Evaluation of the Macula Pigment Optical Density by a Psychophysical Test in Dry Age Related Macula Degeneration

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Research Article

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Abstract

PURPOSE: To evaluate the risk factors, macular pigment optical density (MPOD) and factors associated with MPOD in dry age related macular degeneration (AMD).

METHODS: This prospective study included 68 eyes of 68 dry AMD patients and, as the control group, 90 healthy eyes of 90 healthy volunteers with similar ages and genders. Age, gender, family history, smoking, alcohol use, hypertension, hyperlipidemia, height, weight, dietary lutein intake and multivitamin use were questioned in all participants. A full ophthalmic examination of all eyes were made. Colour fundus photography and fundus autofluorescence (FAF) images were recorded. MPOD was measured by colour perimetry (CP). To test the short-term repeatability of the technique, a total of 27 eyes of 27 young healthy subjects were measured 3 times on 3 consecutive days.

RESULTS: Smoking, obesity, family history, light iris colour, and hyperlipidemia seen more frequently in AMD group. The most frequent fundus finding was hard drusen (79.4%). Average MPOD was measured every three months in the AMD group and values were; 3.69±1.82, 4.74±1.29, 4.99±1.27 ve 5.02±1.35 dB, respectively. In the control group average MPOD measured once at the begining of the study and it was 4.97±1.27 dB. At first visit, the MPOD of the patient group was significantly lower than the control group. Smoking, obesity, poor dietary lutein intake, light iris colour, and hyperlipidemia associated with low MPOD values in both groups.

CONCLUSION: The relationship between MPOD and AMD is controversial in the literature. Depending to our results we think there is a relation between MPOD and AMD. Quit smoking, rich dietary lutein/zeaxhantin intake, having normal blood lipid levels and ideal weight is very important for preventing from AMD or AMD progression.

The results of the CP method are consistent with the results found in other psychophysical tests in the literature.

Introduction

Age related macular degeneration (AMD) is a progressive disease that affects photoreceptors, retina pigment epithelium, Bruch's membrane and choriocapillaris. It is the leading cause of the central vision loss and blindness among the people 60 years and older in the developed countries. AMD accounts for 8.7% of all legal blindness worldwide [1]. The risk factors of AMD are divided into two groups as modifiable and unmodifiable. Unmodifiable risk factors are age, gender, race, family history, genetics, presence of AMD in the fellow eye and light iris color. Modifiable risk factors are smoking, alcohol, obesity, lifetime exposure to sunlight-ultraviolet radiations, nutrition, systemic diseases and environmental factors [2]. Being aware of the risk factors is very important in terms of prevention and treatment of the disease. Oxidative damage to the retina plays a key role in the pathogenesis of the disease [1].

Macular pigment (MP) is thought to play a protective role in AMD by reducing the oxidative damage. MP is composed of lutein (L), zeaxanthin (Z) and mesozeaxanthin (an isomer of zeaxanthin), located in the Henle fiber layer in the fovea centralis and the inner plexiform layer in the parafovea [3]. MP reachs peak concentration in the foveola and drops to inconspicuous levels in the parafoveal region [3, 4]. L and Z levels differ in the various regions of the retina. Centrally, in the fovea, Z level is higher than that of L, with a ratio of 2.3:1 [5]. The level of carotenoids decreases 100-fold per mm in the foveal periphery, where L is more prevalent than Z, with a ratio of 2.4:1. MP has 3 functions including to reduce chromatic aberrations with its filtering effects; to protect the retina from the phototoxic effects of blue light (430–490 nm) and active antioxidant effect such as suppressing oxygen radicals and reducing lipofuscin formation [6]. MP is not synthesized de novo by human body, it is completely of dietary origin. Therefore, the level of MP can be changed by a dietary rich carotenoid intake or supportive treatment. Studies have shown that increased intake of the L and Z or having a diet rich in carotenoids is associated with a decreased risk of AMD [7, 8]. The inability to get the necessary nutrients from the diet is also considered as one of the modifiable risk factors of AMD [9].

Macular pigment optical density (MPOD) is a measure of the concentrations of L and Z in the macula. MPOD can be measured by psychophysical tests (color perimetry (CP), heterochromatic flicker photometry (HFF), motion photometry) or objective methods (fundus autofluorescence (FAF), fundus reflectometry (FR), raman spectroscopy (RS)) [10]. Psychophysical tests are the most common. CP compares colour sensitivity outcomes between two wavelengths (blue and red) differently absorbed by MP. It can measure visual sensitivity with a test wavelength (blue) that is maximally absorbed by MP and a reference wavelength (red) that is not absorbed by MP [11]. CP provides measurements of MP distribution and makes it possible to analyze MPOD across time. This is a simple, rapid and noninvasive method that does not require pupillary dilation. If there is several measurements belongs to same person CP is able to do progression analyzes.

There have been numerous studies investigating the relationship between MPOD and AMD using a variety of measurement techniques and it is controversial in the literature. Some of these studies have showed a MPOD-AMD association, and some have not [7, 12–17].

In this prospective study our aim is to; measure MPOD by CP; to evaluate the effect of the lutein support on the MPOD; to examine the risk factors of dry AMD by evaluating the factors affecting MPOD.

Methods

This prospective study included randomly selected 68 eyes of 68 patients with dry AMD who were followed up in Ankara University Faculty of Medicine Department of Ophthalmology, and 91 healthy eyes of 91, age and gender matched individuals as the control group. All patients gave their verbal and written informed consent before participating in the study. This study was approved by the ethics committee of Ankara University and was carried out according to the rules of the Helsinki Declaration. All patients were followed up for at least 1 year at 3-month intervals. Patients with diabetes mellitus, glaucoma, uveal-

retinal disease, retinal or cataract surgery within six months, ocular or systemic diseases affecting central or paracentral vision were excluded from the study.

All participants were questioned in terms of age, gender, family history, smoking, alcohol use, presence of hypertension-hyperlipidemia, height, weight and multivitamin use. Body mass index (BMI) was calculated by measuring the height and weight of the patients. Those with a BMI of 0-18.4 were considered underweight, 18.5–24.9 were considered normal, 25-29.9 were considered overweight, 30-44.9 were considered obese, over 45 were considered morbid obese. Smoking and alcohol usage were quentionned, the amount (package/day or cubic centimeter (cc)/day) and the duration of usage were recorded. L-Z usage was asked; the amount (gram/day) and duration of usage were recorded. A full ophthalmic examination including best corrected visual acuity (BCVA), biomicroscophic anterior segment-fundus examination and intraocular pressure measurement was performed to all participants. İris colours were recorded. Blue-gray, gray-hazel irises were classified as light; brown-black irises were classified as dark colour. After pupil dilatation (phenylephrine hydrochloride 2.5% and tropicamide 0.5%) lens status and presence of cataract were evaluated and graded. Biomicroscophic fundus examination was performed with a 90 D lens. Presence of drusen, drusen type (hard, soft), retina pigment epithelium (RPE) changes, pigmentation changes and macula in the fellow eye not included in the study were evaluated. After fundus examination, color fundus photographs of all patients were taken at each control. Staging was performed according to the AMD classification reported by the "Age-Related Maculopathy Study Group" [18]. In every control of all patients; fundus autofluorescence (FAF) images were obtained with Heidelberg retinal angiography (II) device using a confocal scanning laser ophthalmoscope with a 488 nm excitation and a 500 nm barrier filter. Patients were evaluated in terms of 8 different drusen patterns determined by the FAM (Fundus Autofluorescence in Age-Related Macular Degeneration) Study Group, and it was noted whether there was a pattern change or progression in the follow-ups [19].

MPOD was measured by Mon CV3 multifunctional perimetry (Metrovision, FR) in all participants. This program evaluates MPOD by comparing the thresholds of perception of blue and red light using a staircase technique similar to the technique used in automated perimetry. Luminance differential thresholds were measured for two stimuli: a blue stimulus (450 to 480 nm), which is absorbed by the MP, and a red stimulus (620 nm), which is not absorbed. The stimuli were presented at the fovea and at six peripheral locations, with an eccentricity of 3 to 10 degree, applied in Goldman III size on a 10 cd/m² illuminated white background. MPOD was estimated as the difference between the thresholds of blue and red stimuli at the fovea and perifovea areas. Blue thresholds show a relative decrease of about 0.6 log units at the fovea, determining the presence of MP, which absorbs blue light, in normal people. A correction for blue light absorption by the lens was made according to the difference between the thresholds of blue and red stimuli at 10 degree eccentricity. Outcomes were provided in units of decibels.

Measurements were performed before pupil dilatation with appropriate near refractive correction and fellow eyes were occluded during the test. The heads of patients were positioned and fixed properly. The position of the tested eye was fixed up to the level of the eye marks. The examiner was able to see whether the tested eye was within the control area thouhgh a camera in the monitor. The visual stimulator

showed a black circle, in the middle of which blue and red stimuli were seen. The examiner wanted the patient to press a button every time a light was seen. In the second step of the test, the visual stimulator showed a central fixation dot. The patient was asked to fixate on the central dot and press the button every time a light was seen on the periphery. All tests were performed in cooperation with the patients with the guidance of the attending physician. All participants were able to understand the tasks and follow the directions. All measurements were made by same ophthalmologist (F.Ç.K). Initial measurements were not recorded as the test was considered as the learning step of the patients.

Short time repeatability of the CP was evaluated by making 3 different measurements on 3 consecutive days in 27 healthy eyes of 27 participants aged between 27–45 (mean 33.1 ± 3.6) .

The dietary habits of all participants were evaluated with a questionnaire consisting of 30 foods rich in L-Z. Diets containing less than 6 mg/day of L-Z are called poor; diets containing 6m/day or more of L-Z were called rich diets.

Statistical analysis

SPSS (Statistical Package for Social Science) for Windows 11.5 (SPSS Inc., Chicago, IL) package program was used for data analysis. Normality of the distribution of continuous and discrete numerical variables was investigated by using the Kolmogorov Smirnov test. The homogeneity of the variances was investigated with Levene's test. Descriptive statistics expressed as mean ± standard deviation or median (minimum-maximum) for continious and discrete numerical variables, and the number of cases and (%) for categorical variables. The significance of the difference between the groups in terms of mean values investigated with Student's t test when the number of independent groups was two, and with One-Way ANOVA in the presence of more than two groups. If the One Way Analysis of Variance result was found to be significant, the condition(s) causing the difference were determined by using the post hoc Tukey HSD test. The significance of the difference between the groups in terms of median values was evaluated with the Mann Whitney U test. Categorical variables were analyzed with Pearson's Chi-Square or Likelihood Ratio test. Whether there was a statistically significant difference in mean MPOD levels between follow-up times in the patient group was evaluated with Repeated Measurements Analysis of Variance using Wilks' Lambda test. If the Wilks' Lambda test statistic result was found to be significant, the Bonferroni Corrected multiple comparison test was used to determine the follow-up times that caused the difference. The Friedman test was used to determine whether there was a statistically significant change in visual acuity in the patient group according to the follow-up times. The presence of statistically significant change in fundus and FAF findings was investigated by the Cochran Q method. Spearman's correlation test was used to determine whether there was a statistically significant correlation between continuous and sortable variables. Multivariate Linear Regression Analysis was used to determine the most predictive risk factors that had a statistically significant effect on MPOD measurements and

the regression coefficient, 95% confidence intervals for each variable were calculated. Repeatability of CP was evaluated by using the intraclass correlation coefficient (ICC) and the Bland-Altman plot. The ICC is

an analysis-of-variance (ANOVA)–type correlation that measures the relative homogeneity within groups (between the repeated measurements) as a ratio to the total variation. The ICC will approach 1.0 when there is no variance within repeated measurements, indicating that the total variation in measurements is caused solely by variability in the parameter being measured. The analysis of intersession repeatability is a calculation of the difference in MPOD obtained for each subject in two test sessions conducted by the same observer. The degree of intersession repeatability is the range over which 95% of the differences—the 95% limits of repeatability—are equal to the mean difference ± 1.96 × standard deviation (SD) of the differences. The limits of agreement were calculated as the mean difference in the measurements obtained by each observation ± 1.96×SD of the differences. The limits of repeatability are shown and were also plotted as the difference versus the mean of the MPOD in the two test sessions. This method uses graphing to assess whether there is agreement between the measurements. [20, 21] A Bland-Altman plot was performed using MedCalc demo version 11.1.1.0 (MedCalc Software, Mariakerke, Belgium). A value of $p < 0.05$ was considered to be statistically significant unless otherwise stated. Bonferroni Correction was performed in this study to control Type I error in all possible multiple comparisons.

Results

In this study, 68 eyes of 68 patients with dry AMD and as the control group 91 eyes of 91 healthy subjects were evaluated. The mean age was 67.3 ± 8.65 and 60.6 ± 6.68 years in the AMD and control groups, respectively. There were 43 (63.2%) women and 25 (36.8%) men in the AMD group and 55 (60.4%) women and 36 (39.6%) men in the control group. Demographic findings of the patient and control groups are shown in the Table 1. There were no statistically significant difference between the groups in terms of age, gender, smoking, alcohol use or hypertension ($p>0.05$). However, in the study group; the amount of smoking, presence of family history, hyperlipidemia and BMI were higher, while the iris color was lighter compared to the control group ($p<0.05$).

Table 1

Demographic and Clinical Features

Variables	Control Group	(n=91)	Patient Group (n=68)	p-value
Age(year)	60,6±6,7		67,3±8,6	>0,001
Gender				0,720
Man	36 (39,6%)		25 (36,8%)	
Women	55 (60,4%)		43 (63,2%)	
BMI (kg/m^2)	23,8±2,2		27,7±4,9	**<0,001**
BMI				**<0,001**
Normal	76 (83,5%)		20 (29,4%)	
Overweight	13 (14,3%)		32 (47,1%)	
Obese	2 (2,2%)		16 (23,5%)	
Smoking				0,433
Not smoke	73 (80,2%)		49 (72,1%)	
Currently smoke	7 (7,7%)		6 (8,8%)	
Quit	11 (12,1%)		13 (19,1%)	
Smoking package/year	20 (2-40)		35 (3-60)	**0,004**
Alcohol use				0,915
Not use	87 (95,6%)		64 (94,1%)	
Currently using	2 (2,2%)		2 (2,9%)	
Quit	2 (2,2%)		2 (2,9%)	
Alcohol Usage Time (year)	18,5 (15-25)		40 (20-50)	0,114
Family history	9 (9,9%)		20 (29,9%)	**<0,001**
Iris Colour				**<0,001**
Light	18 (19,8%)		36 (52,9%)	
Dark	73 (80,2%)		32 (47,1%)	
HPL	17 (18,7%)		39 (57,4%)	**<0,001**

HT	38 (41,8%)	39 (57,4%)	0,052

BMI:Body mass index; HPL: Hyperlipidemia; HT:Hypertension

The median BCVA at the first control was 0.04 (0.00-0.39) log MAR, and 0.00 (0.00-0.05) log MAR in the patient and control groups, respectively. BCVA of control group was significantly higher than patient group ($p>0.05$). There were no significant change in BCVA during the follow-up period ($p>0.05$).

The presence of cataract was evaluated by biomicroscophic examination at each control. In the study group; 43 (63.2%) patients had grade 1, 8 (11.8%) patients had grade 2 cataract, 7 (10.3%) patients were pseudophakic and 10 (14.7%) patients had no cataract. In the control group; 33 (36.3%) patients had grade 1, 2 (2.2%) patients had grade 2 cataract, 4 (4.4%) patients were pseudophakic and 52 (57.1%) patients had no cataract. There were no significant changes in cataract stages in both groups during the follow-up period ($p>0.05$).

The mean MPOD was measured only once at the begining of the study in the control group and was 4.97±1.27 dB. In the patient group, mean MPOD was measured 4 times at 3-month intervals. These values were 3.69±1.82 dB in the 1st, 4.74±1.29 dB in the 2nd, 4.99±1.27 dB in the 3rd, and 5.02±1.35 dB in the 4th visit. MPOD at the first visit was significantly lower compared to the other visits ($p<0.05$); There were no significant differences between the values in the second, third and fourth visits ($p>0.05$).

When the study group evaluated with a nutrition questionnaire consisting of 30 foods rich in L-Z; 46 (67.6%) patients were had a diet rich in L-Z, and 22 (32.4%) patients had a diet poor in L-Z.

In the study group 58 (85.3%) patients had hard drusen, 33 (48.5%) patients had soft drusen, 29 (42.6%) patients had RPE changes and 19 (27.6%) patients had RPE atrophy. There were no significant changes in fundus findings during the study.

At each visit, color fundus photographs of the patients were taken and staging of AMD was performed. At first visit 54 (79,4%) patients had early, 14 (20,6%) patients had intermediate AMD. There was no significant progression in the disease stage during the follow-up period ($p>0.05$).

At first visit 22 (32,4%) patients had normal, 10 (14,7%) patients had minimal change, 10 (14,7%) patients had focal increased, 14 (20,6%) patients had patchy, 2 (2,9%) patients had linear, 5 (5,9%) patients had lacelike, 6 (8,8%) patients had reticular, 5 (5,9%) patients had speckled patterns. Progression and new pattern formation were noted. During the follow-up period, 33 patients showed progression in the current FAF pattern. Focal increased pattern developed in 2 patients, reticular pattern developed in 2 patients and patchy pattern developed in 1 patient during the follow up.

Patients were evaluated for the presence of wet AMD or geographic atrophy (GA) in the fellow eyes that were not included in the study. Wet AMD was detected in 13 (19.1%), and GA was found in 12 (17.6%) eyes.

When the patient group was evaluated in terms of multivitamin use, 35 (51.4%) patients were not using L-Z at the first visit; 22 (32.4%) patients were using 6mg/day lutein and 11 (16.2%) patients were using 10mg/day of lutein and 2mg/day of zeaxanthin. 6 mg/day lutein was given to 12 and 10 mg/day lutein and 2mg/day zeaxanthin was given to 19 patients who did not use multivitamins . 4 patients in the patient group refused multivitamin use and did not receive during follow-up period. At the end of the follow-up, the mean duration of multivitamin use of the patients was 24 (12-56) months. There were no correlation between the mean BCVA and the mean MPOD or cumulative multivitamine use ($p>0.017$).

The mean baseline MPOD's were 4.54±1.46 dB in the lutein-rich diet group and 1.89±0.99 dB in the lutein-poor diet group. Baseline MPOD was lower in the lutein-poor diet group than lutein-rich diet group, and the MPOD increase after lutein supplementation was more dramatic ($p<0.001$). In the lutein rich diet group MPOD variation between 2^{nd} and 1^{st}, 3^{th} and 1^{st}, 4^{th} and 1^{st} visits were 0.47±1.05, 0.55±1.76 and 0.59±1.82 dB, respectively ($p<0.001$). In the lutein poor diet group MPOD variation between 2^{nd} and 1^{st}, 3^{th} and 1^{st}, 4^{th} and 1^{st} visits were 2.29±1.40, 2.87±1.42 and 2.89±1.43 dB, respectiveley ($p<0.001$) (Figure 1)

When the patients were divided into those with and without wet AMD in the fellow eye; at first visit, the mean MPOD of the patients with wet AMD in the fellow eye was 3.07±1.53 dB, while it was 3.83±1.86 dB in those without. When the patients were divided into those with and without GA in fellow eye; at first visit, the mean MPOD of those with GA in the fellow eye was 4.39±2.24 dB, and 3.53±1.70 dB in those without. There was no statistically significant difference between the groups ($p>0.025$).

MPOD values of the patients with or without wet AMD in the fellow eye at first visit were significantly lower than the 2^{nd} (5.03±1,21 dB / 4.68±1.31 dB), 3rd (5.03±1.21 dB / 4.68±1.31 dB) and 4th (5.21±1.19 dB / 4.99±1.40 dB) visits ($p<0.025$); There was no significant difference between the 2, 3, and 4th visit values ($p>0.025$). MPOD values of the patients with or without GA in the fellow eye at first visit were significantly lower than the 2^{nd} (5.17±1.75 dB / 4.66±1.17 dB), 3rd (5.63±1.27 dB / 4.86±1.25 dB) and 4th visits (5.68±1.31 dB / 4.89±1.34 dB) ($p<0.025$); There was no significant difference between the 2, 3, and 4th visit values ($p>0.025$). There were no significant effect of wet AMD or GA in the fellow eye on MPOD ($p>0.05$).

In the patients with and without GA in the fellow eye groups MPOD variation between 2^{nd} and 1^{st}, 3^{th} and 1^{st}, 4^{th} and 1^{st} visits were 0.77±0.86 / 1,12±1,54, 1.23±1.26 7/ 1,32±2,11, 1.28±1,17 / 1,35±2,16 dB, respectively. In the patients with and without wet AMD in the fellow eye groups MPOD variation between 2^{nd} and 1^{st}, 3^{th} and 1^{st}, 4^{th} and 1^{st} visits were 1,95±1,55/ 0,84±1,35, 1,92±1,75/1,16±2,01, 2,13±1,77/ 1,15±2,03dB, respectively. MPOD variation between 2^{nd} and 1^{st} visit in the patients with wet AMD in the fellow eye group was statistically significant than patients without wet AMD in the fellow eye ($p=0,012$).

The mean baseline MPOD was 3.67±1.86 dB in early AMD and 3.76±1.67 dB in intermediate AMD group. In the early AMD group MPOD variation between 2^{nd} and 1^{st}, 3^{th} and 1^{st}, 4^{th} and 1^{st} visits were 1.07±1.47, 1.35±1.75 and 1.36±1.79 dB, respectively. In the intermediate AMD group MPOD variation between 2^{nd}

and 1st, 3th and 1st, 4th and 1st visits were 1.02±1.39, 1.12±2.76 and 1.25±2.80 dB, respectively. No statistically significant difference was found in terms of baseline MPOD and MPOD change between early and intermediate AMD patients (p>0.0083).

The mean baseline MPOD was 4,30±1,31 dB, 3,70±1,88 dB, 3,42±1,79 dB and 3,67±1,87 dB in the patients with hard drusen, soft drusen, pigment epithelium atrophy and pigment epithelium changes groups, respectively. In patients with hard drusen MPOD variation between 2nd and 1st, 3 th and 1st, 4th and 1st visits were 1.12±1.51, 1.37±2.10, 1.39±2.15dB, respectively. In patients with soft drusen MPOD variation between 2nd and 1st, 3 th and 1st, 4th and 1st visits were 1.04±1.58, 1.32±2.35, 1.40±2.41 dB, respectively. In patients with pigment epithelium atrophy MPOD variation between 2nd and 1st, 3 th and 1st, 4th and 1st visits were 0.88±1.52, 1.03±2.56, 1.10±2.59, respectively. In patients with pigment epithelium changes MPOD variation between 2nd and 1st, 3 th and 1st, 4th and 1st visits 0.88±1.07,1.14±1.33, 1,.10±1.32 dB, respectively. There were no statistically significant correlation between fundus findings and MPOD values or MPOD changes during the study (p> 0.05).

Mean baseline MPOD was 3.81±1.41 dB in the normal pattern group, 4.11±2.07 dB in the minimal change group, 3.12±1.37 dB in the focal increased group, 3.15±1.58 dB in the patchy group, 3.76±1.10 dB in the linear group, 4.55±3.52 dB in the lacelike group, 4.03±2.61 dB in the reticular group and 3.54±2.25 dB in the speckled group. FAF patterns were found to have no effect on the mean baseline MPOD and MPOD changes during the study (p> 0.05). (Table 2).

Table 2

MPOD changes according to FAF patterns in the patient group

Variables	2nd – 1stmeasurement	3th – 1st measurement	4th-1st measurement
Normal Pattern1			
No	1,04±1,56	1,21±2,20	1,20±2,23
Yes	1,10±1,20	1,49±1,43	1,63±1,46
p-value †	0,871	0,596	0,413
Minimal Change 1			
No	1,09±1,47	1,38±2,06	1,45±2,07
Yes	0,89±1,36	0,84±1,40	0,67±1,55
p-value †	0,693	0,429	0,261
Focal Increased 1			
No	1,04±1,51	1,17±2,05	1,25±2,13
Yes	1,14±1,00	2,10±1,33	1,84±1,03
p-value †	0,839	0,171	0,400
Patchy 1			
No	1,02±1,38	1,29±2,03	1,30±2,07
Yes	1,21±1,73	1,34±1,84	1,47±1,85
p-value †	0,659	0,941	0,789
Reticular 1			
No	1,08±1,39	1,41±1,63	1,48±1,72
Yes	0,77±2,07	0,17±4,27	-0,11±3,93
p-value †	0,614	0,509	0,371
Speckled 1			
No	1,08±1,43	1,32±1,93	1,36±1,93
Yes	0,79±1,81	1,05±2,82	1,07±3,17
p-value †	0,668	0,772	0,761

† According to Bonferroni Correction, the results for $p<0.0083$ were considered statistically significant.

, a: The difference between Grade 1 and pseudophakic was statistically significant ($p<0.0083$) 1. FAF patterns at first visit

During the follow-up period, progression in the FAF pattern was observed in 33 patients and new pattern formation was observed in 5 patients. The new patterns formed were patchy in 1 patient, reticular in 2 patients, and focal increased in 2 patients. The mean baseline MPOD was 3.55±2.03 dB in those with progression in the FAF pattern and 3.81±1.61 dB in those without. There was no statistically significant effect of progression or new pattern formation on baseline MPOD or MPOD change (p>0.083). In the patients with and without progression in the FAF pattern MPOD variation between 2^{nd} and 1^{st}, 3^{th} and 1^{st}, 4^{th} and 1^{st} visits were 1.16±1.24/0,95±1,65, 1.36±1.38/1,25±2,48, 1.63±1.54/1,00±2,42 dB respectively.

Mean MPOD at first visit was 2.41±1.19 dB in the group not using L; 4.25±1.13 dB in the group using 6 mg/day L and 5.68±1.62 dB in the group using 10 mg/day L and 2 mg/day Z. The mean MPOD of the group not using L was significantly lower compared to the groups using 6 mg/day or 10 mg/day L and 2 mg/day Z (p<0.05). MPOD levels in the groups using multivitamins from the beginning of the study remained stable throughout the study (p>0.05). MPOD values in the 2nd, 3rd and 4th visits were significantly higher than baseline in both 6 mg/day and 10 mg/day L groups (p<0.05). However, there was no significant difference between the values in the 2nd, 3rd and 4th visits (p>0.05) (Figure 2, 3).

A decrease in MPOD values was observed during the follow-up period in 4 patients who did not use multivitamins (Figure 4).

Table 3 shows the MPOD changes according to the multivitamin use of the patients in 4 controls. Patients using L 6 or 10 mg/day had a significant increase in MPOD during follow-up compared to patients not using L (p<0.001). The mean MPOD at the last visit of 4 patients who did not use L was significantly lower than the first visit (p<0.001). Figure 5 shows the MPOD values of a one patient before and after 10 mg/day L and 2 mg/day Z use.

Table 3

MPOD changes according to multivitamin use

Variables	2nd – 1st measurement	3th – 1st measurement	4th-1st measurement
Multivitamin use			
Not use	-1,32±1,62 [a]	-2,87±4,11 [a,b]	-2,93±4,36 [a,b]
6 mg/day	0,77±1,06	1,11±1,40 [b]	1,19±1,50 [b]
10 mg/day	1,63±1,51 [a]	1,94±1,77 [a]	1,94±1,77 [a]
p-value †	<0,001	<0,001	<0,001

†

According to Bonferroni Correction, the results were considered statistically significant for p<0.0083., a: The difference between the non-

user group and 10 mg/day was statistically significant ($p<0.001$), b: The difference between the non-user group and 6 mg/day was statistically significant ($p<0.001$).

There were no significant correlation between the presence or grade of the cataract, pseudophakia and baseline MPOD ($p>0.0125$). The mean MPOD increased in patients with grade 1 cataract however decreased in pseudophakic patients ($p<0.0083$).

The mean baseline MPOD was significantly lower in smokers, overweight or obeses and patients with family history, light iris color or hyperlipidemia. Gender, alcohol use or hypertension has no effect on baseline MPOD. (Table 4).

Table 4

MPOD levels according to clinical and demographic characteristics in patient and control groups

Variables	MPOD 1 (dB)	p-value
Gender		0,832
Man	4,39±1,73	
Woman	4,45±1,61	
Smoking		**<0,001**
None	4,69±1,65[a,b]	
Yes	3,15±0,97[a]	
Quit	3,77±1,50[b]	
Alcohol use		0,409
No	4,47±1,58	
Yes	3,64±2,65	
Family History		**<0,001**
Yes	3,43±1,41	
No	4,65±1,63	
İris Colour		**<0,001**
Light	3,77±1,63	
Dark	4,76±1,57	
Hyperlipidemia		**0,009**
Yes	3,96±1,66	
No	4,68±1,60	
Hypertension		0,063
Yes	4,17±1,79	
No	4,66±1,48	
Body/mass index		**<0,001**
Normal	4,85±1,46[c]	
Overweight	3,67±1,87[c]	
Obese	4,06±1,35	

a: The difference between the non-smoker group and the current smoker group was statistically significant (p=0.003), bThe difference between the non-smoker group and the ex-smokers group was statistically significant (p=0.027), c: The difference between the normal weight group and the overweight group was statistically significant (p<0.001). MPOD1: MPOD at first visit

When adjusted for other risk factors with linear regression analysis, baseline MPOD was continued to be statistically significantly lower: in the patient group compared to the control group; those with a family history compared to those without; current smokers or those who quit smoking compared to those who have never smoked, and those with light iris color compared to those with darker iris (p<0.01). In our study, when correction was made for other possible factors in all cases with multivariate linear regression analysis, the most determinant factors on MPOD measurements were; being in the patient group, presence of family history, light iris color and smoking (p<0.05) (Table 5).

Table 5

The most determinant factors on MPOD measurements

Variables	**Regression coefficient**	**%95 Confidence interval**		**p-value**
		Lower limit	*Upper limit*	
Patient group	-1,151	-1,751	-0,551	**<0,001**
Age	0,019	-0,014	0,052	0,247
Body mass index	0,023	-0,043	0,090	0,494
Family history	-0,698	-1,323	-0,072	**0,029**
Light iris colour	0,571	0,025	1,118	**0,041**
Hyperlipidemia	0,154	-0,444	0,751	0,612
Hypertension	-0,281	0,768	-0,206	0,255
Curent smoker	-1,335	-2,212	-0,458	**0,003**
Quit smoking	-0,711	-1,387	-0,036	**0,039**

According to the multivariate linear regression analysis, the most determinant factors on the difference in the measurements of MPOD between visits in the AMD group were; diet and multivitamin use and it was observed that the degree of cataract was also effective on the difference in the 3rd and 4th controls (p<0.0083). (Table 6).

Table 6

Defining the most determinant factors on the change in MPOD measurements in the patient group according to multivariate linear regression analysis

Variables	Regression coefficient	%95 Confidence interval		p-value
		Lower limit	*Upper limit*	
2nd – 1st measurement				
Poor diet	1,433	0,829	2,038	**<0,001**
Using 6 mg/day multivitamin	2,567	1,086	4,048	**<0,001**
Using 10 mg/day multivitamin	2,849	1,380	4,319	**<0,001**
Multivitamin usage time	-0,028	-0,054	-0,002	0,032
3th – 1st measurement				
Poor diet	1,875	1,138	2,612	**<0,001**
Cataract degree	-0,524	-0,768	-0,280	**<0,001**
Using 6 mg/day multivitamin	3,849	2,238	5,460	**<0,001**
Using 10 mg/day multivitamin	4,049	2,394	5,704	**<0,001**
4th-1st measurement				
Poor diet	1,861	1,128	2,594	**<0,001**
Cataract degree	-0,598	-0,841	-0,356	**<0,001**
Using 6 mg/day multivitamin	4,018	2,416	5,620	**<0,001**
Using 10 mg/day multivitamin	4,134	2,488	5,779	**<0,001**

† According to Bonferroni Correction, the results were considered statistically significant for p<0.0083.

In order to evaluate the intra-observation repeatability of the CP, the results of the measurements we made on healthy controls on 3 consecutive days were examined, and the ICC of the method was found to be 0.669 (ICC of; first-second measurement was 0,696, second-third measurement was 0,822, first-third measurement was 0,485). The differences described in the Bland-Altman plots of days 1 to 2, days 1 to 3, and days 2 to 3 did not show a systematic distribution around the zero point, and no relationship was prominent between the averages and differences. The graph of the measurement results from the Bland-Altman plots of the first and second measurements and the first and third measurements indicates that the mean difference was 0.5 dB. Also the width of the limits of agreement was 3.3 dB for first to second, 4.7 dB for first to last, and 3.1 dB for second to last measurement, so the best agreement was between the last two. This was an indication of a learning period of the test.

Discussion

The MP is thought to play a protective role against AMD by reducing the phototoxic effects of short wavelength blue light and oxidative damage.

In our study, we found that baseline MPOD values were significantly lower in the patient group than control group.

In the linear regression analysis, this decrease continued to be when adjustments were made for other risk factors that may cause low MPOD.

Based on these results, we concluded that MPOD is associated with AMD. There are many studies in the literature that have found similar results. Beatty et al. compared healthy eyes of patients with advanced AMD in the fellow eye and healthy eyes of healthy controls. They found the MPOD lower in the first group compared to the healthy controls and reported that low MPOD could cause the development of AMD. They showed that 3 of 9 patients in the first group developed soft drusen and 3 developed wet AMD in the 18-month follow-up after the measurement [22]. Nolan et al. reported that age, family history, smoking, diet poor in carotenoids and hypercholesterolemia might have association with low MPOD and AMD development [10]. Obana et al. demonstrated that MPOD was lower in patients with AMD compared to healthy individuals and it was lower in cases with advanced AMD than in cases with early AMD. In addition, it was observed that the disease progressed more rapidly in cases with low MPOD [23]. Seddon et al. showed that highly dietary L-Z intake reduces the risk of AMD. [24] Richer et al. reported that lutein and antioxidant support provided improvement in MPOD, visual acuity, contrast sensitivity and amsler grid test in cases with AMD, and emphasized that MPOD is associated with AMD. [25] In the literature, there are also studies reporting that MPOD is not associated with AMD [26].

Berendschot et al. compared patients with AMD at different stages and healthy controls, and reported that there was no significant difference between the groups in terms of MPOD, therefore MPOD was not associated with AMD. [27] Kanis et al. argued that MPOD is not protective against early AMD and there is no relationship between MPOD and the development of early AMD [28]. Although many studies have been conducted to investigate the relationship between MPOD and AMD, no consensus has been reached yet, and the relationship between MPOD and AMD is still controversial.

In this study, we evaluated the factors affecting MPOD. Different results were found in many studies conducted to investigate the effect of gender on MPOD. In our study, we found that gender had no effect on MPOD. There are several studies in the literature that have same results [27, 29]. However, there are also studies reporting that MPOD is higher in men than in women [10, 30].

Different results have been reported in the studies about the effect of age on MPOD. In our study, when the patient and control groups were evaluated together, MPOD decreased with advancing age. Just like us, Hammond et al. examined 217 cases between the ages of 17–92 and showed that MPOD decreased with advancing age [28]. Kaya et al. emphasized that MPOD is not affected by age in healthy individuals, but decreases with advancing age in patients with AMD [31]. Demirel et al. reported that there was no relationship between MPOD and age in healthy individuals [32].

Today, it is known that smoking increases oxidative stress in tissues and a risk factor for AMD. It has been suggested that smoking can reduce MPOD by increasing oxidative stres. We found that MPOD was significantly lower in current smokers and ex-smokers compared to those who never smoked. However, we did not observe a significant difference between those who quit smoking and still smoke. These results were compatible with the literature. In most of the studies MPOD was found to be lower in smokers than in non-smokers [10, 33].

It is known that alcohol use accelerates atherosclerotic changes in tissues and increases oxidative stress. Therefore, it was thought that it could be a risk factor for AMD as well as be related to MPOD level. However, studies on this subject have not found a relationship between alcohol use, AMD and MPOD levels [2, 10]. We also did not found any difference between the patient and control groups and did not found a relation between alcohol use and MPOD in our study.

Most of the carotenoids in our body are stored in adipose tissue. Therefore, BMI is thought to affect the carotenoid concentration in the retina. In our study, the mean BMI in the patient group was significantly higher than the control group, and the majority of the patient group was overweight. This finding was consistent with the literature and supported the idea that obesity is a risk factor for AMD [2]. In our study, we also evaluated the relationship between BMI and MPOD. We found that MPOD in overweight and obese individuals were significantly lower than normal weight individuals.

Many studies in the literature have reported that obesity affects MPOD [34–36]. In the studies, the effect of obesity on MPOD was attributed to two reasons: Adipose tissue is thought to compete with the retina for uptake of L-Z from the blood and the diets of obese people contain less L-Z due to poor eating habits [35, 36].

We found that the iris color was significantly lighter in the patient group compared to the control group, and we concluded that light iris color is a risk factor for AMD.

This finding is compatible with the literature [2]. When we examine the relationship between iris color and MPOD; we found that MPOD in light iris colors was significantly lower than in dark iris colors. Like us Hammond et al. showed that MPOD decreased significantly as the iris color lightened [37].

L-Z are not synthesized in the body, they are only taken by food. In this study we found that MPOD was significantly lower in those fed a diet low in L-Z than those fed a diet rich in L-Z, and there was a more dramatic increase in MPOD in the group fed a poor diet after lutein supplementation. There are many studies in the literature reporting similar results [38–40].

The information obtained from all these studies suggests that a diet rich in L-Z will increase MPOD, reduce oxidative damage to the retina and have a very important place in protection from AMD.

Today, family history is a known and accepted risk factor for AMD. In this study, the presence of family history in the patient group was significantly higher than in the control group. We found that MPOD was lower in those with a family history of AMD than in those without. Just like us, Nolan et al. in their study

on the relationship between AMD risk factors and MPOD on 828 healthy individuals; reported that MPOD was significantly lower in individuals with a family history of early or late stage AMD compared to individuals without [10].

As known, the source of MP is foods and diet is very effective on MPOD. Nowadays, the only treatment approach recommended for dry AMD is multivitamin support. Based on this, many studies have evaluated the effect of L-Z supplementation on MPOD in AMD patients. In this study, we found that MPOD increased significantly in both 6mg/day and 10mg/day lutein groups compared to pretreatment, and the increase was more dramatic in cases with low baseline MPOD values.

In different studies conducted with HFF, which is the most commonly used psychophysical method today, there was a significant increase in MPOD with lutein support of 5 mg/day and above, and disease progression slowed down. It has been reported that there is a greater increase in MPOD as the dose of lutein used increases [8, 25, 41–46] .In the 'LUNA' study using the FAF method, a significant increase was found in the mean MPOD of AMD patients with 12mg/day lutein and 1m/day zeaxanthin supplementation. The authors also noted that individuals with low baseline MPOD show more dramatic increases after treatment [46]. Also in different studies conducted with objective methods such as RS and FR, a significant increase in MPOD was reported with lutein supplementation of 10 mg/day and above [47–49].

In our study, we did not detect any relationship between FAF patterns, progression of these patterns, or new pattern formation and MPOD. There are no studies on this subject in the literature.

It was observed that the type or density of drusen and the accompanying pigment epithelial atrophies did not have any effect on MPOD and MPOD changes during the study. In the literature, there are studies reporting that MPOD in early AMD is higher than in advanced AMD [23]. One of the limitations of our study is patients with advanced AMD were not included in the study, and therefore the evaluation could not be made.

No significant difference was found between the mean MPOD of the patients who had advanced AMD (GA or wet) in the fellow eye and who had not. However, baseline mean MPOD of both groups were lower than healthy controls. Beatty et al. reported that MPOD of the healthy eyes of the patients who had advanced AMD in the other eye was lower than healthy controls [22].

In our study, we attributed the absence of any difference between the groups to the dry AMD in all eyes.

We did not observe any progression in disease stage during the study.

We think that this situation is related to the lutein support patients receive. Similar results have been found in important studies such as LAST and LUTEGA in the literature [25–29].

When we compared individuals without cataracts, with grade 1 or 2 cataracts and pseudophakic, we found no significant difference about baseline MPOD. However, when we evaluated the MPOD changes

after lutein supplementation in the 1 year follow-up; we observed that MPOD increased in individuals without cataracts and had grade 1 or 2 cataracts, and decreased in pseudophakics. We found that there is a statistically significant difference between the increase in grade 1 cataracts and the decrease in pseudophakics. Demirel et al. reported that MPOD was lower in pseudophakic individuals than in phakics, and decreased more as the time passed after the operation increased [32].

There are many psychophysical methods used for measurement of MPOD. These methods work on the same basic principle. Compare the patient's sensitivity to light of wavelengths that are absorbed (blue) and not absorbed (red, green) by the macular pigment. One of these methods is HFF and it has been widely used in related studies up to now. CP is a newer psychophysical method. There are few studies conducted with this method in the literature. Reliability and reproducibility are very important in evaluating the effectiveness of these methods. Reliability is defined as the consistency of repeated measurements or the repeatability of measurements. Intra- and inter-assessment reproducibility is evaluated with the intra-class correlation coefficient and the Bland Altman chart. In our study, we evaluated the reproducibility of the CP technique with 3 different measurements made in healthy controls and found the ICC of the method as 0.669 that demonstrates low agreement between the results. Bland-Altman plots of data for days 1 to 2 indicated low agreement between the measurements. The best agreement was between the last two measurements and the changes between days 1 and 3 were in the 95% confidence interval. We tought that this moderate agreement might be related to the learning effect of the test. The test-retest variability with CP could be variable in AMD patients until they get used to do it. In a study conducted with the same method in the literature, MPOD was found to be 5.59 ± 2.06 dB in dry AMD patients and 5.97 ± 2.14 dB in the healthy controls [50]. Studies have reported that MPOD is 0.44 log units with HFF, 0.41 log units with FAF, and 0.60 log units with FR [12, 13, 15].

CP is a quick and easy method; no pupillary dilatation is required. Using more than one test point in the periphery is another advantage. In the presence of more than one measurement of the same patient, it is possible to perform follow-up and progression analysis. The fixation of the patient can be followed by the person performing the test by the camera system. However, just like in other psychophysical tests, it is not possible to apply it to every patient since it is a method dependent on the patient's compliance. It cannot be applied to children, individuals with mental problems or who do not have sufficient visual acuity and visual field.

Learning to do the test takes time. In this respect, objective methods are superior to all psychophysical tests, including CP. In this study, in which we used the CP, we obtained results consistent with studies in the literature in which other psychophysical tests or objective methods were used.

Declarations

Conflict of interest statement:

The authors declare that there is no conflict of interest regarding the publication of this paper.

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All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Feyza Çalış Karanfil], The first draft of the manuscript was written by [Feyza Çalış Karanfil] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Informed Constent:

Written and verbal informed consent was obtained from all individual participants included in the study

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Figures

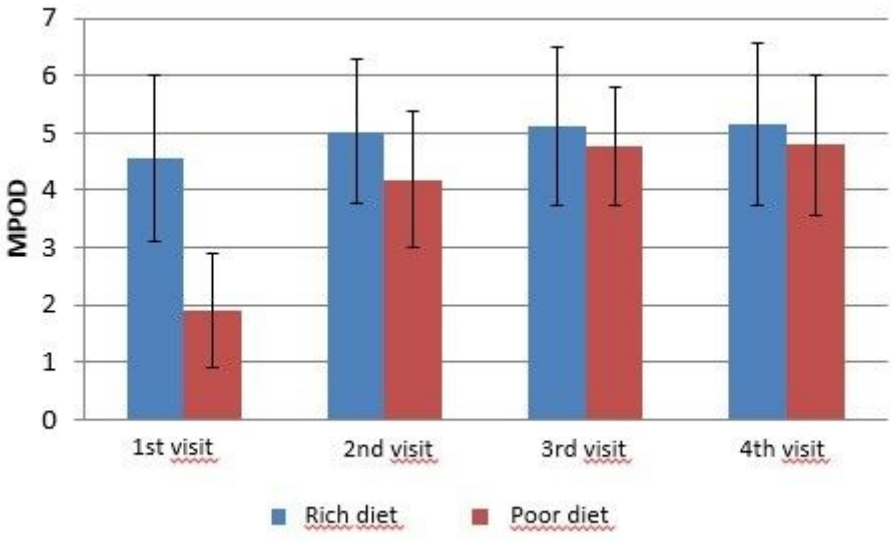

Figure 1

Mean MPOD in patients fed lutein rich and poor diets

Figure 2

Mean MPOD values of the 6mg/day or 10 mg/day lutein users and patients

newlys started lutein

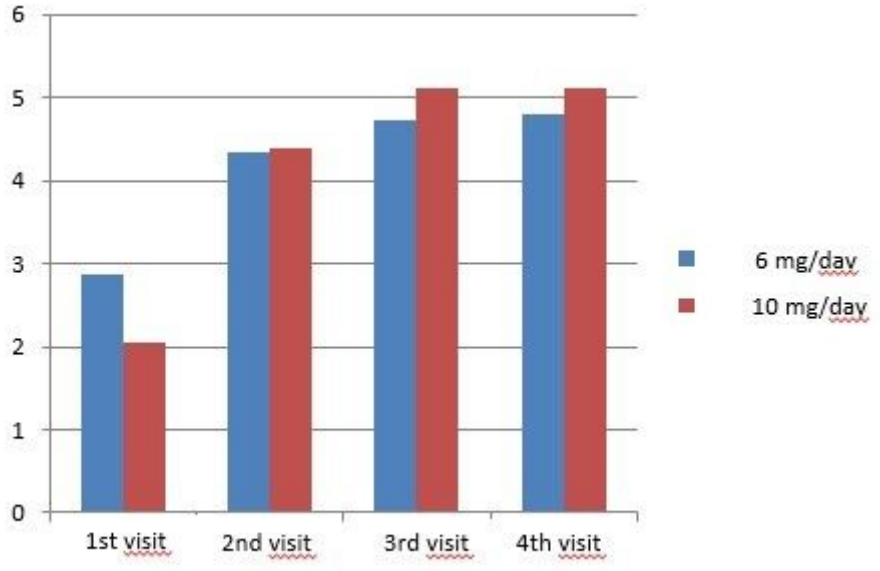

Figure 3

MPOD changes in the 6 and 10 mg/day lutein groups

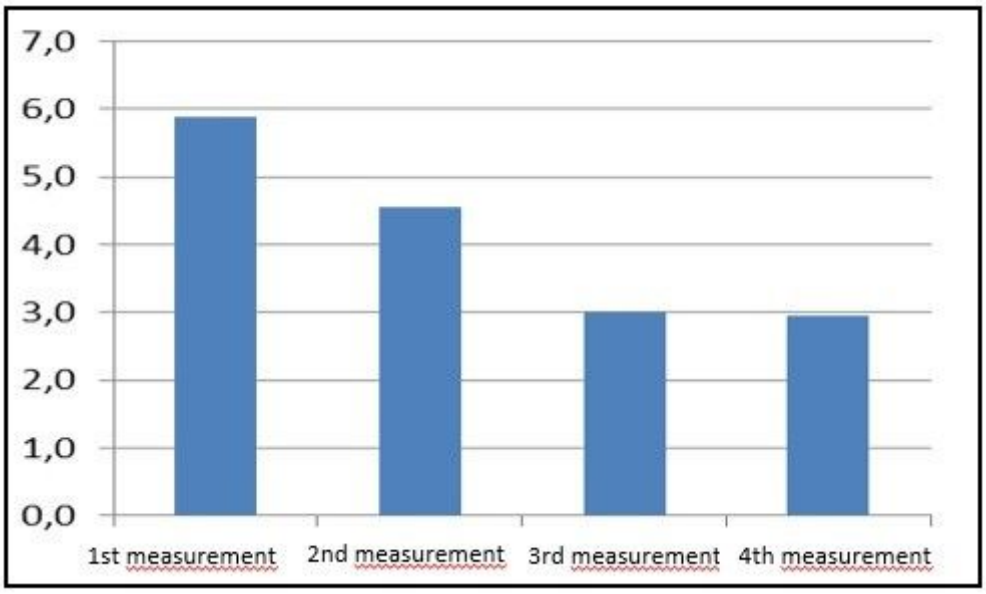

Figure 4

MPOD of 4 patients who did not use multivitamins

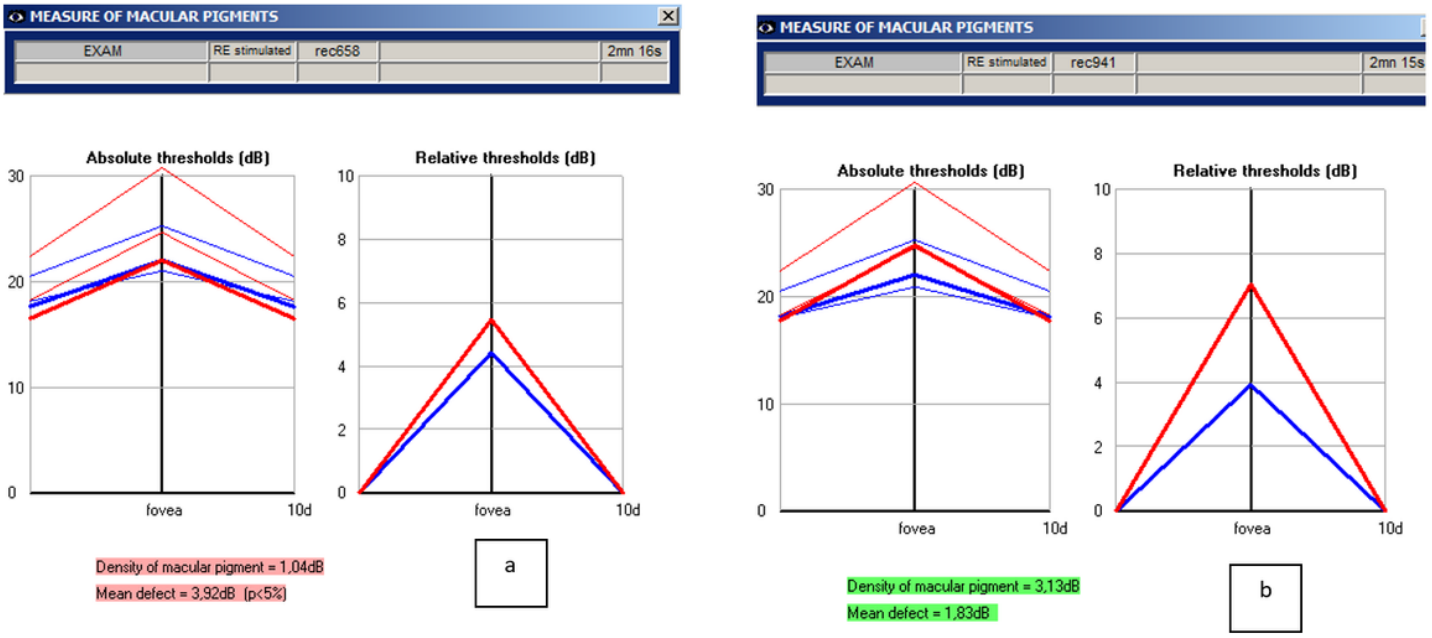

Figure 5

a MPOD of a patient fed a diet low in lutein before multivitamin use

b MPOD of the same patient fed with a lutein-poor diet after 6 months of multivitamin use