ORIGINAL ARTICLE

Arquivos Brasileiros de Oftalmologia

Evaluation of fundus autofluorescence ımaging of diabetic patients without retinopathy

Avaliação do exame de imagem de autofluorescência do fundo do olho em pacientes diabéticos sem retinopatia

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ABSTRACT | Purpose: To evaluate the usefulness of fundus autofluorescence imaging of diabetic patients without retinopathy to investigate early retinal damage. **Methods:** Fundus autofluorescence images of patients with type 2 diabetes mellitus without retinopathy (diabetic group) and age-sex matched healthy patients (control group) were recorded with a CX-1 digital mydriatic retinal camera after detailed ophthalmologic examinations. MATLAB 2013a software was used to measure the average pixel intensity and average curve width of the macula and fovea. **Results:** Fifty-six eyes of 28 patients, as the diabetic group, and 54 eyes of 27 healthy patients, as the control group, were included in this study. The mean aggregation index was 168.32 ± 37.18 grayscale units (gsu) in the diabetic group and 152.27 ± 30.39 gsu in the control group (p=0.014). The mean average pixel intensity value of the fovea was 150.87 ± 35.83 gsu the in diabetic group and as 141.51 ± 31.10 gsu in the control group (p=0.060). The average curve width value was statistically higher in the diabetic group than in the control group (71.7 ± 9.2 vs. 59.4 ± 8.6 gsu, respectively, p=0.03). **Conclusion:** Fundus autofluorescence imaging analysis revealed that diabetic patients without retinopathy have significant fluorescence alterations. Therefore, a noninvasive imaging technique, such as fundus autofluorescence, may be valuable for evaluation of the retina of diabetic patients without retinopathy.

Keywords: Diabetic retinopathy; Diabetes mellitus; Optical imaging; Fundus oculi

RESUMO | Objetivo: Avaliar a utilidade da autofluorescência do fundo de olho de pacientes diabéticos sem retinopatia para investigar lesões precoces na retina. **Métodos:** Imagens de autofluorescência do fundo de olho de pacientes com *diabetes mellitus* do tipo 2 sem retinopatia (grupo diabético) e indivíduos saudáveis pareados por idade e sexo (grupo controle) foram registrados com uma câmera retiniana digital midriática CX-1 após exames oftalmológicos detalhados. O software MATLAB 2013a foi usado para medir a intensidade média do pixel e a largura média da curva da mácula e fóvea. **Resultados:** Cinquenta e seis olhos de 28 pacientes, como o grupo diabético, e 54 olhos de 27 indivíduos saudáveis, como grupo controle, foram incluídos neste estudo. O índice médio de agregação foi de 168,32 ± 37,18 unidades de escala de cinza (gsu) no grupo diabético e em 152,27 ± 30,39 gsu no grupo controle (p = 0,014). O valor médio da intensidade de *pixel* na fóvea foi de 150,87 ± 35,83 gsu no grupo diabético e de 141,51 ± 31,10 gsu no grupo controle (p=0,060). O valor médio da largura da curva foi estatisticamente maior no grupo diabético do que no grupo controle (71,7 ± 9,2 vs. 59,4 ± 8,6 gsu, respectivamente; p = 0,03). **Conclusão:** A análise por imagens de autofluorescência de fundo de olho revelou que pacientes diabéticos sem retinopatia apresentam alterações significativas de fluorescência. Portanto, uma técnica de imagem não invasiva, como a autofluorescência de fundo de olho, pode ser valiosa para a avaliação da retina de pacientes diabéticos sem retinopatia.

Descritores: Retinopatia diabética; Diabetes mellitus; Imagem óptica; Fundo de olho

INTRODUCTION

Diabetes mellitus (DM) is a common systemic disease with microvascular pathology that can cause visual loss unless diagnosed and treated early[1,2]. Diabetic retinopathy (DR) develops in 56.0% of type 1 and in 30.3% of patients with type 2 DM [3]. Fluorescence is the term in which certain light-excited molecules of a given wavelength emit light at longer wavelengths. It can be indu-

Submitted for publication: August 31, 2018
Accepted for publication: December 29, 2018

Funding: No specific financial support was available for this study.

Disclosure of potential conflicts of interest: None of the authors have any potential conflicts of interest to disclose.

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Approved by the following research ethics committee: Sakarya University Education and Research Hospital (#71522473/050.01.04/190).

 http://dx.doi.org/10.5935/0004-2749.20190078

ced by some exogenous substances, such as fluorescein drugs, or endogenously by some structural molecules, called autofluorescence. Lipofuscin, the main flourophore responsible for fundus autofluorescence in the retinal pigment epithelium (RPE)[4,5], contains the oxidative degradation products of fatty acids, retinoid, and some proteins, resulting in the products of phagocytosis of the photoreceptor outer segment[6,7], also accepted as an indicator of oxidative stress[8].

Fundus autofluorescence (FAF) imaging is a noninvasive method for the diagnosis and follow-up of various retinal and choroidal diseases, such as age-related macular degeneration, hereditary fundus dystrophies, and choroiditis[7,8], which gives information about the pathophysiology and progression of the disease based on the quantity of fluorescence and distribution of fluorophores, especially lipofuscin, in the RPE[9]. In a normal FAF image, the foveola appears hypofluorescent because of the presence of lutein and zeaxanthin in the cone cells[9]. It is known that DM induces inflammation and oxidative stress by disrupting vascular permeability[1]. Many studies in the literature report that DR and diabetic macular edema cause hyperautofluorescence in FAF imaging due to the increased amount of lipofuscin and decreased amount of lutein and zeaxanthin[7,8,10,11]. To the best of our knowledge, no study has yet to evaluate FAF imaging of diabetic patients without retinopathy.

Therefore, the aim of the present study was to evaluate and compare FAF images of diabetic patients without retinopathy to those of healthy patients.

METHODS

Patients

Patients with type 2 DM without retinopathy and healthy age- and sex-matched patients were included in this 3-month study. The study protocol was approved by the Ethics Committee of Sakarya Training and Research Hospital (Sakarya, Turkey) and written informed consent was obtained from each subject in accordance with the World Medical Association Declaration of Helsinki.

The demographic characteristics of the patients, the duration of DM, comorbid diseases with DM, and glycosylated hemoglobin (HbA1c) levels were obtained from patient records. Fundus fluorescein angiography was performed to investigate the presence of DR among patients with no evidence of microaneurysms, hemorrhages, or vessel leakage who were recruited to participate in this study. Healthy patients with no ocular pathology were randomly selected from among patients who visited the Ophthalmology Clinic for various reasons.

Patients with macular diseases, such as age-related macular degeneration, but no previous laser photocoagulation therapy, intravitreal injection, or any ocular surgery, as well as additional systemic disease, chronic drug use, or ocular media opacity were excluded from this study. All the patients underwent detailed ophthalmological examinations, which included best corrected visual acuity, as measured with a Snellen chart, and slit-lamp biomicroscopy. Optical coherence tomography, color fundus imaging (CFI), and FAF imaging were performed and evaluated by the same ophthalmologist. Fasting blood glucose measurements were performed between 8 and 10 am and HbA1c levels of all the patients were measured.

Evaluation of CFI and FAF ımaging

CFI and FAF imaging were performed with a CX-1 digital mydriatic retinal camera (Canon Inc., Tokyo, Japan). Pupillary dilation was performed with 1% tropicamide in all cases before imaging. After CFI, FAF imaging was performed in FAF mode (exciter filter, 530-580 nm; barrier filter, 640 nm; field, 30º) in the same room under standard environmental light. A single FAF image was recorded at high quality for each patient. In the acquired FAF images, 5.5 mm diameter area between the superior and inferior temporal vascular arcuates, as the macula, and an area of 1.5 mm in diameter in the center of the macula, as the fovea, were manually marked by two experienced ophthalmologists (Figure 1). After marking the macula and fovea, all images were recorded as 512×512-pixel, 16-bit, grayscale, tagged image file format, and analyzed with MATLAB 2013a software (MathWorks, Inc., Natick, MA, USA) and the average pixel intensity (API) and average curve width (ACW) of the macula and fovea were measured.

Statistical analysis

IBM SPSS Statistics for Windows, version 22.0 (IBM Corporation, Armonk, NY, USA) was used to perform all analyses. Parametric data with normal distributions or equal variance were compared with the Student's t-test, while non-parametric data with abnormal distributions or unequal variance were compared with the Mann-Whitney *U* test. The results are expressed as the mean ± standard deviation (SD). A probability (p) value of <0.05 was considered statistically significant.

RESULTS

The diabetic group consisted of 12 males and 16 females with a mean age of 57.38 ± 8.28 (range, 47-70) years, while the control group was comprised of 12 males and 15 females with a mean age of 58.26 ± 8.65 (range, 45-71) years. The groups were matched for age and sex (p=0.826 and 0.524, respectively). The patients in the diabetic group were controlled in the Internal Medicine Clinic and received oral antidiabetic agents. No patient received insulin therapy. The mean duration of DM was 4.2 ± 0.6 years and the mean HbA1c level was 94 ± 1.43 mmol/mol. There was a significant difference in the mean fasting blood glucose levels between the diabetic and control groups (162 ± 45 vs. 87 ± 16 mg/dl, respectively, p=0.001). No patients had DM-related complications or any additional systemic disease, such as hypertension or hypercholesterolemia. Table 1 shows the characteristic features of the diabetic and control groups. In each case, best corrected visual acuity was measured as 1.0 in decimals. There was no abnormal ophthalmological finding in any subject. CFI revealed no abnormality in any of the patients. On FAF imaging evaluation, the mean API value was 168.32 ± 37.18 grayscale unit (gsu) in the diabetic group and 152.27 ± 30.39 gsu in the control group (p=0.014). There was no statistically significant difference between the mean API values of the fovea between the diabetic and control groups (150.87 ± 35.83 vs. 141.51 ± 31.10 gsu, respectively, p=0.060). The macular ACW value was greater in the diabetic group than in the control group (161.86 ± 38.34 vs. 151.57 ± 34.42 gsu, respectively, p=0.015). The mean foveal ACW value was slightly higher in the diabetic group than in the control group; however, the difference was not statistically significant (151.00 ± 37.68 vs. 140.88 ± 34.38 gsu, respectively, p=0.146) (Table 2). Patients in the diabetic group had broader curves than those in the control group (Figure 2).

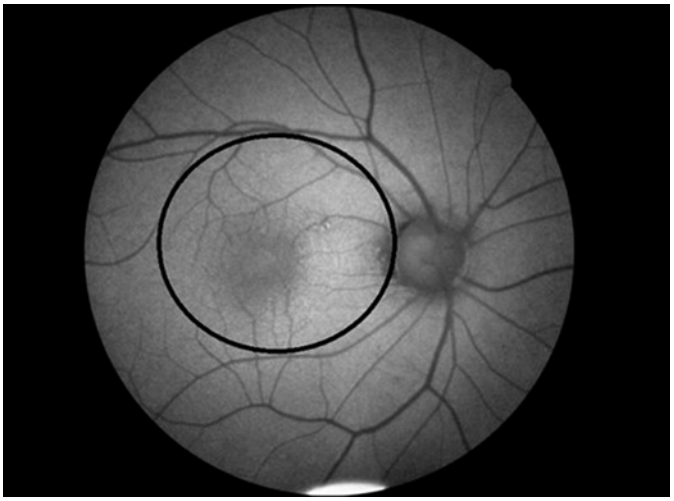


Figure 1. A representative FAF image.

Table 1. Characteristic features of the diabetic and control groups

	Diabetic group (n=56 eyes)	Control group (n=54 eyes)	*p*
Sex (male/female)	12/16	12/15	0.826
Age (years)	57.38 ± 8.28	55.26 ± 8.65	0.524
DM duration (years)	4.2 ± 0.6		
HgA1c levels (%)	7.94 ± 1.43	4.3 ± 1.25	**0.001**
Fasting blood glucose levels (mg/dL)	162 ± 45	87 ± 16	**0.001***

(DM, diabetes mellitus; data are expressed as the mean ± SD; p<0.05 was considered significant).

Table 2. Comparison of fundus autofluorescence data between groups

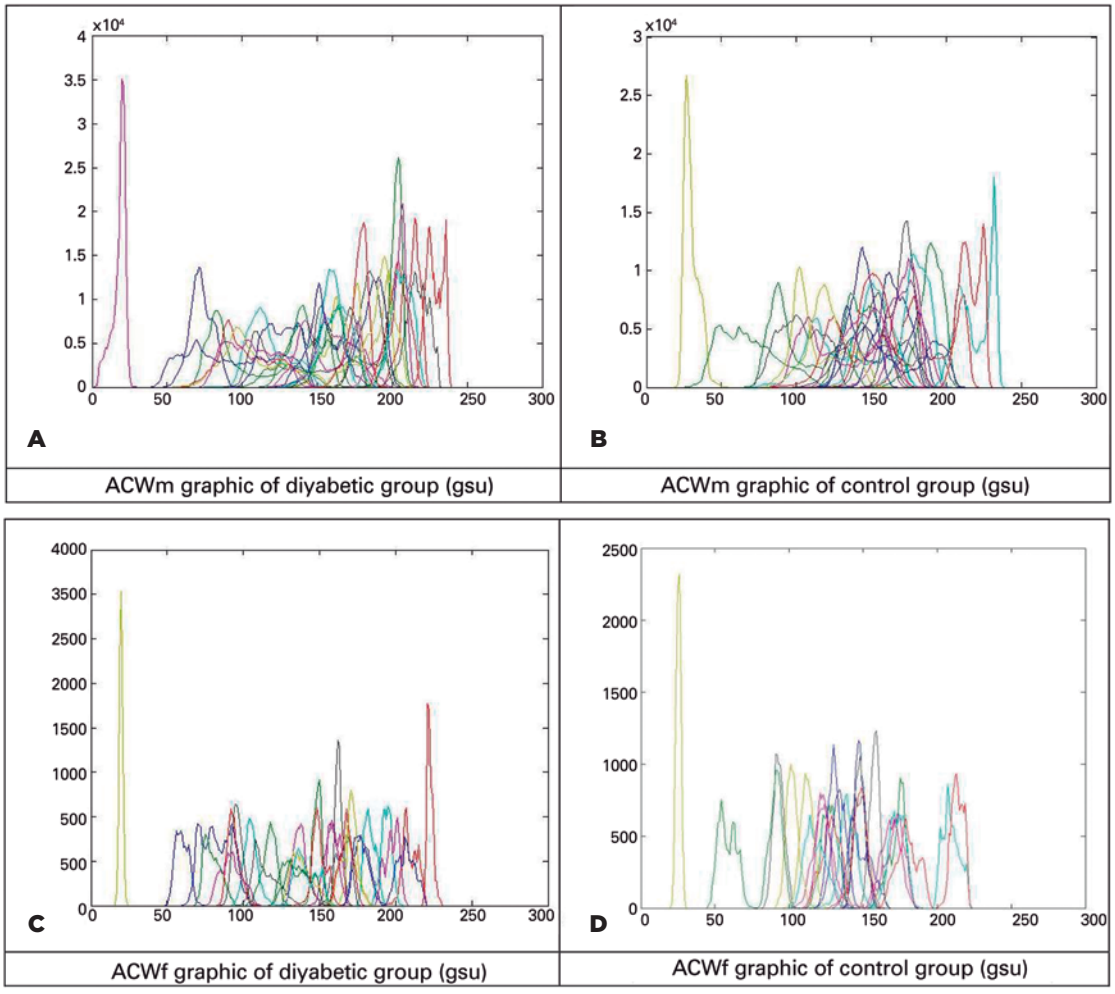
	Diabetic group (n=56 eyes)	Control group (n=54 eyes)	*p*
API macula (gsu)	168.32 ± 37.18	152.27 ± 30.39	**0.014***
API fovea (gsu)	150.87 ± 35.83	141.51 ± 31.10	0.060
ACW macula (gsu)	169.03 ± 9.2	152.37 ± 8.5	**0.015***
ACW fovea (gsu)	146.66 ± 8.7	141.33 ± 8.4	0.146

(API, average intensity; ACW, average curve width; data are expressed as the mean ± SD; p<0.05 was considered significant).

DISCUSSION

DM is a common systemic disease characterized by microvascular damage, caused by oxidative stress, polyol accumulation, advanced glycation end products, activation of protein kinase-c and caspase 3, mitochondrial damage, and apoptosis of the retinal pericytes and endothelial cells[2,12,13]. Damage to the RPE in DR is frequently associated with microvascular damage and perfusion defects, vascular leakage due to increased expression of vascular endothelial growth factors, and oxidative stress induced by the accumulation of iron ions[14-17]. Disruption of the metabolic activities of RPE cells leads to the induction of apoptosis and subsequent increase in RPE degradation products and lipofuscin granules[16].

CFI is not suitable for demonstrating early retinal changes[18]. Fundus fluorescein angiography is an effective, but invasive, method for detecting retinal and vascular damage. FAF is a noninvasive technique that provides information about the severity and extent of retinal damage due to metabolic changes to the RPE. Since autofluorescence varies with age[19], the patient and control groups were age-matched in this study. Several studies



ACWm: average curve width of macula; ACWf: average curve width of fovea.
Figure 2. The graphics of macular and foveal ACW.

have reported that FAF imaging provides beneficial data on retinal pathology in various diseases, such as DR, diabetic macular edema, and age-related macular degeneration[5,8,10,20]. Calvo-Maroto et al. reported that FAF imaging revealed increased alteration of retinal autofluorescence, although color imaging was normal in patients with early stage DR[18]. They suggested that FAF imaging may be useful to detect early retinal changes in patients without DR.

In DR, lipofuscin granule accumulation might occur in the microglial cells rather than RPE cells so the increase in FAF might reveal deteriorated function of the neurosensory retina[8,21]. In the current study, macular API and ACW values were higher in the diabetic group than in the control group, suggesting that the function of the neurosensory retina might be impaired in diabetic patients before the occurrence of retinopathy. However, there have been relatively few studies on FAF imaging of DM patients without retinopathy. Nonetheless, Elner et al. reported that ACI and ACW values were directly related to the severity of retinopathy in DR and that hyperautofluorescence was increased in diabetic patients as compared with healthy patients[19]. Schweitzer et al. also showed that the duration of fluorescence is significantly prolonged patients with in type 2 DM without retinopathy[22]. In addition, other studies have suggested that hyperautofluorescence is associated with elevated apoptosis-associated lipofuscin, and these regions may present hypoautofluorescence due to the progression of atrophy[23,24].

In the present study, diabetic patients without retinopathy had increased macular hyperautofluorescence on FAF images, as compared to those of the healthy patients. However, there was no significant difference

in foveal autofluorescence between the two groups, which may have been due to the foveal avascular zone and the presence of lutein and zeaxanthin pigments in this region. Since cone cells are mainly responsible for fine resolution, it is thought that the fovea is preserved in patients with early stage DR without vision loss[24,25].

In conclusion, the results of this study revealed an increase in macular autofluorescence but no change in foveal autofluorescence in diabetic patients without DR. Autofluorescence intensity and distribution may have a predictive value for the early detection of retinal changes in diabetic patients. However, further studies are needed to confirm these finding.

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