean optical density. Right eye of a healthy participant: Macular pigment optical density measurement: maximum optical density = 0.462, mean optical density = 0.163 (b)

The level of the AMD was the most effective parameter on maximum MPOD ($P = 0.041$). Correlation analysis for mean MPOD and maximum MPOD levels is shown in Table 2.

Discussion

There is a growing body of evidence in support of the view that MP protects against AMD.^[20] Several epidemiologic studies have indicated that lutein and zeaxanthin intake is associated with a lower risk of AMD development; however, the findings are inconsistent.^[19,21,22] A previous meta-analysis concluded that no significant relationship was found between the dietary intake of lutein and zeaxanthin and early AMD, whereas an increase in the dietary intake of these carotenoids was reported to be associated with a 26% risk reduction in late AMD progression, suggesting that lutein supplementation may be more effective in preventing the progression from early to late stage of AMD.^[23]

The hypothesis that MP protects against ARM is based on the assumption that MP acts as a direct antioxidant role as well as a filter for blue light and high energy radiation in the human retina.^[9] MPs accumulate in high concentrations in the retina and generally peak at the center of the macula.

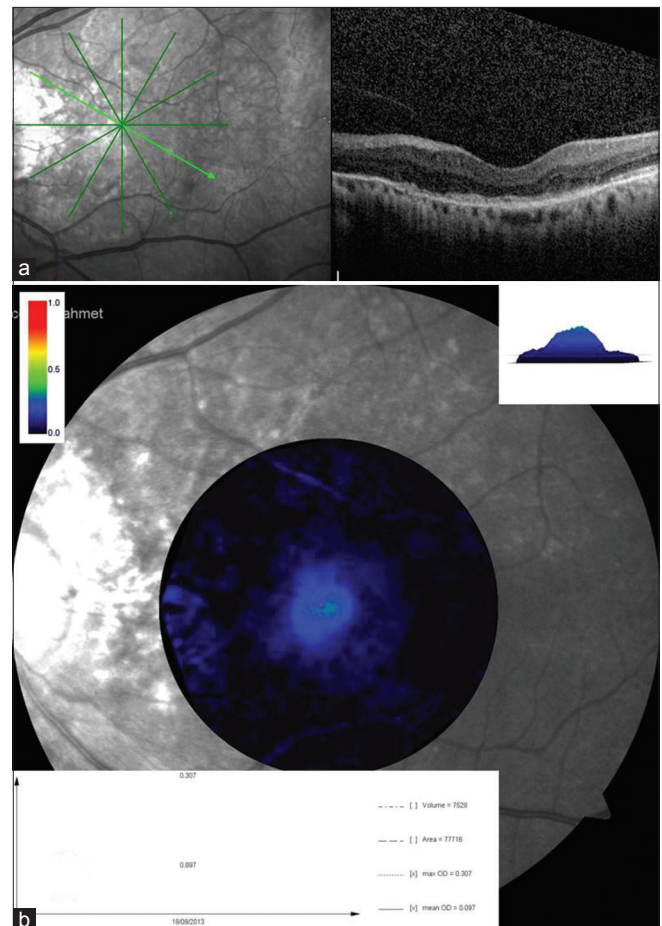


Figure 2: Spectral domain-optical coherence tomography imaging of dry age-related macular degeneration (a). Left eye of a patient with dry age-related macular degeneration: Macular pigment optical density measurement: maximum optical density = 0.307, mean optical density = 0.097 (b)

Table 2: Overall correlation analyses (Spearman's rho) between mean macular pigment optical density and maximum and macular pigment optical density age, gender, serum lutein, serum zeaxanthin, level of age-related macular degeneration, and significant correlations ($P \leq 0.05$) highlighted

	Mean MPOD		Maximum MPOD	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Age	0.063	0.282	0.058	0.339
Gender	0.002	0.975	0.081	0.164
Serum lutein	0.020	0.736	0.005	0.926
Serum zeaxanthin	0.174	0.062	0.019	0.825
Level of AMD	0.180	0.056	0.184	0.041*

MPOD: Macular pigment optical density, AMD: Age-related macular degeneration, * $P < 0.05$ was considered statistically significant

Typically, the MPOD reaches its half-peak OD at an average of only 1.03° (0.3 mm) retinal eccentricity.^[24] Although wet AMD constitutes only 10%–20% of the patients with AMD, it is the most visually disabling form of such a disease. Many studies have suggested that the risk of wet AMD development is not correlated with serum and dietary levels of MP.^[25,26] The dry and wet subtypes of AMD may have different etiologies and risk factors, and the need to examine the density of MP in wet AMD separately has been emphasized.^[19]

To the best of our knowledge, our study is the first study that evaluated the MPOD levels separately in eyes with wet and dry AMD. In our study, we found that serum lutein and zeaxanthin concentrations were higher in the control group when compared with dry and wet AMD groups. There was no difference between dry and wet AMD groups with regard to serum lutein and zeaxanthin concentrations. Mean MPOD level was found to be similar in all of the three study groups. However, the maximum MPOD level was higher in control group when compared to dry and wet AMD groups. No significant difference was found in maximum MPOD levels between dry and wet AMD groups. In light of these findings, we feel that the main problem of MPOD in AMD occurs in especially central fovea, and the maximum MPOD level is a more important parameter than mean MPOD level in such cases. It should also be emphasized that neither serum lutein and zeaxanthin concentrations nor mean and maximum MPOD levels showed any difference between dry and wet AMD groups. It is well known that lutein and zeaxanthin concentrations peak in the central fovea and zeaxanthin is the dominant carotenoid at this location. This specific distribution of the xanthophyll carotenoids suggests that zeaxanthin may play an essential role in the center of the retina, but until recently, the research specifically concerning the efficacy of zeaxanthin is still limited.^[27,28] In Japanese quails fed supplemental zeaxanthin, the number of apoptotic photoreceptors in light-damaged eyes was inversely correlated with retinal zeaxanthin concentration, and zeaxanthin supplementation for 6 months markedly decreased the levels of light-induced photoreceptor apoptosis.^[29,30] In the current study, there was no relation between maximum MPOD levels and serum lutein concentration, whereas there was a weak correlation between maximum MPOD level and serum zeaxanthin concentration. Furthermore, the improvement of

BCVA was significantly related to maximum MPOD level and serum zeaxanthin concentration. Hence, the level of zeaxanthin in the dietary supplemental treatments should be reconsidered in patients with early AMD findings to decrease the risk of progression. The MP is considered to enhance good visual performance, including contrast sensitivity, by reducing chromatic aberrations and the "blue haze" caused by small particles in the atmosphere.

A variety of techniques can be used to estimate the MPs. Based on whether a response is required from the participant, these criteria can be divided into two categories: the psychophysical technique and the objective technique. Heterochromatic flicker photometry is the most commonly used psychophysical method to date. Fundus reflectometry, fundus autofluorescence, and resonance Raman spectroscopy constitute three traditional objective techniques. In the present study, VISUCAM 500, which is based on reflectometry, measuring MPOD through reflectance of a single 460 nm wavelength, was used to measure the MPOD level. There are some studies about the reliability of this method. Dennison *et al.*^[31] reported that MP values obtained using the Heidelberg Spectralis were comparable to MP values obtained using the densitometer. In contrast, MP values obtained using the Zeiss VISUCAM were not comparable with either the densitometer or SPECTRALIS MP measuring devices, and the Zeiss VISUCAM appears to underestimate MP measurement. They concluded that densitometer and SPECTRALIS were suitable for measuring MP in both the clinical and research settings, whereas the VISUCAM was not. However, Delori *et al.*^[32] reported that MPOD obtained with fundus reflectance spectroscopy correlates well with values obtained with autofluorescence and heterochromatic flicker photometry, and there are many reports that support the results of this study.^[33–35] Nevertheless, it should be emphasized that there is still no standard method to measure the MP density. Various limitations to the present study should be acknowledged: our data would have been strengthened had we compared the one-wavelength reflectometry method with other methods such as heterochromatic flicker photometry and fundus autofluorescence. It has been shown that current cigarette smokers had significantly less MPOD. We did not record the smoking condition of the cases, and this is another limitation of our study.

Conclusion

Maximum MPOD level was found to be lower in patients with AMD when compared with control cases. The relation of maximum MPOD level with serum zeaxanthin concentration rather than serum lutein concentration is an interesting finding. However, we could not find any difference between wet and dry AMD groups with regard to MPOD levels or serum lutein and zeaxanthin concentrations. We believe that despite a strong relation between MPs and AMD, more prospective studies with broader series are warranted to better understand the role of the MPs in the pathogenesis of AMD.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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