Fundus autofluorescence in the assessment of diabetic macular edema

Autofluorescencia de retina en la evaluación del edema macular diabético

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Abstract

Objective: To assess the association between fundus autofluorescence (FAF) and optical coherence tomography (OCT) and fluorescein angiography (FA) parameters in patients with diabetic macular edema (DME). **Methods:** Study design: non-comparative case series. Thirty-seven eyes of 20 patients with DME were included. Patients underwent best-corrected visual acuity and contrast sensitivity determination, assessment of central subfield thickness (CST), macular volume (MV), and macular cube average thickness (MCAT) by spectral-domain OCT, as well as FAF and FA imaging. SD-OCT EMD and foveal FAF patterns (decreased, normal, increased single-spot, and increased multiple-spot) were evaluated. Fisher exact test, interobserver variability, and Kruskal-Wallis test were used for statistical analysis. **Results:** A statistically significant difference was observed between the four FAF foveal patterns in the MV ($p = 0.03$), and in the MCAT groups ($p = 0.01$). Post-hoc analysis with the U-Mann-Whitney test showed a statistically significant difference between decreased FAF and normal FAF patterns, and between decreased FAF and increased multiple-spot FAF patterns ($p < 0.05$) in the MV and MCAT groups. Foveal capillary leakage by FA was observed in a higher proportion of eyes in the increased single-spot and multiple-spot FAF patterns ($p = 0.03$). **Conclusions:** Flash camera FAF shows changes regarding MV, MCAT, and the presence of foveal capillary leakage in patients with DME. It is relevant to establish the value of FAF in the assessment of DME.

Key words: Diabetic retinopathy. Diabetic macular edema. Fundus autofluorescence. Optical coherence tomography. Fluorescein angiography.

Resumen

Objetivo: Evaluar la asociación entre la autofluorescencia de retina (AF) y las variables estructurales de la tomografía de coherencia óptica (OCT) y de la angiografía con fluoresceína (FA) en pacientes con edema macular diabético (EMD). **Métodos:** El diseño del estudio fue una serie de casos no comparativa. A 37 ojos de 20 pacientes con EMD se les realizó la determinación de la agudeza visual mejor corregida y la sensibilidad al contraste, y la determinación del grosor foveal central, grosor promedio macular (GPM), volumen macular (VM) y patrones de EMD mediante OCT de dominio espectral

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(OCT-DE). Se tomaron imágenes de AF y de FA. Se evaluó el patrón de AF foveal (disminuida, normal, aumentada unifocal y aumentada multifocal). Se efectuó prueba exacta de Fisher, variabilidad interobservador y prueba de Kruskal-Wallis como análisis estadístico. **Resultados:** Se encontró una diferencia estadísticamente significativa entre los cuatro patrones de AF en el VM ($p = 0.03$) y en el GPM ($p = 0.014$). El análisis post hoc mostró, en el VM, una diferencia estadísticamente significativa entre el patrón de AF disminuida y el de AF normal ($p < 0.05$), así como entre el patrón de AF disminuida y el de AF aumentada multifocal ($p < 0.05$). La fuga de la red capilar perifoveal por FA se presentó en mayor proporción en los patrones de AF aumentada tanto uni como multifocal ($p = 0.032$). **Conclusiones:** Existen cambios en la AF con cámara de flash, en pacientes con EMD asociados con el GPM, el VPM y a la fuga de red capilar perifoveal. Es importante establecer el valor de la AF en la evaluación del EMD.

Palabras clave: Retinopatía diabética. Edema macular diabético. Autofluorescencia de retina. Tomografía de coherencia óptica. Angiografía con fluoresceína.

Introduction

Diabetic macular edema (DME) is the leading cause of visual loss in diabetic patients¹⁻³. It constitutes one of the alterations observed in diabetic retinopathy and is an important cause of visual disability in Mexico and the world⁴.

A comprehensive evaluation of DME, especially in the era of the multimodal diagnostic paradigm of vitreoretinal pathology includes the assessment of visual function, posterior segment biomicroscopy, fluorescein angiography (FA), and optical coherence tomography (OCT)^{5,6}.

A more recent test in the evaluation of DME is fundus autofluorescence (FAF). However, FAF has not been extensively used in the diagnosis and follow-up of patients with DME, especially with FAF imaging systems with a *flash* camera. *Flash* camera systems have the advantage of being more affordable compared to confocal scanning laser systems⁵⁻¹⁶. Several authors have explored the utility of FAF in the evaluation and response to DME treatments⁶. Vujosevic, et al.¹³ found a correlation between FAF, using a confocal scanning laser imaging system, and functional variables such as the sensitivity obtained by microperimetry. Therefore, there is a need to evaluate the correlation between FAF with the *flash* camera-based systems and the existing parameters in other imaging modalities such as OCT, as well as variables to evaluate retinal function, in order to determine their role in DME assessment.

Objective

The purpose of this study was to assess the association between FAF images obtained using a *flash* camera system and the structural variables obtained using spectral-domain OCT (SD-OCT), functional tests and FAF, in patients with DME.

Methods

The study design was observational, prospective, cross-sectional, and analytical. All the patients were recruited from the Retina Service of Clínica David, in Morelia City, Michoacán, Mexico, from October 2017 to May 2018. Patients with DME detected clinically or by OCT were included.

Patients with significant cataract (determined by the presence of OCT image quality with a signal strength less than 6), edema with a tractional component determined by OCT, and other macular pathologies (including age-related macular degeneration and retinal vein occlusion), previous treatment with intravitreal anti-VEGF therapy, previous treatment with laser applied within the macular area, were excluded.

All patients underwent a complete ophthalmological examination that included best-corrected visual acuity (BCVA), contrast sensitivity (CS) and biomicroscopy. A *flash* camera-based system was used to obtain FAF and FA, and clinical images, SD-OCT and clinical pictures of the posterior segment were also performed.

Informed consent was obtained from all patients and the study was approved by the Ethics Committee of the Clínica David, Ophthalmology Unit. The study was performed in accordance with the regulations of the General Health Law in Health Research regarding the ethical aspects of research in humans.

Also, the study was performed in accordance with the Nuremberg code, the tenets of the Declaration of Helsinki and following the guidelines established by the official Mexican standards NOM 04 and NOM 012 of the Ministry of Health.

Procedures performed on patients

Best-corrected visual acuity (BCVA). Distance BCVA for each eye was measured using the standard protocol

of the Early Treatment Diabetic Retinopathy Study (ETDRS) at a distance of 4 meters with the modified ETDRS chart (Precision Vision, La Salle, IL, USA). BCVA was recorded as the total number of letters read correctly for each eye separately.

Contrast sensitivity (CS). CS was measured in all patients using the Hamilton-Veale CS chart (Hamilton Veale, Canterbury, New Zealand). CS was recorded as the total number of letter pairs read correctly at a 1-meter distance for each eye separately.

FAF qualitative analysis. FAF images were obtained with a fundus camera (fundus camera model Visucam NM/FA, Carl Zeiss Meditec, Oberkochen, Germany). A wavelength of 510-580 nm was used for excitation, while the emitted light was detected above 640 nm due to a barrier filter. Several images were taken, and those with artifacts, such as the presence of shadows or poor visualization, were excluded. The selected digital images were saved on the computer for further analysis, classification, and processing. The pictures were improved with the automatic editor of the Visucam camera software. A classification for FAF patterns was designed, modifying the one published by Vujosevic, et al.¹³. FAF images were classified according to different FAF patterns in the foveal area: decreased FAF, normal FAF, increased single-spot FAF, and increased multiple-spot (Fig. 1). The classification of FAF images was performed independently by two expert evaluators in a masked manner.

Fundus photography and FA. In all patients, after pupil dilation, a photographer trained with the same fundus camera (fundus camera model Visucam NM/FA, Carl Zeiss Meditec, Oberkochen, Germany) took both color pictures and FA pictures of the posterior pole. FA images of the macula were classified according to the presence or absence of ischemia and dye leakage. FAF images were taken before the other imaging studies.

Optical coherence tomography (OCT). A SD-OCT scan (Cirrus 5000 SD-OCT; Carl Zeiss Meditec, Oberkochen, Germany) of the macular area was performed. The scanning protocol used for this study was fast macular thickness, program. structural image analysis of the PDF report was performed. For this study, the central millimeter's retinal thickness was used as a measure of central subfield thickness (CST) by OCT. Macular volume (MV) in mm³ and macular cube average thickness (MCAT) in microns (μm) were measured. These images were also saved on the hard disk for further analysis. DME was classified into four patterns according to the classification described by Otani, et al.¹⁷: focal, diffuse

Figure 1. Fundus autofluorescence (FAF) patterns in the foveal area. **A:** Decreased AF; **B:** Normal AF; **C:** Increased single-spot FAF; **D:** Increased multiple-spot FAF. The image of each pattern is accompanied at the bottom by its corresponding clinical image.

cystic, diffuse spongiform (non-cystoid), and diffuse with subfoveal serous neuroretinal detachment.

Statistical analysis. Descriptive statistics were performed, and the Kolmogorov-Smirnov normality test was subsequently applied to assess the normality of data distribution. Kruskal-Wallis test was used to compare groups and U-Mann-Whitney test was used as a *post-hoc* test. Fisher's exact test was used to compare small expected frequencies. The κ coefficient was determined to assess inter-observer variability. The minimum level of significance considered was $p < 0.05$. The SPSS program for Mac was used as statistical software (IBM Corp. Released 2012 IBM SPSS Statistics for Mac, Version 21.0. Armonk, NY: IBM Corp).

Results

This study included 37 eyes of 20 patients with a mean age of 62.8 ± 7.2 years (range, 53-79). Twelve patients were male, and 8 patients were female. Mean BCVA and CS were 31.5 ± 16 letters and 7.04 ± 3.5 letter pairs, respectively.

Mean CST, MV, and MCAT were $356.3 \mu\text{m} \pm 123.5$, $10.9 \text{ mm}^3 \pm 3$ and $363.9 \mu\text{m} \pm 93.9$, respectively. FAF patterns were: decreased (13%), normal (40%), increased single-spot (17%) and increased multiple-spot (30%) (Fig. 1).

The following edema patterns were observed by OCT: focal (17.9%), diffuse cystoid (15.4%), diffuse non-cystoid or spongiform (51.3%), and diffuse with subfoveal serous neuroretinal detachment (15.4%) (Table 1).

Inter-observer reliability between the two investigators for AF classification (grades 1 to 4) was evaluated with a κ coefficient of 0.906 ($p = 0.000$).

Table 1. Characteristics of the patients with DME.

Sex of the patients, n (%)		FAF pattern, n (%) [95 CI]*	
Male	12 (60)	Decreased	5 (13) [3-27]
Female	8 (40)	Normal	15 (40) [23-59]
Age, years		Increased single-spot	6 (17) [3-30]
Average $\pm$ SD	62.8 $\pm$ 7.2	Increased multiple-spot	11 (30) [13-47]
Range (years)	53-79	Presence of capillary leakage by FA, n (%)*	
DM diagnosis (in years)	11 $\pm$ 5.6		
Associated SH (% of patients)	80		
BCVA, number of letters	31.5 $\pm$ 16	Absent	8 (30.8)
CS, number of letter pairs	7 $\pm$ 3.5	Present	19 (69.2)
DME patterns by OCT, n (%)*			
Focal			7 (17.9)
Diffuse cystoid			6 (15.4)
Diffuse non-cystoid (spongiform)			20 (51.3)
Diffuse with serous subfoveal neuroretinal detachment			6 (15.4)

FAF: fundus autofluorescence; BCVA: best-corrected visual acuity; FA: fluorescein angiography; DME: diabetic macular edema; SH: systemic hypertension; OCT: optical coherence tomography; CS: contrast sensitivity

Table 2. Description of OCT and functional characteristics by FAF Pattern (central subfield thickness [CST], macular volume [MV], macular cube average thickness [MCAT]) (best-corrected visual acuity [BCVA] and contrast sensitivity [CS])

	Foveal FAF pattern				p (Kruskal- Wallis)
	Decreased FAF	Normal FAF	Increased single-spot FAF	Increased multiple-spot	
BCVA (number of letters)	25 $\pm$ 13.2	28.4 $\pm$ 17.3	20 $\pm$ 14.1	25.6 $\pm$ 18.4	0.70
CS (number of pair of letters)	6 $\pm$ 3.1	7.2 $\pm$ 3.5	5.5 $\pm$ 6.3	5.8 $\pm$ 3	0.70
CST (μ)	474 $\pm$ 144.2	385.2 $\pm$ 159.7	463.5 $\pm$ 177.5	400 $\pm$ 142	0.31
MV (mm ³)	8.8 $\pm$ 2.2	10.9 $\pm$ 2.7	13.6 $\pm$ 0.7	13.5 $\pm$ 2.62	0.03
MCAT (μ)	437 $\pm$ 35.3	341.8 $\pm$ 29.5	378.5 $\pm$ 21.9	374.1 $\pm$ 73.8	0.01

Analysis with Kruskal-Wallis test showed a statistically significant difference among the four FAF patterns for MV ($p = 0.03$) and MCAT ($p = 0.014$) (Table 2).

Post-hoc analysis with the U-Mann-Whitney, showed that, for MV, there were statistically significant differences between the group of decreased FAF and normal FAF ($p = 0.024$) and between the group of decreased FAF and increased multiple-spot FAF ($p = 0.022$).

For MCAT, a statistically significant difference was observed between the pattern of decreased FAF and

that of normal FAF ($p = 0.012$), as well as between the pattern of decreased FAF and that of increased multiple-spot FAF ($p = 0.003$).

There were no statistically significant differences between FAF patterns and DME patterns by OCT ($p = 0.21$).

In contrast, leakage from the perifoveal capillary network by FA was observed in a more significant proportion in both single- and multiple-spot increased FAF patterns ($p = 0.032$) (Table 3). Figure 2 shows a case

Table 3. Differences among the different DME patterns by OCT and the presence or the absence of perifoveal capillary network leakage evaluated by FA (fluorescein angiography)

Foveal FAF pattern					
	Decreased FAF n (%)	Normal FAF n (%)	Increased single- spot FAF n (%)	Increased multiple- spot FAF n (%)	p (Fisher's exact test)
OCT pattern					p = 0.21
Focal	-	6 (85.7)	1 (14.3)	-	
Cystoid	2 (33.3)	1 (16.6)	2 (33.3)	1 (16.6)	
Spongiform	3 (16.6)	6 (33.3)	2 (11.1)	7 (38.8)	
DSRNS	1 (16.6)	1 (16.6)	-	4 (66.6)	
Presence of leakage by FA					p = 0.034
Perifoveal capillary network leakage	3 (17.6)	5 (29.4)	4 (23.5)	5 (29.4)	
No perifoveal capillary network leakage	1 (12.5)	6 (75)	-	1 (12.5)	

FAF: fundus autofluorescence; DSRNS : serous detachment of the sensorineural retina; FA: fluorescein angiography; OCT: optical coherence tomography.

Figure 2. Diabetic macular edema with an increased single-spot FAF foveal pattern. **A:** Clinical image of the posterior pole (the arrow indicates a more reflective hard exudate). **B:** FAF image (the arrow indicates increased unifocal FAF that corresponds to the reflective exudate from the clinical image). **C:** FA image. **D:** Infrared image. Focal area of macular thickening that corresponds to the increased FAFspot observed in image C.

of increased single-spot FAF and the correlation with its multimodal images: clinical, SD-OCT and FA.

Discussion

FAF images are very useful in the diagnosis and monitoring of different retinal diseases, such as inherited retinal disorders like Stargardt's disease, age-related macular degeneration, and DME⁸.

In DME, although FAF has shown benefits, its widespread use has been more limited among physicians dedicated to treating macular disorders^{5,6,10-16,18,19}.

Different authors^{11,13,16} have described several patterns of FAF changes in patients with DME. Pece et al.¹⁶ described two types of FAF patterns in DME: type 1, which they called *multilobulated*, characterized by the presence of multiple areas of increased AF, similar to that of increased multiple-spot FAF in our classification; type 2, also called *uni-lobulated*, that corresponds to the presence of a large cyst in the OCT image, and type 3 or mixed, with characteristics of both type 1 and type 2.

Vujosevic et al.¹³ described a classification with the following FAF patterns within the foveal area: normal, increased single-spot FAF, and increased multiple-spot FAF.

To obtain FAF images, they used a confocal scanning laser system. The authors considered the images with decreased FAF a consequence of the blockade produced by macular pigments on FAF, and classified them as a normal FAF pattern.

In FAF images obtained by *flash* camera systems, both the excitation and the emission of the FAF signal occurs at a higher wavelength, which translates into a lower color density appearance of the macular pigments in the image obtained.

There were cases in our study where we observed a more decreased FAF, especially in the periphery of the fovea and in the area between the fovea and the parafovea, which we did not consider as part of the normal FAF pattern.

The irregular-looking, paracentral characteristics that corresponded to areas of focal thickening by OCT, in these cases of decreased FAF, made us consider that they were probably not due to the presence of macular

pigments. For this reason, we decided to modify the classification described by Vujosevic et al.¹³ and we added a new pattern: decreased FAF. We hypothesized that reduced FAF in this pattern could be due not to the macular pigments, but to the presence of an increased thickness in the periphery of the foveal area, which should be considered as a different category or pattern from foveal FAF.

Since decreased FAF is related to increased retinal thickness in the parafoveal area, Yoshitake et al.¹⁸ hypothesized that decreased FAF in these eyes reflects autofluorescence of the retinal pigment epithelium (RPE) blocked by the edematous retinal parenchyma, mainly in the DME spongiform pattern. This could explain the fact that MCAT was higher in the group of decreased FAF compared to the other groups.

In contrast, in the cystoid pattern of DME, cysts located in the outer plexiform and inner nuclear layers in the foveal area, especially in the center of the fovea (an area that is thinner compared to the surrounding retina), where there is a maximum accumulation of luteal pigment, produce an increased FAF image. The cysts displace the luteal pigment and prevent normal blockade of the foveal FAF signal, giving patterns of increased single- and multiple-spot FAF.

Chung, et al.¹¹ described another classification system for FAF, but exclusively foveola: eyes without increased FAF in the foveola were classified as grade 1, eyes with FAF increased by less than half of the foveola as grade 2, eyes with increased FAF between one-half and three-fourths of the foveola as grade 3, and when increased FAF occupied the entire foveola, as grade 4.

These classifications of the different FAF patterns, especially with confocal systems, helped to design studies in which the correlation between FAF and retinal anatomical-functional variables has been evaluated^{10-16,18}.

Reznicek et al.¹⁵, for example, evaluated the role of green wavelength FAF in DME. Their results indicated a weak correlation of the FAF intensity with CST, the integrity of the retinal ellipsoid layer and the thickness of the layer-RPE complex-ellipsoid in diabetic patients with or without DME. In our study, we observed a correlation with MV and MCAT, but no significant correlation with CST.

Pece et al.¹⁶ evaluated FAF images in patients with diabetic retinopathy and cystoid DME and its correlation with FA and OCT findings. They observed that FA and OCT findings had a positive correlation with the increase of FAF. In our study, we also found a higher proportion of cases with perifoveal capillary network

leakage in eyes with increased FAF patterns, both single- and multiple-spot.

Chung et al.¹¹ described that FAF correlated well with quantitative data. They evaluated BCVA, FAF and SD-OCT images that were obtained before and after injection of intravitreal bevacizumab for the treatment of DME. They described that DME showed a significant correlation with increased foveolar FAF, especially regarding imaging biomarkers, such as disruption of the outer layers of the retina as the external limiting membrane. Like Reznicek et al. (and unlike our study), Chung, et al found a positive correlation between CST and the degree of FAF.

Chung et al.¹¹ also observed that foveolar FAF was 4.2 times more likely to occur in DME with intraretinal cysts. They concluded that the correlation between FAF and SD-OCT parameters, as well as visual status in patients with DME, could predict the restoration of photoreceptor integrity and subsequent visual recovery, especially in cases of severe DME where the integrity of photoreceptors before treatment cannot be adequately assessed by SD-OCT.

Vujosevic et al.¹³, by evaluating the role of structural and functional macular imaging in the characterization of DME patterns, correlated FAF better with central field microperimetry and OCT patterns than with visual acuity. There was a significant difference in BCVA average between three different groups of FAF patterns (normal, single-spot, and multiple-spot).

In the same study by Vujosevic et al.¹³, macular thickness was not significantly different among the FAF groups. On the contrary, in our study, we found a correlation between the different FAF patterns for MV and MCAT, mainly between the pattern with decreased FAF and the pattern with normal FAF, as well as between the pattern with decreased FAF and the pattern with increased multiple-spot FAF.

In another study, Vujosevic et al.¹⁰ evaluated changes in specific retinal imaging biomarkers, such as intraretinal hyperreflective spots, subfoveal neuroretinal detachment, and increased foveal autofluorescence after treatment with intravitreal dexamethasone or ranibizumab in DME, as possible indicators of the retinal inflammatory condition. They found that the mean area of increased FAF decreased in both groups, both in the dexamethasone and ranibizumab groups.

In ranibizumab-treated eyes, they observed a significant inverse correlation between the area of increased FAF at baseline and BCVA. After both treatments, dexamethasone and ranibizumab, the mean area of increased FAF decreased significantly¹⁰.

One of the limitations of our study is the use of a *flash* camera to obtain FAF images. Fundus cameras capture more reflected and scattered light compared to confocal systems. Scattered light from structures outside the retinal plane can falsely increase the FAF signal, a phenomenon called pseudoautofluorescence⁸. Furthermore, there is variability when obtaining the FAF signal, so it is possible that some cases that are observed with increased FAF at one time, could actually have decreased FAF and viceversa. According to Frampton et al.²⁰, the sensitivity and specificity of the *flash* camera-based system for obtaining FAF images in DME is variable (sensitivity from 43 to 67% and specificity from 86 to 99%).

Fundus cameras also produce low-contrast images that can lead to image misinterpretation due to possible confusion of foveal FAF patterns. To overcome misinterpretation of images, it would be interesting to evaluate the role of quantitative FAF methods.

To classify the DME pattern by OCT, we used only the image of the PDF report. This could induce bias due to inadequate assessment of the images and, therefore, the correlation could be affected.

Several methods have attempted to quantify FAF. Yoshitake et al.¹⁸ described a method for quantifying the relative levels of FAF intensity in individual subfields of the ETDRS grid in DME and assessed the clinical relevance compared to OCT findings. However, this method does not represent quantitative analysis of FAF.

Delori et al.²¹ developed another method to perform standardized quantitative measurements of FAF, that has been used in several retinal pathologies²¹⁻²⁴. This technique is applicable to scanning laser ophthalmoscopy and probably also to fundus cameras.

The method incorporates a standard fluorescent reference into the imaging device. The reference compensates for changes in laser power and detector gain and allows comparison of images obtained longitudinally or using devices at different sites. Therefore, FAF can be compared longitudinally between eyes and images obtained with different devices.

Lastly, due to the lack of equipment standardization (mainly due to different image acquisition qualities) and of a standardized normative database for FAF, FAF is not currently a widely used examination method for diseases of the retina and the RPE.

The reproducibility and consistency of FAF measurements have not been systematically examined in any of the FAF studies. However, FAF has a significant potential as a tool to provide answers to many questions in the area of macular diseases.

Conclusions

In patients with DME, there are different FAF patterns observed with *flash* camera systems that correlate with structural variables by SD-OCT and FA, mainly MV and MCAT, as well as perifoveal leakage. The greater the FAF, the greater the average macular thickness and the perifoveal capillary network leakage by FA. Moreover, a more accurate FAF phenotypic classification could help to determine prognostic factors for visual loss or the design of other clinical trials for DME.

FAF has a significant potential to evaluate the RPE and to obtain a more comprehensive evaluation of the retinal function in the current era where diagnosis paradigm is based on multimodal retinal imaging is so prevalent.

Finally, longer-term studies with a larger number of patients are warranted to determine the role of FAF in patients with DME.

Conflicts of interest

The authors declare no financial interests regarding this work.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

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