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The Prognostic Role of Early Hyperfluorescence in Cystoid Diabetic Macular Edema Treatment: Angiographic Study

Eyyup KARAHAN [1], Melek KOROĞLU CANLI [1], Gözde ŞAHİN VURAL [1], Sami YILMAZ [2], Ercan GÖMLEKSİZ [3], Cenap GÜLER [1]

[1] Balikesir University, Faculty of Medicine, Department of Ophthalmology.
[2] Bursa Retina Eye Hospital, Department of Ophthalmology.
[3] Yozgat Sorgun State Hospital, Department of Ophthalmology.

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ABSTRACT

Objective: To evaluate the predictive value of fluorescence level in fundus fluorescein angiography (FFA) images for the prognosis of diabetic macular edema (DME). **Material and Methods:** In this retrospective study, 21 eyes of 21 patients who have been treated with intravitreal injection (ranibizumab, aflibercept) for DME were evaluated. In addition to demographic features, pre/post-treatment best-corrected visual acuity (BCVA) and central retinal thickness (CRT), early-stage reflectance of fluorescence in FFA were also quantified. The prognostic role of early angiographic reflectance in response to treatment were evaluated. Groups were defined as high or low early fluorescence according to the reflectance level in early angiographic phase. **Results:** After treatment, mean BCVA was increased and mean CRT was decreased significantly ($p<0.05$). There was no significant difference between groups regarding BCVA and CRT change ($p=0.716$, $p=0.809$, respectively). The change in CRT in eyes with higher early fluorescence was significantly higher than eyes with lower fluorescence. Eyes with higher fluorescence had a wider foveal avascular zone. **Conclusion:** This study demonstrated that more vascular and cellular damage is related to higher hyperfluorescence level in the early period of FFA. The prognostic significance of this finding deserves to be evaluated with further studies.
Keywords: Diabetic Macular Edema, Fundus Fluorescein Angiography, Early Hyperfluorescence, Intravitreal Injection.

Anjiografik Erken Hiperfloresansın Kistoid Diyabetik Maküler Ödem Tedavisindeki Prognostik Rolü: Anjiyografik Çalışma

ÖZ

Amaç: Çalışmanın amacı, fundus floresein anjiografi (FFA) görüntülerindeki floresans seviyesinin diyabetik maküler ödem prognozundaki öngördürücü değerini saptamaktır. **Gereç ve Yöntem:** Retrospektif tasarımlı bu çalışmada, diyabetik maküler ödem için intravitreal enjeksiyon (ranibizumab, aflibersept) tedavisi alan 21 hastanın 21 gözü değerlendirilmiştir. Demografik özelliklere ek olarak, tedavi öncesi ve sonrası en iyi düzeltilmiş görme keskinliği (EİDGK), santral retinal kalınlık (SRK) ve FFA'da erken evre floresans düzeyi kaydedilmiştir. Erken evre anjiografik florasansın tedaviye yanıt prognozunu gösterme etkisi değerlendirilmiştir. Gruplar reflektans düzeyine gore düşük ya da yüksek floresans olarak gruplandırılmıştır. **Bulgular:** Tedavi sonrası EİDGK anlamlı olarak artarken, ortalama SRK anlamlı olarak azalmıştır ($p<0,05$). Gruplar arasında EİDGK ve SRK değişimi açısından anlamlı fark saptanmamıştır ($p=0.716$, $p=0.809$, sırasıyla). Yüksek erken floresansı olan gözlerde SRK'daki değişim düşük floresan olan gözlerden daha yüksek saptanmıştır ($p<0,05$). Yüksek erken floresansı olan gözlerde daha geniş foveal avasküler zon ölçülmüştür. **Sonuç:** Bu çalışma sonucunda FFA'nın erken döneminde yüksek floresansı olan gözlerde daha fazla vasküler ve hücresel hasar bulunduğu gösterilmiştir. Bu bulgunun prognostik önemi ileride yapılacak çalışmalarda irdelenmelidir.
Anahtar Kelimeler: Diyabetik maküler ödem, Fundus floresein anjiyografi, Erken hiperfloresans, İntravitreal enjeksiyon.

Sorumlu Yazar / Corresponding Author: Gözde ŞAHİN VURAL, Balikesir University, Faculty of Medicine, Department of Ophthalmology, Balikesir, Türkiye.
E-mail: gozdejcgrl@hotmail.com

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INTRODUCTION

Diabetic macular edema (DME) is the most common cause of visual loss in patients with diabetic retinopathy (Yau et al., 2012). Even though several hypotheses have been declared about the pathogenesis of DME, the most important mechanism is the accumulation of intravascular fluid and proteinous material in retinal interstitial tissues through vascular endothelial injury. This injury ultimately causes macular edema in various configurations (Ozaki et al., 1997). The cystic formation in retinal layers results in cystoid macular edema (CME) which is one of the macular edema types. Fundus fluorescein angiography (FFA) had been a gold standard method to diagnose DME, to detect the severity of macular edema and, to follow-up patients for years. However, after 2000s, the widespread use of optic coherence tomography (OCT) has been started and the requirement of FFA has been decreased distinctly (Wells et al., 2016). In addition to be a non-invasive method, OCT has been a breakthrough to give the opportunity to evaluate all details of retinal layers and to estimate the disease in all aspects. However, FFA is still the best method that enables to evaluate the status of retinal vascular structure dynamically. It is postulated that cystoid spaces fill rapidly with fluorescein in the early stages of FFA and higher hyperfluorescence occurs if vascular endothelial damage is severe. Beyond this, if the vascular endothelial injury is mild, the fluid in cystoid spaces blocks choroidal fluorescence and hypofluorescence occurs in the early stages (Ryan & Ogden, 1989). We hypothesized that the fluorescence level in the early stages of FFA could be related to prognosis and response to intravitreal treatment, so it could be used as a prognostic criterion in patients with CME. In the light of these findings, we aimed to evaluate the relationship between the density of early fluorescence with functional and anatomical outcomes of intravitreal injection in patients with CME.

MATERIALS AND METHODS

Study type

This retrospective study was conducted in Balıkesir University Department of Ophthalmology between May 2019 and September 2020

Study group

FFA images and recordings of 83 Diabetes Mellitus patients who have been treated with intravitreal injection for diabetic CME were included. According to strict exclusion criteria; patients with media opacities, high spherical equivalent (> ±6D), glaucoma, and retinal or ocular surgery history in the past 6 months, eyes with vitreomacular interface abnormalities were excluded. In addition to that, if the latest intravitreal injection time has not been more than 4 weeks yet, patients were excluded.

Dependent and independent variables

The independent variables of this research are gender, sex, age, pre/post-treatment best-corrected visual acuity (BCVA), central retinal thickness (CRT). The dependent variable is the mean value of fluorescence in fundus fluorescein angiography.

Procedures

Pre/post-treatment best-corrected visual acuity (BCVA), central retinal thickness (CRT) via spectral-domain OCT (SD-OCT), and early-stage (25-35 seconds) FFA images in the period of seven-days before treatment were recorded. The FA images were obtained by using FA (Visucam 500; Carl Zeiss Meditec, Jena, Germany) in early and late phases (6-10 minutes after intravenous contrast injection). There were spaces with both heterogenic and homogenous reflectivity. The optimal FFA image was chosen by two ophthalmologists (GSV, CG), and a retina specialist (EK) settled any disagreements. There was no retinal hemorrhage above the cystoid spaces, and the attention was paid not to have any hemorrhage in the cross-sectional images. The images were transferred to the Image-J program, (http://rsb.info.nih.gov/ij) (Figure 1a) and converted to 8-bit grayscale image to enhance image quality. The Image-J programme developed at the National Institutes of Health and the Laboratory for Optical and Computational Instrumentation (LOCI, University of Wisconsin) to process Java-based images (Collins, 2007; Schneider et al., 2012). The scientists may edit, analyze, process, save, display, and print 8-bit color and grayscale, 16-bit integer, and 32-bit floating point images. The quantitation of the area and pixel value statistics of user-defined selections and intensity-thresholded objects can be performed. The distances and angles are measured, and the density histograms and line profile plots can be created. The retinal vein thickness in the exit area from the optic disc was considered 150µm and a set-scale was performed. The fovea was accepted as the center and an area with a diameter of 5,500µm was determined. To quantify the reflectivity levels, the margin of each space was manually traced using the images with inverted grayscale. Within this region, the areas showing hyperfluorescence due to the cystoid spaces in the late period were determined with the polygon tool and recorded in the region of interest (ROI) tab (Figure 1a). The fluorescein pooling 3 intensity then was divided into two levels. Then, in the early period pictures, the same areas which recorded in ROI tab were marked using the 'show overlay' option (Figure 1b). The association between the level of the fluorescence and pre/post-treatment BCVA and CRT were elucidated. For post-treatment analysis, the values at the 4 to 6 weeks visit, where the CRT was the thinnest were used as post-treatment data in the study. The area of the foveal avascular zone (FAZ) was measured by using the polygon tool in Image-J program (Figure 2).

The mean value of fluorescence was determined and patients were divided into two groups according to the level of early fluorescence. Group 1 was included 12 eyes that have the reflectance value below average fluorescence level while group 2 was included 9 eyes that have the reflectance equal or above average. Group 1 and 2 were compared in terms of pre-treatment BCVA

and CRT, post-treatment BCVA and CRT, the difference of BCVA and CRT after treatment. Groups were also compared for the area of the FAZ. Structural SD-OCT findings, including ellipsoid zone (EZ) damage, external limiting membrane (ELM) damage were evaluated.

The band above the RPE-choriocapillaris line is described as the EZ, and the ELM locates above the EZ line. In this study, the "EZ damage" was defined as the "irregularity/discontinuity/absence of the EZ", and "ELM damage" as "irregularity/discontinuity/absence of the ELM" in the area of 5,500µm centered with foveal center.

Post-treatment SD-OCT images were used for evaluation of the continuity of EZ and ELM (Figure 3a and 3b).

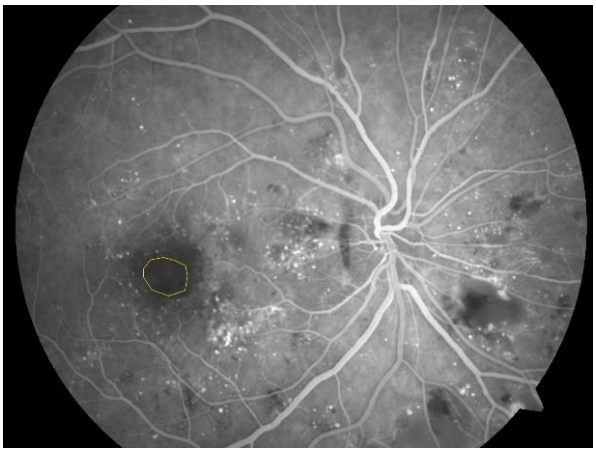

Figure 2. The foveal avascular zone bounded by the polygon tool in the Image-J program.

Figure 1a. Areas of hyperfluorescence due to cystoid edema in the late period of fundus fluorescence angiography (evaluated with Image-J).

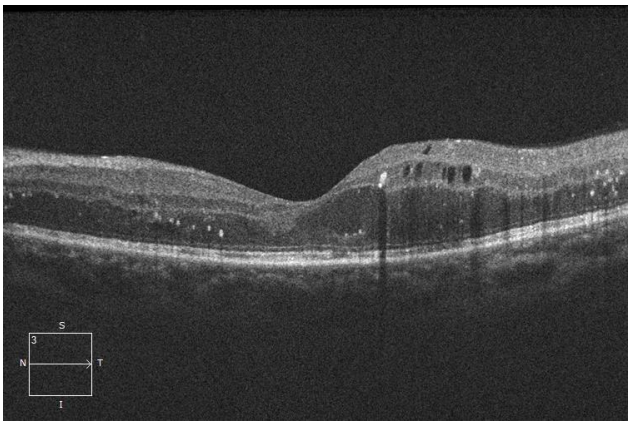

Figure 3a. Optical coherence tomography image with intact external limiting membrane and ellipsoid zone.

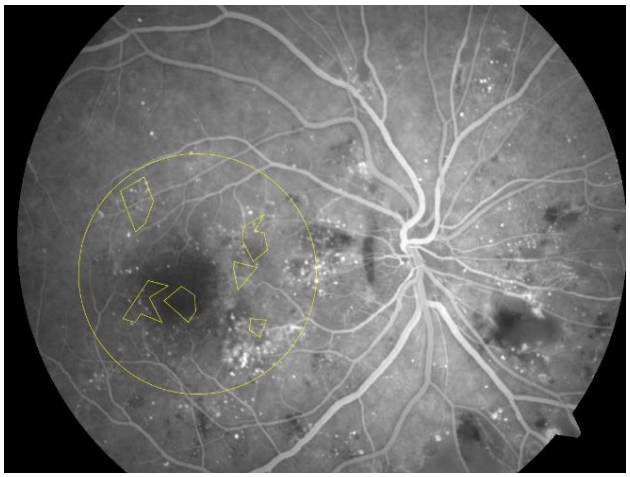

Figure 1b: Early appearance of hyperfluorescence areas marked in the late period

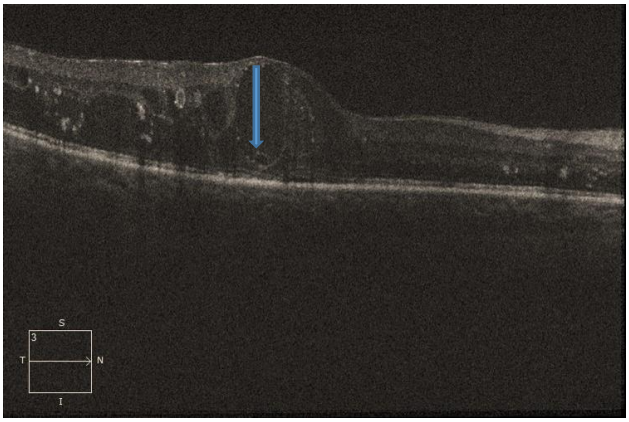

Figure 3b. Optical coherence tomography image demonstrates disrupted external limiting membrane (arrow).

Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences, for Windows V.11.5 (SPSS, Inc, Chicago, Illinois, USA). All values were reported as mean ± standard deviation (SD). BCVA in Snellen was transformed to log MAR. The association between reflectance and BCVA, CRT, area of the FAZ were evaluated by linear regression analysis. For the difference between the parameters in pre- and post-treatment examination, the paired t-test was used in dependent groups, t-test was used for differences in independent groups related to reflectance level. Chi-square test was used to compare groups for the damage of EZ and ELM.

Ethical considerations

All procedures applied in this study were in accordance with the ethical standards of the Balıkesir University Ethical Committee (Clinical Trial Number: 2020/148; 09/09/2020). The study adhered to the tenets of the Declaration of Helsinki and its later amendments or comparable ethical standards. The written informed consent was confirmed from all patients at the beginning of the procedure.

RESULTS

The mean age of 21 patients (11 men and 10 women) was 60.8±7.6 years (47 to 74 years). Nine of the eyes were treated with ranibizumab, 12 of them were treated with aflibercept. None of the evaluated data was different between the patients treated with ranibizumab and aflibercept. The mean pre-treatment BCVA was 0.50±0.24 (0.05-0.7), it was 0.32±0.29 (0.05-1.0) at post-treatment visit (p<0.001). The mean pre-treatment CRT was 430.3±134.2µm (257 to 808µm), the mean post-treatment CRT was 249.1±80.6µm (130 to 463µm), (p<0.001). There was no significant correlation between the change in CRT and the change in BCVA (r^2=0.049, p=0.802).

The mean reflectance level of 21 eyes was 43.1±27.5 (14.8-116.6). No significant correlation of reflectance with pre-treatment BCVA (r^2=0.052, p=0.931) and also with CRT (r^2=0.067, p=0.922) were obtained. There was no significant correlation between reflectance and the difference in BCVA or CRT after treatment (r^2=0.045, p=0.716, r^2=0.049, p=0.809, respectively).

There was no significant difference between two groups in terms of change in BCVA (p=0.122). The average change of CRT in group 2 was significantly higher in group 2 (p=0.024). There was no significant difference between group 1 and group 2 regarding the discontinuity of EZ and ELM (p=0.350, p=0.062 respectively). The mean area of FAZ was significantly larger in group 2 compared with group 1 (p=0.018). A moderate correlation was found between fluorescence level and the area of the FAZ (r^2=0.187, p=0.014) (Figure 4).

The comparison of evaluated data has been shown in Table 1.

Table 1. Comparison of evaluated data in group 1 and group 2.

	Group1 (n=12)	Group 2 (n=9)	p value
Age	60.7±7.1	61.1±8.6	0.972
FFA image taken at (sec)	18.9±2.7	18.0±2.4	0.471
Pre-treatment BCVA	0.34±0.26	0.30±0.21	0.167
Post-treatment BCVA	0.46±0.27	0.38±0.32	0.277
Pre-treatment CRT (µm)	411.7±99.6	456.3±173.3	0.829
Post-treatment CRT (µm)	237.4±74.1	264.7±90.6	0.706
Change in BCVA	0.12±0.22	0.09±0.16	0.122
Change in CRT (µm)	174.3±78.7	191.7±20.7.6	0.024*
EZ damage (%)	3 (25.0)	4 (44.4)	0.350
ELM damage (%)	3 (25.0)	6 (66.7)	0.062
FAZ area (µm^2)	10.8±6.1	19.3±9.1	0.018*

FFA fundus fluorescein angiography, *BCVA* best-corrected visual acuity, *CRT* central retinal thickness, *EZ* ellipsoid zone, *ELM* external limiting membrane, *FAZ* foveal avascular zone
* Statistically significant

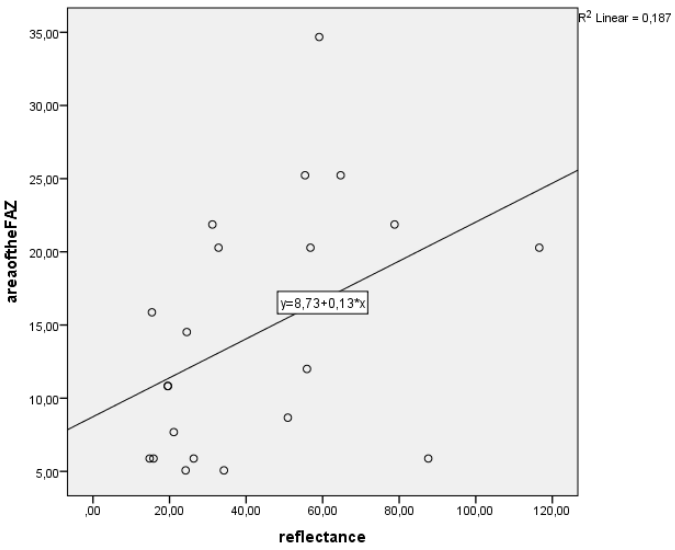

Figure 4. Regression analysis of reflectance and area of the foveal avascular zone.

DISCUSSION

Diabetic macular edema is generally treated with intravitreal anti-VEGF and corticosteroids (Boyer et al., 2014; Wells et al., 2016) Unfortunately, about half of patients do not respond to the intravitreal treatment and

the visual prognosis is not as good as predicted (Erdurman, 2013). To define the prognostic factors in response to intravitreal treatment in DME and to understand whether the patients would respond to the treatment is extremely significant for the management of the process. There are also previously defined prognostic factors that predict the transformation from non-proliferative to proliferative stage in diabetic retinopathy (Balaratnasingam et al., 2016; "Early Treatment Diabetic Retinopathy Study Design and Baseline Patient Characteristics: ETDRS Report Number 7," 1991; Fickweiler et al., 2018; Kulikov et al., 2017; Lee et al., 2016; Otani et al., 1999; Roh et al., 2010; Shimura et al., 2011; Shin et al., 2012).

Beside the benefits in diagnosis and follow-up, SD-OCT findings can also be used to predict the prognosis. Diabetic macular edema can be divided into three types according to SD-OCT findings; *i)* diffuse retinal thickness, *ii)* CME and *iii)* serous retinal detachment (Otani et al., 1999). Previous studies showed that the prognosis changes in various subtypes but results are still controversial (Fickweiler et al., 2018; Roh et al., 2010; Shimura et al., 2011). Hyperreflective bands in outer retinal layers especially EZ and ELM in OCT have a prognostic value for visual acuity outcomes. The defect in these layers gives an idea about the worse prognosis (Shin et al., 2012). Hyperreflective dots in SD-OCT shows that much more hard exudates will occur and the prognosis is poorer. In addition to the prognostic value, hyperreflective dots guide the treatment algorithm, and adding steroids to the treatment is recommended in these eyes (Shin et al., 2012). Disorganization of retinal inner layers (DRIL) in SD-OCT is also a poor prognostic factor and that appears predominantly in ischemic retinal areas. In patients with wider FAZ, DRIL is more common (Balaratnasingam et al., 2016). Tractional membranes or vitreomacular traction in OCT is a precursor sign for inadequate response to intravitreal injection and surgical approach should be given priority to eliminate tractions (Kulikov et al., 2017). Recently, optic coherence tomography angiography (OCT-A) has been performed frequently in retinal disease and it reveals the condition of inner-outer capillary plexus distinctly. There is a correlation between vascular density in OCT-A and structural stability of the retina. It can be predicted that ischemia is severe and the prognosis will be poorer in patients with reduced vascular density (Lee et al., 2016). The width of FAZ and capillary non-perfusion areas in FFA suggests that the ischemic component is excessive, so the visual prognosis will not be good. In addition, eyes with wider FAZ are tend to transform more frequently into the proliferative phase ("Early Treatment Diabetic Retinopathy Study Design and Baseline Patient Characteristics: ETDRS Report Number 7," 1991). Macular ischemia and peripheral non-perfusion in the retina guide the prognosis in conventional FFA. It is known that the visual prognosis is worse in patients with impaired FAZ, and peripheral nonperfusion is important for transition to proliferation. Both FFA and OCT-A provide information about the vascular structure, but FFA is a dynamic device and it is possible to detect fluorescent material leakage from the vessel wall in this method. This leakage occurs in cases of macular edema and neovascularization, and these findings have been used frequently. In the above-mentioned types of leakage, hyperfluorescence is more common in the late period. On the other hand, early hyperfluorescence has a value that was not considered much before. The fluorescein leakage level is related to the retinal vascular endothelium dysfunction. If the leakage is absent, the cystoid spaces fill quitely and become evident in late phases of angiography. The area of CME may become early hypoflurescence in the angiography because the fluid in the space prevents the underlying choroidal fluorescence like a barrier. If the fluorescein leakage is dense, the cystoid spaces fill rapidly (Ryan & Ogden, 1989). We excluded the images that contain hemorrhage blockage, so the hemorrhage has minimal effect on the reflectance. In the light of this information, we planned to carry out this study considering that early fluorescence level may have a prognostic significance in patients with CME.To the best of our knowledge, this issue has not been discussed previously in the literature. Even any significant correlation between the density of hyperfluorescence and the difference in BCVA and CRT after treatment has not been declared, the greater decrease in CRT after intravitreal injection in patients with high fluorescence density might be a significant finding. This finding shows that the treatment to improve endothelial permeability provides much more decrease in CRT in patients with more severe endothelial damage other than less severe endothelial damage. However, in the eyes with high fluorescence, the improvement in visual acuity does not accompany to the reduction in CRT. It is well known that there is a modest correlation between OCT-measured center point thickness and visual acuity. (Browning et al., 2007) Nevertheless, less improvement in visual acuity could be associated with more severe macular ischemia in patients with a more severe vascular endothelial injury. To reveal this relationship, the association between hyperfluorescence and area of the FAZ was evaluated and a moderate correlation was found (p=0.014). The average area of the FAZ in group 2 was 19.3±9.1 μm^2 and it was 10.8±6.0 μm^2 in group 1 (p=0.018). The greater impairment of vascular permeability may indicate that cellular functions in these patients are further impaired. In order to verify this finding, the integrity of ELM and EZ was compared between 2 groups simultaneously at the time of FFA was performed. The difference between the two groups was not statistically significant, but the damage on ELM in group 2 was higher than in group 1 at the limit of significance (p=0.062). This difference in ELM damage and the wider FAZ in group 2 gave us the impression that there was a relationship between early hyperfluorescence and vascular or cellular damage. The limitation of the study was a small number of patients and retrospective, cross-sectional design. Included

patients were not naïve. However, early fluorescence level has a potential to be a reliable factor to detect the function of the inner blood-retina barrier and may leads to determine the frequency of treatment and follow-up which has not been described yet. Because of the retrospective design, we could not reach the same FA image sections in the OCT, so we could not conclude about the relationship between OCT and FA. OCT-A is a method that can show the structure of the inner and outer plexuses, but it is not possible to show the leak with OCT-A. OCT-A also has the disadvantages of being expensive and less accessible.

CONCLUSION

As a result, we believe that conducting other studies to evaluate the importance of this parameter of FFA, which has not been used much in today's world, where the value of FFA compared to OCT is very low, may provide us useful information for monitoring the diabetic retinopathy.

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Conflict of Interest

The authors have no potential conflicts of interest related to the research, authorship and/or publication of this article.

Author Contributions

Plan, design: EK, GSV, SY; **Material, methods and data collection:** MKC, EG, SY; **Data analysis and comments:** CG, GSV; **Writing and corrections:** GSV, EK, SY.

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