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Macular pigment optical density after panretinal photocoagulation

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Mustafa Doğan* MD
Bünyamin Kutluksaman† MD
*Department of Ophthalmology, Afyonkarahisar Health Sciences University, Afyonkarahisar, Turkey
†Department of Ophthalmology, Kahramanmaras Necip Fazil City Hospital, Kahramanmaras, Turkey
E-mail: mustafadogan@yahoo.com

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Clinical relevance: Panretinal photocoagulation, an important treatment method in diabetic retinopathy, can affect macular pigment optical density, which has protective and antioxidant properties. As a result of this effect, the retina may become more sensitive to high-energy visible light.

Background: The current study assesses the effect of panretinal photocoagulation treatment on macular pigment optical density, which has essential functions for the retina.

Methods: In this prospective clinical study, the colour perimetry method was used to measure macular pigment optical density. Thirty-six eyes of 36 participants with severe non-proliferative diabetic retinopathy without macular involvement were included in the study. Conventional panretinal photocoagulation treatments were applied at baseline, one month, two months, and at three months to the participants who clinically required this treatment. Macular pigment optical density and retinal thickness measurements were performed at baseline, months one, two, three and six.

Results: The mean macular pigment optical density reduction in the fovea over the six months was 0.02 ± 0.02 logarithmic units ($p < 0.001$). Similarly, the pericentral areas declined by 0.04 ± 0.03 logarithmic units ($p < 0.001$). Mean central macular thickness and foveal thickness increased by 5.03 ± 5.02 μm and 2.78 (interquartile range 2–4) μm, respectively. In this study, correlation analysis shows that the laser energy applied was significantly and strongly correlated with reductions in macular pigment optical density (for the fovea and pericentral area respectively: $r = -0.855$, $p < 0.001$; $r = -0.895$, $p < 0.001$). Further, there were significant and strong correlations between the applied laser energy, and central macular thickness and fovea thickness ($r = 0.751$, $p < 0.001$; $\rho = 0.718$, $p < 0.001$, respectively).

Conclusion: Panretinal photocoagulation may potentially cause a decrease in macular pigment density in proportion to the laser energy applied.

Key words: central macular thickness, diabetic retinopathy, foveal thickness, macular pigment optical density, panretinal photocoagulation

Diabetic retinopathy is one of the leading causes of visual impairment and blindness, especially in developing countries.[1] The main features of diabetic retinopathy are increased vascular permeability, capillary congestion, and new vessel formations.[2] Diabetic retinopathy may result in severe irreversible complications such as macular oedema, vascular vitreoretinal bands, or retinal detachment in later stages. The generally accepted classification of diabetic retinopathy is 'the international clinical disease severity scale for diabetic retinopathy'.[3] With this classification there are five stages; the first stage that requires photocoagulation is severe non-proliferative diabetic retinopathy.[4] There are main fundus criteria to determine in the diagnosis of severe non-proliferative diabetic retinopathy: microaneurysm and haemorrhage in all four fundus quadrants, venous beading in two quadrants, and intraretinal microvascular abnormality in one quadrant.[3] However, long-term visual acuity can be good in severe non-proliferative diabetic retinopathy patients who are treated and have reasonable glycaemic control.[5]

Panretinal photocoagulation (PRP), one of the essential treatment methods for the prevention of diabetic retinopathy progression, reduces the need for intravitreal injection and vitreoretinal surgery.[6] Although PRP is a widely accepted treatment method, some undesirable results may occur.[7] In the literature, there have been suggestions that PRP treatment increases oxygen flow through the inner retinal layers, but it may also cause a decrease in retinal blood flow.[5] PRP treatment can cause serious complications such as macular oedema which can affect visual acuity, or severe retinal detachment which can cause loss of vision.[8,9] Numerous reports in the literature have suggested that complications may occur after PRP, such as the mechanical or thermal effect of PRP to the vitreous gel, or increased permeability in vascular vessels secondary to retinal inflammation.[8,9]

The macular pigment, which is one of the important structures of the retina, is located very intensely around the fovea, and its density decreases gradually toward the periphery.[10] The human macular pigment consists of isomeric carotenoids containing lutein,

zeaxanthin, and meso-zeaxanthin.[10] Lutein and zeaxanthin cannot be synthesised in the human body, whereas meso-zeaxanthin can be synthesised from lutein in humans.[11] Macular pigment resides in photoreceptor axons, inner plexiform layer, retinal pigment epithelium, and outer photoreceptor layers. They protect the retina by its antioxidant effect against high-energy visible lights and by absorbing blue light that has a destructive impact on the retina.[12,13] They also play a role in functional outcomes such as contrast sensitivity and reduction of night glare.[14] Recently, literature has suggested that macular pigment optical density (MPOD) can be affected in diabetic retinopathy as well as many conditions.[15]

One of the methods that can measure MPOD is heterochromatic flicker photometry. There are many studies performed with this method in the literature, and they show that heterochromatic flicker photometry is one of the most preferred ways with its non-interventional and reproducible features in clinical studies.[16,17] For detecting the change in a certain process, these trials have been supporting that heterochromatic flicker photometry is a safe method.[17]

Although PRP has an essential place in diabetic retinopathy, it contains many secondary retinal effects. One of these is the possible potential effects on macular pigment, which has substantial functions on the macula and retina. The potential indirect effects of PRP, including the direct thermal effect or secondary inflammation on the retina are known.[9] In this study, we aimed to investigate the effect of PRP on MPOD in cases with severe non-proliferative diabetic retinopathy without macular pathology and to determine changes in some ocular parameters.

Methods

Ethics approval

The clinical research ethics committee approved this study, and it was performed following the Helsinki Declaration principles (2013, Fortaleza, Brazil) (2011-KAEK-22015/323). This study was also recorded with the number of NCC03150654 by www.clinicaltrials.gov, the United States National Library of Health clinical research database affiliated to the National Institutes of Health. All participants received detailed information about the clinical practices and tests to be performed before participating in the clinical trial, and all participants who agreed to participate signed informed consent forms for the study.

Participants

Between January 2015 and July 2016, 36 eyes of 36 participants who had been newly diagnosed with severe non-proliferative diabetic retinopathy and scheduled to be treated with PRP, were included in this study. If both eyes of volunteers met the inclusion criteria, only one eye of each participant was included in the assessment. Side selection was made to ensure the equality of sides in turn.

This clinical trial was carried out in the Department of Ophthalmology at Afyon Kocatepe University Hospital. Severe non-proliferative diabetic retinopathy was diagnosed based on 'the international clinical disease severity scale for diabetic retinopathy.' Heidelberg Spectralis fundus fluorescein angiography (Heidelberg Engineering Inc, Baden-Württemberg, Germany) provided characteristic findings of diabetic retinopathy. Eyes with macular fluid or oedema as assessed by optical coherence tomography (Cirrus HD 4000; Carl Zeiss Meditec AG, Thuringia, Germany) were not included in the study.

Ophthalmological examinations

Best-corrected visual acuity examination was measured as the logarithm of the minimum angle of resolution (logMAR) for the convenience of statistical analysis (VisiChart; Topcon Medical Systems, Tokyo, Japan). Later, slitlamp examination was performed after maximum pupil dilation was achieved with 1% tropicamide and 2.5% phenylephrine eye drops. The eye examination was performed using a 90-dioptre indirect non-contact fundus lens, and the findings were recorded.

After application of mydriatic agents to achieve pupil dilatation with a diameter of at least 7 mm, MPOD levels were measured using luminance differential threshold test (colour perimetry technique) at baseline, before PRP, and every month until the end of the study (Metrovision Inc, Nord, France).

The colour perimetry measurement method, which is a technique developed in France, is very similar to the heterochromatic flicker photometry technique. Both techniques are based on similar operating principles. One of the essential differences

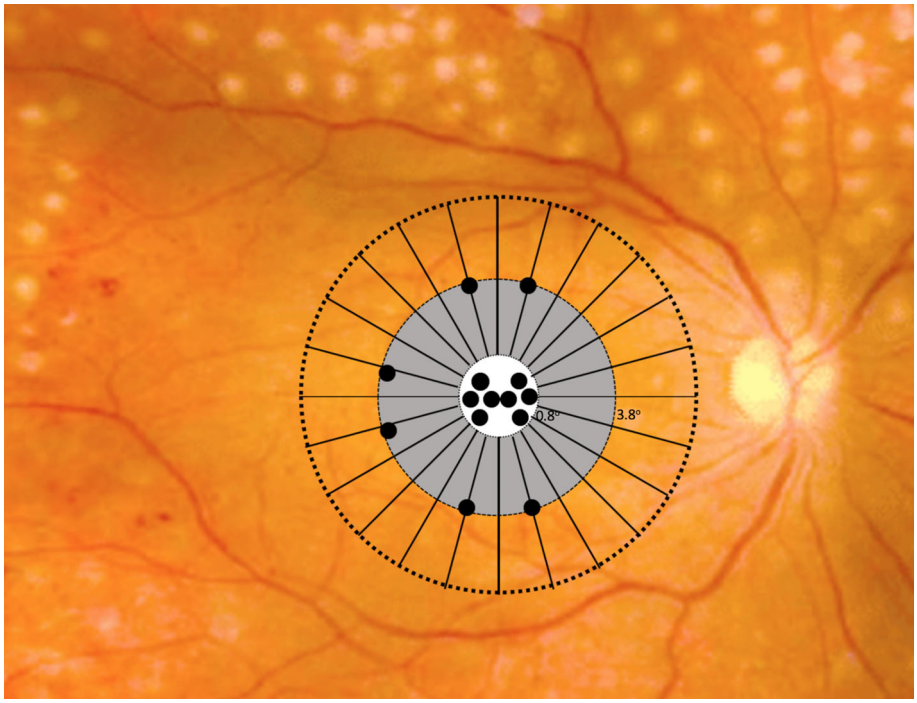

Figure 1. The localisations of the lights reflected on the retina to measure macular pigment density. There are eight reference points in the foveal area in the centre and six reference points in the surrounding pericentral area.

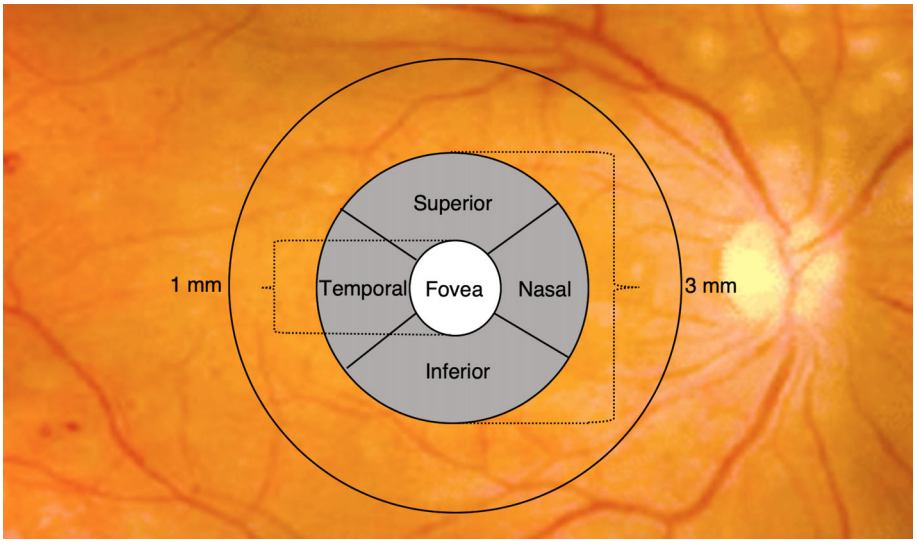

Figure 2. Representation of macular thicknesses measured by optical coherence tomography. The retinal thickness of the 1 mm diameter circular area centred in the foveola is represented as foveal thickness. In comparison, the retinal thickness of the annular region, including the 2 mm wide circular pericentral area, is referred to as the central macular thickness.

Age, mean (range), years	58.1 (IQR 54.5–63.0) (40–65)
Male/female, n (%)	18 (50) /18 (50)
Right eye selected for study, n (%)	19 (53)
Duration of diabetes mellitus, mean (range), years	7.86 ± 2.21 (4–12)
Best-corrected visual acuity, mean (range), logMAR	0.25 (IQR 0.2–0.3) (0.2–0.3)
MPOD, mean (range), log units	
Fovea	0.63 ± 0.06 (0.54–0.78)
Pericentral	0.54 ± 0.06 (0.45–0.69)
Refractive error,† mean (range), dioptres	
Spherical	0.52 ± 0.56 (0–2.25)
Cylinder	0.25 ± 0.33 (0–1.25)
Central macular thickness, mean (range), µm	293.0 ± 19.9 (237–323)
Central foveal thickness, mean (range), µm	152.70 ± 6.19 (142–164)

IQR: interquartile range, MPOD: macular pigment optical density.
†Absolute value of refractive error (dioptre).

Table 1. Baseline characteristics of the enrolled patients (n = 36)

between them is that while the colour perimetry technique uses blue and red light to measure macular pigment, the heterochromatic flicker photometry uses wavelengths of green to yellow instead of red light.[17,18] In summary, this MPOD measurement method is based on the principle of comparing the absorbable blue light and non-absorbable red light reflected from the device on the retina and back onto the device. In the first stage, to evaluate the effect of intraocular structures on the measurements, back reflections from the peripheral retina where macular pigment is lacking are measured. After the impact of intraocular structures is measured, the same procedure is performed for the fovea and pericentral regions where macular pigment is present. Therefore, two stimuli measure the luminance differential thresholds: while macular pigment absorbs blue light reflected on the retina (450–480 nm), those do not absorb red light (615 nm). Reflections were applied to eight points in the fovea and six points in the pericentral region (Figure 1). While the 0° retinal eccentric ring was the referred fovea, the mean values of 0.8°–3.8° eccentric rings were accepted as the pericentral region reference point. Finally, similar to automatic perimetry, the standard deviation from the mean gender and age matched from the community values in the comparative measurements database is calculated.

The macular thickness of the participants was measured using an optical coherence tomography device. The device's software automatically calculated foveal and central macular thickness. According to the retinal map of the software, the retinal thickness of the 1 mm diameter circular area centred foveola was presented as the foveal thickness, and the retinal thickness of the circular area, including the pericentral region around the 2 mm wide fovea, was presented as the central macular thickness (Figure 2). Optical coherence tomography measurements were measured and recorded at first, second, and third months before PRP laser applications and finally in the sixth month.

Panretinal photocoagulation

Conventional PRP treatments (Ellex Medical Lasers, Adelaide, Australia) were performed using a quadraspheric lens (Volk Optical Inc, Mentor, OH, USA) under topical anaesthesia at baseline, first, second, and third months. PRP parameters were the power of range between 100 to 250 mW, spot sizes of 200–400-500 µm, and 0.1–0.2 seconds pulse options. The spot separation was set at 0.5 times the burn width. When necessary, temporary changes in parameters were made, including laser power. All total values of laser parameters, including the laser energy applied, were recorded in all applications. The resulting millijoules value was converted to joules (J) and used for statistical evaluation.

Values of MPOD and applied laser energy of all participants at baseline, first, second, third months, and only the MPOD value in the sixth month were measured and recorded before the laser applications. During study visits, changes in patients' eating habits were questioned. All participants were told to continue their regular diet, and it was confirmed that they did not use lutein- or zeaxanthin-containing supplements.

Number of shots,[†] n, mean (range)	3,882 ± 264 (3,448–4,327)
Total area of laser spots,[‡] mm^2, mean (range)	762.0 ± 51.9 (677–849)
Total duration,[§] seconds, mean (range)	388.0 ± 26.4 (344–432)
Total energy,[¶] joules, mean (range)	97.0 ± 6.7 (86–108)

PRP: panretinal photocoagulation.
[†]Total number of laser shots applied to retina during the study.
[‡]Total area of laser spots applied to retina during the study.
[§]Total duration of laser PRP shots applied to retina during the study.
[¶]Total energy of laser PRP applied to retina during the study.

Table 2. PRP parameters of the enrolled patients during the study (n = 36)

	Mean, log unit	p-value
MPOD fovea		
Baseline	0.64 ± 0.06	
1 month	0.62 ± 0.06	< 0.001[†]
2 months	0.62 ± 0.06	< 0.001[†]
3 months	0.62 ± 0.06	0.27[†]
6 months	0.61 ± 0.06	0.12[†] – < 0.001[‡]
MPOD pericentral		
Baseline	0.55 ± 0.06	
1 month	0.52 ± 0.07	< 0.001[†]
2 months	0.51 ± 0.07	< 0.001[†]
3 months	0.51 ± 0.07	< 0.05[†]
6 months	0.50 ± 0.07	< 0.05[†] – < 0.001[‡]

MPOD: macular pigment optical density.
[†]Mean change from previous control.
[‡]Mean change from baseline at 6 months.

Table 3. The mean MPODs in the fovea and pericentral region during the study (n = 36)

	Mean, µm	p-value[†]
Central macular thickness		
Baseline	293.02 ± 19.99	
6 months	296.86 ± 21.48	< 0.001
Central foveal thickness		
Baseline	239.75 ± 6.19	
6 months	242.53 ± 6.37	< 0.001

[†]The mean change from baseline.

Table 4. The mean central macular and foveal thicknesses during the study (n = 36)

Inclusion and exclusion criteria

Inclusion criteria in the study were: age of volunteer participants between 45–65 years (40 ≤ age ≤ 65), the first time to be diagnosed as having severe non-proliferative diabetic retinopathy, planning of initiation of PRP treatment, best-corrected visual acuity ≤ 0.4 logMAR. Exclusion criteria were: the presence of a corneal scar, cataract or intravitreal haemorrhage affecting the appearance of the fundus, presence of macular pathology such as age-related macular degeneration or choroidopathy, detection of macular fluid or oedema in optical coherence tomography or fundus fluorescein angiography, focal or grid photocoagulation requirement, refractive or vitreoretinal surgery history, spherical refractive error ≥ 6.00 dioptres or cylindrical refractive error ≥ 3.00 dioptres, supplemented food consumption containing lutein or zeaxanthin, apparent changes in dietary habits (vegan or vegetarian diet or special diet), and the presence of chronic gastrointestinal disease, which may affect food absorption.

Statistical analysis

The mean values of the normally distributed data in the text and tables are presented with the standard deviation (mean ± standard deviation). Mean values that did not fit the normal distribution are shown as the median and interquartile range (IQR). The normality in the variables was tested using the Shapiro–Wilk test as the number of participants was less than 50. Repeated measures of variance analysis was performed to assess the intra-group change in the process where there were more than three measurements in the dependent group (with Bonferroni correction), and the p-value of significance was accepted as 0.05 ($p < 0.05$). The paired comparison of the mean values of the groups that conform to the normal distribution was made using the paired sample t-test. Correlations between parameters were evaluated by bivariate correlation analysis. In cases where both parameters had normal distribution features, the Pearson test was used as a parametric test for correlation analysis (Pearson's r). In cases where at least one of the groups did not have a normal distribution feature, the Spearmen test was used for correlation analysis (Spearmen's ρ). Correlation co-efficient value > ±0.6 was considered as a strong relationship, and the p-value of significance below 0.5 level was considered as a significant relationship. The data collected from the participants were coded and transferred to the computer software. SPSS 20.0 software (SPSS Inc, Chicago, IL, USA) was used for statistical evaluation.

Results

Thirty-six eyes of 36 participants who had no previous PRP treatment and were diagnosed with severe non-proliferative diabetic retinopathy for the first time were

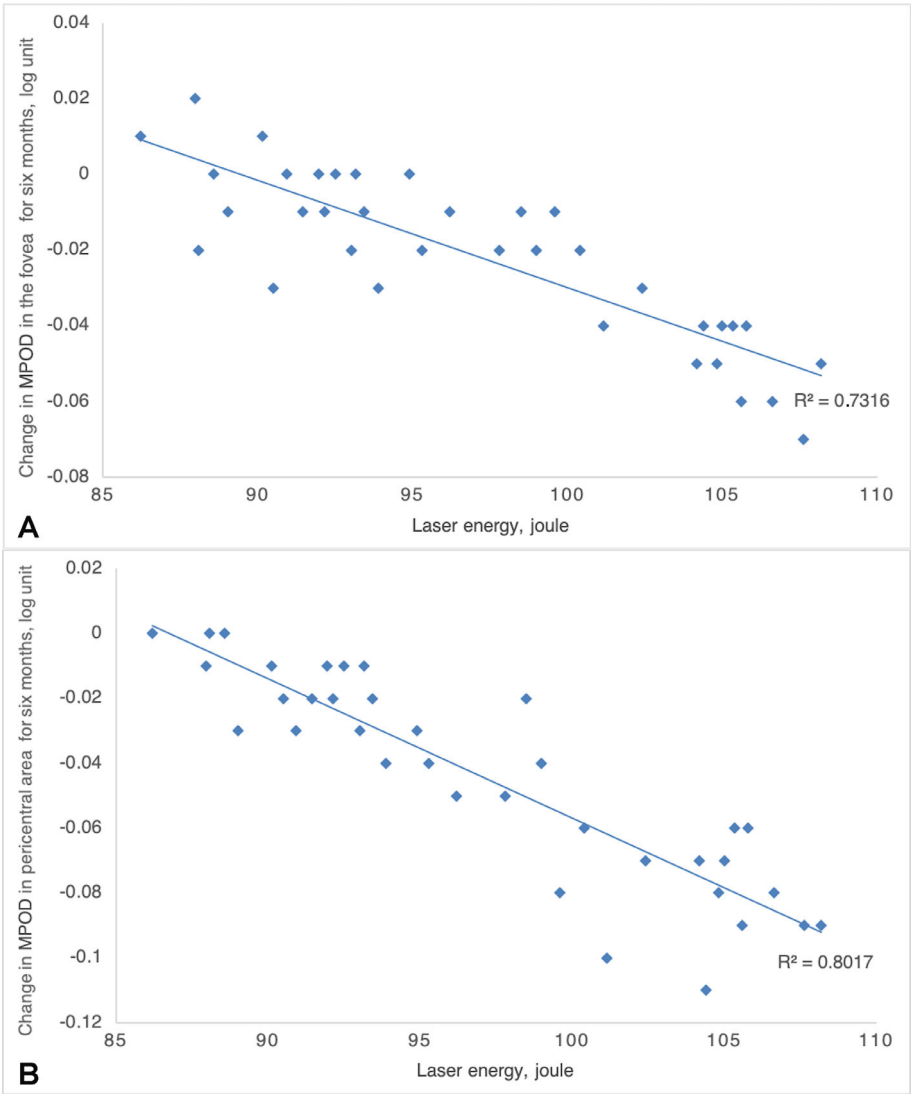

Figure 3. A: Correlation graph between change in macular pigment optical density (MPOD) in the fovea over six months and total laser energy applied (Pearson's r = −0.855, p < 0.001, n = 36). B: Illustration indicating the relationship between change in MPOD in pericentral area for six months and total laser energy applied (Pearson's r = −0.895, p < 0.001, n = 36).

evaluated, and all participants completed the planned PRP treatments and follow-up controls. The average follow-up time of the participants was 184.2 ± 3.36 days (range 180–191 days). Table 1 shows the initial characteristics of the participants evaluated.

Table 2 presents the PRP laser parameters. During the study, undesirable side effects such as retinal detachment, cystoid macular oedema, secondary retinal inflammation, or intraocular haemorrhage which may be seen after PRP treatment were not observed in any of the participants. During the study, two patients experienced temporary increased intraocular pressure after PRP, but the treatment of brinzolamide 1% eye drops two times a day for three days reduced increased intraocular pressures.

Macular pigment optical density

Table 3 shows the average MPOD values in the process for the foveal and pericentral region. Repeated measures of variance analysis suggested that mean MPOD values in the fovea and pericentral regions decreased significantly over the six months ($p < 0.001$) (Table 3). The reductions in MPOD values for the foveal and pericentral areas during the six months were 0.022 ± 0.02 logarithmic units and 0.041 ± 0.03 logarithmic units, respectively. While the MPOD decrease in the fovea was significant during the first two months, it did not change significantly during the next four months. The mean MPOD values in the pericentral region continued to decrease continuously during the six months; this reduction was more evident in the first two months.

Central macular thickness and foveal thickness

Table 4 depicts the mean central macular thickness and foveal thickness values during the study. In this study, statistical analysis demonstrated significant increases in the mean central macular thickness and foveal thickness values from baseline to six months ($p < 0.001$). For the six months, the average increase in central macular thickness was 5.03 ± 5.02 µm, while the average increase in foveal thickness was 2.78 (IQR 2–4) µm.

Correlations

In this study, there was no significant and robust relationship between the foveal and pericentral MPODs and the duration of diabetic retinopathy (for both $r = -0.3$ and $p = 0.075$). However, correlation analysis presented in Figure 3 showed significant and strong correlations between changes in foveal and pericentral MPODs in the six-month period and total laser energies applied (respectively; Pearson's $r = -0.855$, $p < 0.001$, $n = 36$; Pearson's $r = -0.895$, $p < 0.001$, $n = 36$). When retinal thickness changes were examined, significant and strong correlations were found between changes in central macular thicknesses during the six months and total laser energies applied (Pearson's $r = 0.751$, $p < 0.001$, $n = 36$). There was also a significant and strong relationship between the changes in foveal thickness and total applied laser energies in this study (for six months: Spearmen's $\rho = 0.718$, $p < 0.001$, $n = 36$). In this prospective study, at six months, significant correlations were observed between the changes in MPODs for both regions and the changes in central macular thickness and foveal thickness (Figure 4).

Discussion

Macular pigment, which is one of the essential structures in the retina, assumes an

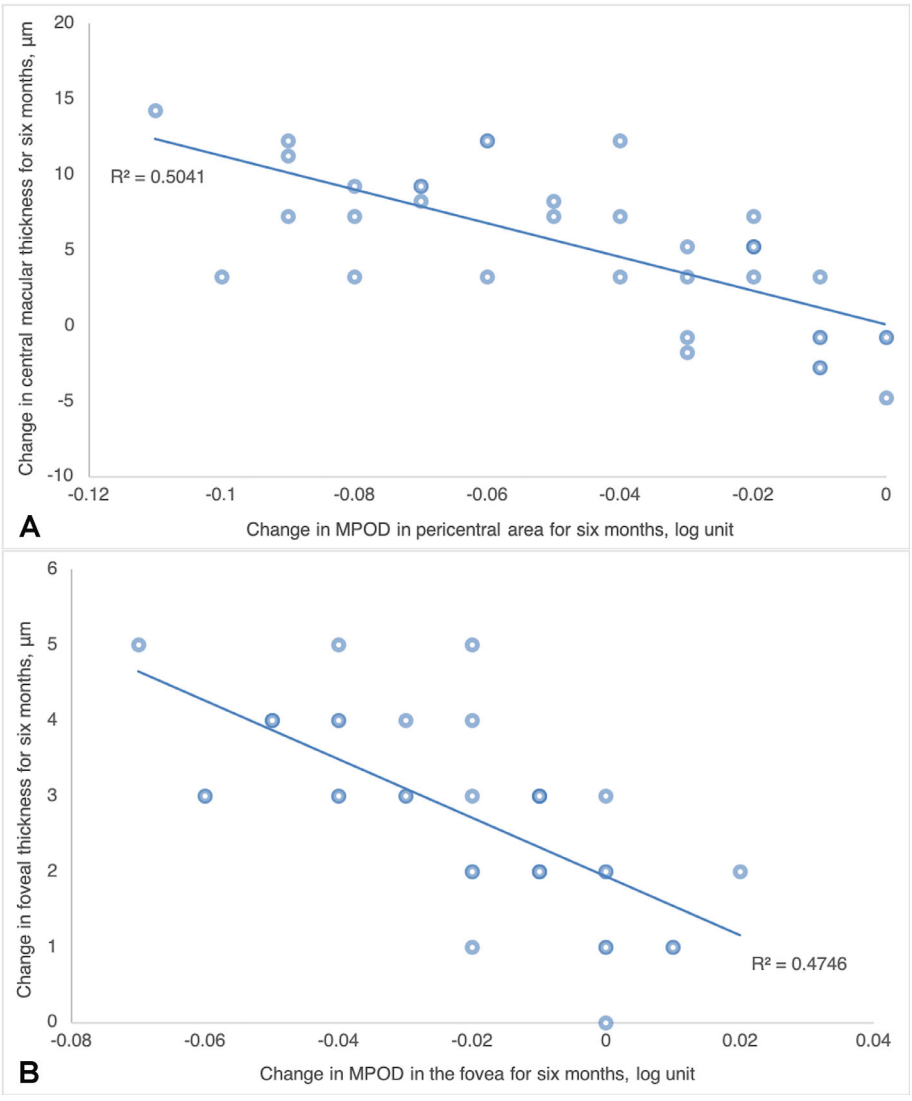

Figure 4. A: Correlation graph between changes in central macular thickness and pericentral macular pigment optical density (MPOD) over six months (Pearson's r = −0.71, p < 0,001, n = 36). Double-ring circles represent more than one case. B: Graph showing the relationship between changes in MPOD in the fovea and changes in foveal thickness. Circular rings of increasingly darker colours represent a more significant number of cases (Spearmen's ρ = −0.685, p < 0,001, n = 36).

increasingly important role in macular diseases, with its antioxidant and protective properties. Many previous studies reported the critical role of macular pigment in macular diseases such as age-related macular degeneration. In the literature, studies related to the factors that cause a reduction in MPOD except for age-related macular degeneration are limited. Recent research has shown that there is a decrease in MPOD in diabetic retinopathy.[15] Since its complex and susceptible structure, many factors that can affect macular pigment may be expected. The possibility of not only a numerical, but also structural effect on macular pigment should be borne in mind.

PRP is one of the main treatment methods in diabetic retinopathy with its feature of preventing vision loss.[6] Clinicians should note the possibilities of the indirect effect of vibration wave in the vitreous caused by PRP treatment or the direct impact of the thermal stress of laser application. As a result, it has been observed in this study that a significant decrease occurs in MPOD values after laser applications due to the effects of PRP, whether direct or indirect.

The reason for selecting cases with severe non-proliferative diabetic retinopathy in the study was to evaluate the participants who require PRP treatment and to minimise the effect of neovascularisation on the macula. Thus, the potential impact of diabetic retinopathy on macular pigment was minimised. Although PRP was applied to the non-macular retina in this study, it was observed that MPOD decreased in both the foveal and pericentral regions in participants who showed no previous macular findings. Considering this result, one of the critical findings of the study is that the MPOD decreased in the pericentral and foveal regions and correlated with the total laser energy applied.

An important topic in this study is the significant correlation between the total laser energy applied and the reduction in MPOD. This supports the thesis that the effect of laser energy used, rather than diabetic retinopathy, is more important in the decrease of MPOD.[15] Despite this relationship, it is not easy to exclude the possible contribution of diabetic retinopathy in these cases. It should be considered that diabetic retinopathy can contribute to this process. To reach a definitive conclusion in this regard, studies examining the change in MPOD over time in diabetic retinopathy at different stages and glycated haemoglobin levels are needed. Also, the average central macular thickness and foveal thickness values increased significantly at six months. In the evaluation of central macular thickness and foveal thickness changes, it seems more likely that the increase is due to increased permeability from vascular structures and secondary inflammation. Distinguishing whether reduced MPOD occurred due to either retinal inflammation or thermal damage was difficult.

In this study, many opinions can be presented about the decrease in MPOD values and the level of significance being higher in the pericentral area, compared with the fovea. (a) The reduction in MPOD in the pericentral area may be due to the direct effect of the laser, whereas the decrease in the fovea may be secondary to the impact in the pericentral region. (b) Macular pigment in the fovea may be derived from the peripheral region, and the deformed macular pigment may migrate toward the fovea from the pericentral area. Thus, the mass and density of the macular pigment in the

fovea may decrease. (c) The decrease in MPOD without a numerical and structural change in macular pigment may be due to the relative decline associated with the oedema increase in central macular thickness and foveal thickness.

Correlations detected between the changes in central macular pigment, foveal thickness, and MPOD after laser treatment may be indicative of increased oxidative stress in the macula. The issue of pathologies, such as macular atrophy or age-related macular degeneration, associated with increased oxidative stress after PRP, should also be explored in more detailed and long-term studies. Another issue that needs to be investigated is significant relationships between other pathologies or atrophy occurring in the macula and the total laser shot, area, and energy. For example, in this study, when particular values were exceeded in total laser energy, MPOD change was observed to be higher, but the transformation of this condition into macular pathology in the long term is the subject of a long-term study requiring follow-up. Further, strategies to reduce possible increased oxidative stress in the macula in the post-laser application period are worth investigating.

One of the limitations of this study is the number of participants. However, for statistical evaluation, the evaluation group conformed to the normal distribution and allowed variance analysis, which is a parametric test in terms of its ability to analyse the change in a particular process. Another limitation is that there was no gold-standard method accepted in the literature regarding the technique of measuring MPOD. However, it can be said that the heterochromatic flicker photometry method is one of the popular methods for measuring MPOD. As a variation of heterochromatic flicker photometry, it is advantageous for the colour perimetry to calculate the deviation of the determined value from the mean age- and gender-matched data. Although the device in the study has a database of approximately 8,000 people, the average deviation from the age- and gender-matched data in this study was not evaluated due to the inadequate homogeneity of the age and gender distributions in the database. This was also one of the limitations of this study. Despite those limitations, considering that the primary purpose of this study is to evaluate the change in a particular process after the application and that the strength of the statistical method used and the distribution within the group fit to the normal distribution, it can be said that the evaluation was made with a generally accepted safe method for the purpose of the study. Another limitation in the study is that although the reduction in MPOD in the process correlated highly with the laser energy applied, this situation cannot precisely exclude the effect of diabetic retinopathy. There is a need for comparative studies.

Conclusion

There are very few studies that have investigated the effect of PRP on MPOD. Our study found that PRP has a proportional impact on MPOD levels in the central and paracentral macula. The higher the amount of laser energy applied on the MPOD in the fovea and pericentral macular region the greater the loss in MPOD. It is also seen that this significant and associated decrease correlates with an increase in central macular thickness and foveal thickness, which may be compatible with secondary inflammation and increased oxidative stress.

Given the results in this study, clinicians may be advised to be aware of the risk of MPOD being affected after PRP treatment. Clinical trials with longer duration and more participants are required in terms of the clinical significance of this condition.

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