

Imaging Evidence of Diabetic Choroidopathy In Vivo: Angiographic Pathoanatomy and Choroidal-Enhanced Depth Imaging

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Abstract

Purpose: To describe the pathoanatomy of diabetic choroidopathy (DC) in pre-diagnosed diabetic retinopathy (DR) cases and to provide angiographic and optical evidence for DC using indocyanine green angiography (ICGA) and enhanced depth imaging spectral-domain optical coherence tomography (EDI SD-OCT).

Methods: A retrospective analysis of 80 eyes from 40 DR patients was conducted. In Group One, choroidal vascular abnormalities were evaluated by comparing angiographic findings from simultaneous ICGA with those from fundus fluorescein angiography (FFA). In Group Two, EDI SD-OCT was used to evaluate the subfoveal choroidal thickness (SFCT) and define the choroid boundary in order to acquire the bilateral and symmetric choroidal area (CA). Data were then analyzed by Image Pro Plus 6.0.

Results: In Group One, choroidal abnormalities that were evident using ICGA but not FFA included early hypofluorescent spots in 47 eyes (75.81%), late hyperfluorescent spots in 37 eyes (59.68%), and late choroidal non-perfusion regions in 32 eyes (51.61%). In particular, a significant difference between proliferative DR (PDR) in 17 of 23 eyes (73.91%) and non-PDR in 16 of 39 eyes (41.03%) was observed in late choroidal non-perfusion regions. Eighteen of 31 eyes (58.06%) also exhibited “inverted inflow phenomena.” In Group Two, both the SFCT and CA of eyes with diabetic macular edema and serous macular detachment were significantly greater than those in the other eyes. The CA in panretinal photocoagulation (PRP) treated cases was also greater than that in non-PRP treated cases.

Conclusions: Early hypofluorescent spots, late choroidal non-perfusion regions, inverted inflow phenomena, higher SFCT, and larger CA are qualitative and quantitative indexes for DC. Moreover, the late choroidal non-perfusion region is a risk factor for DC with DR. Our study suggests that the supplemental use of ICGA and EDI SD-OCT with FFA is a better choice for DR patients.

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Introduction

Diabetic choroidopathy (DC) is defined as various choroidal abnormalities in diabetics. The obliterated retinal zones in normal diabetic retinas typically present as diffuse background fluorescence. In DC, any of the following are possible: hyperfluorescent lobules in the choriocapillaris, hypofluorescent lobules from delayed filling of some focal ischemic inner choriocapillaris regions, choroidal aneurysms, neovascularizations, varicose and tortuous choroidal vessels, and late phase hypoperfusion[1,2]. Histopathologically, DC may consist of disclosed choriocapillaris and other small

choroidal blood vessels with thickened basement membranes. In addition, choroidal arteries partially resemble the arteriosclerotic arteries of diabetic glomerulosclerosis, i.e., Kimmelsteil-Wilson disease[3]. The unexplained loss of visual acuity in diabetic patients who do not exhibit any retinopathy may be due to DC[4].

Thus far, no systematic studies have used imaging to qualitatively and quantitatively assess DC at a pathological level in vivo. Indocyanine green angiography (ICGA) and fundus fluorescein angiography (FFA) have been used together to obtain simultaneous images of choroidal abnormalities in the same area[5]. Recently, enhanced depth imaging spectral-

domain optical coherence tomography (EDI SD-OCT) has become more popular as well as acceptable for choroidal analysis, especially for cases of chronic central serous chorioretinopathy (CCSCR) [6] and for identifying subfoveal choroidal thickness (SFCT) abnormalities in patients with Vogt-Koyanagi-Harada syndrome[7].

Some pathological similarities in serous macular detachments (SMDs) exist between diabetic retinopathy (DR) and CCSCR patients. However, one study found no significant correlations between SFCT and total choroidal or subfoveal choroidal blood flow in healthy young subjects[8]. Recently, an association between a slight increase in SFCT among diabetes mellitus patients and higher glycosylated hemoglobin values has been observed[9]. No association was found between abnormal SFCTs and the presence or severity of DR, however. In this study, we introduce choroidal area (CA; μm^2) as a new parameter for analyzing choroidal quantity by EDI SD-OCT. CA can be used to interpret the choroidal factors that contribute to SMD in non-proliferative diabetic retinopathy (NPDR) patients with diabetic macular edema (DME). In addition, CA can also be used to quantitate choroids in diabetic eyes.

Materials and Methods

Patients

A retrospective study was conducted in the ophthalmology outpatient clinic of China Medical University. DR patients were diagnosed and classified according to ETDRS standards[10]. The subjects were enrolled and then divided into Groups One and Two for either a qualitative and quantitative study, respectively. Patients with systematic diseases were excluded from this study. These diseases included hypertension, systemic lupus erythematosus, anemia, leukemia, and other ocular diseases, such as tractional retinal detachment (TRD) or exudate lesions. The study included 80 eyes from 40 patients. These patients included 17 males and 23 females and had a mean age of 53.78 ± 11.50 years (range: 36-74 years).

Group One consisted of 62 eyes from 31 DR cases, which were qualitatively assessed. This group included 13 eyes with slight NPDR, 11 with moderate NPDR, 15 with severe NPDR, and 23 with proliferative diabetic retinopathy (PDR).

Group Two consisted of 18 DME eyes from nine NPDR cases, which were quantitatively assessed for choroids. In each case, one eye had SMD and was placed in the SMD sub-group while the other eye did not have SMD and was placed in the non-SMD sub-group. Three of the nine cases in Group Two had a history of biocular panretinal photocoagulation (PRP) and were designated as the PRP-treated sub-group. The other 6 cases were placed in the non-PRP treated sub-group.

The study adhered to the tenets of the Declaration of Helsinki and was approved by the Medical Research Ethics Committee of First Hospital of China Medical University. Written informed consent was obtained from all participants.

Methods

All patients received indirect ophthalmoscopy and slit-lamp fundus biomicroscopy. In Group One, ICGA (excitation 787 nm;

emission 800 nm; field of view: $30^\circ \times 30^\circ$; image resolution: 768×768 pixels; dye: indocyanine green) and FFA (excitation 488 nm; emission 500 nm; field of view: $30^\circ \times 30^\circ$; image resolution: 768×768 pixels; dye: fluorescein sodium) were performed simultaneously using a Heidelberg multi-modality imaging system (Spectralis HRA+OCT; Heidelberg Engineering). Movie mode, beginning at 30 seconds, was used to record 31 eyes (six eyes with slight NPDR, six with moderate NPDR, eight with severe NPDR, and 11 with PDR) and observe whether "inverted inflow phenomena" (Figure 1), i.e., whether choroidal vessel filling time (CVFT) was longer than retinal vessel filling time (RVFT), occurred. Late stages occurred between 36 to 40 minutes. Angiographic findings from both methods were compared to identify choroidal vascular abnormalities, including: 1) early hypofluorescent spots, due to a delay in dye filling or choriocapillaris defects; 2) late hyperfluorescent spots, resulting from nodules in the choriocapillaris/stroma, choroidal aneurysms, or intrachoroidal microvascular abnormalities; and 3) late choroidal non-perfusion regions, secondary to choroidal vascular occlusion.

In Group Two, EDI SD-OCT, consisting of 1024 A scans per line (Spectralis Acquisition and Viewing Modules, version 5.3.2; Heidelberg Engineering), was used to evaluate SFCT and define the choroidal boundary between the outer retinal pigment epithelium (RPE) and inner scleral borders.

Choroidal area

A 30° horizontal EDI SD-OCT scan (6 mm) through the center of the fovea was used to acquire the choroidal boundaries. In this study, CA was defined as the region between the outer RPE and inner scleral borders (the choroidal boundary) and the vertical line drawn at the end of the choroidal boundary (three lines in all; Figure 2). CA was then measured in unit pixels (Image Pro Plus 6.0) and converted into μm^2 using a factor of 18. The factor of 18 was the result of a comparison between Image Pro Plus 6.0 and Heidelberg Eye Explorer. Image Pro Plus has been widely used as a measuring tool for choroidal neovascularization[11], retinal vessels[12], retinal ganglion cells[13], diabetic retinopathy[14], and glaucoma[15].

Statistical analyses

Statistical analyses were performed using SPSS, version 14.0. The data were expressed as the median (min-max). The Pearson Chi-square test was used to assess DC severity. The relationship between DR and "inverted inflow phenomena" was evaluated by Spearman rank correlations. The bilateral ocular differences between SFCT and CA were compared using Wilcoxon matched-pairs signed-ranks tests. Mann-Whitney-Wilcoxon tests were performed to analyze CA differences between the PRP-treated and non-PRP treated groups. A P value < 0.05 was considered statistically significant.

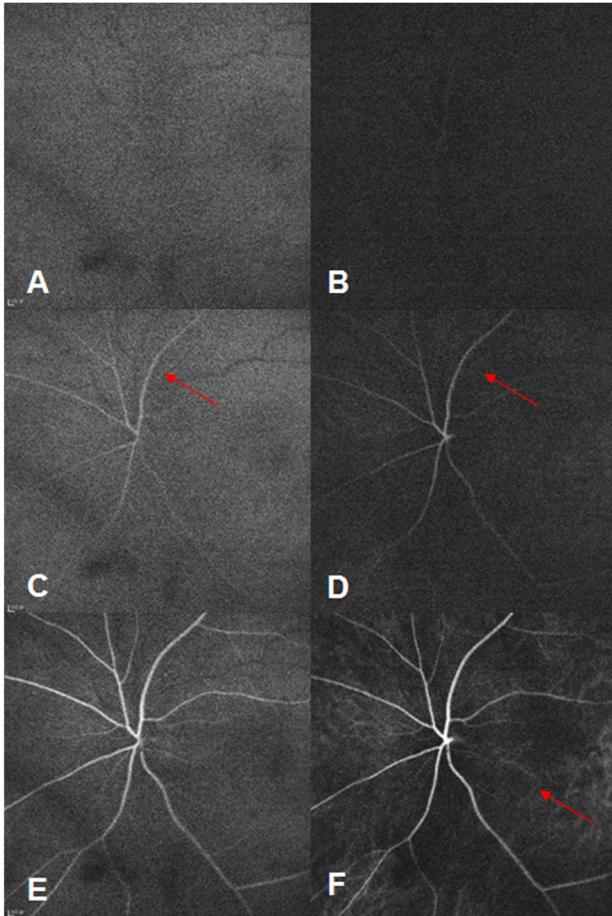


Figure 1. Inverted inflow phenomena. At 18.96 s, no dye was detected by FFA (A) and ICGA (B). At 20.00 s, retinal arteries (red arrow) started to fill in both FFA (C) and ICGA (D) images, without the perfusion of choroidal vessels in ICGA (D). At 21.28 s, ICGA (F) showed the filling phase of choroidal vessels (red arrow), and FFA (E) showed the filling phase of retinal vessels at the same time.

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Results

Morphological DC changes (Group One)

Compared to FFA, choroidal abnormalities detected by ICGA alone included early hypofluorescent spots in 47 (75.81%) eyes, late hyperfluorescent spots in 37 (59.68%) eyes, and late choroidal non-perfusion regions in 32 eyes (51.61%; Figure 3).

The incidence rate of late choroidal non-perfusion regions detected by ICGA was 73.91% (17 of 23 eyes) for PDR, which was significantly larger than the incidence of 41.03% (16 of 39 eyes) for NPDR ($X^2=6.285$, $P=0.012$).

Additionally, 18 of 31 (58.06%) eyes exhibited inverted inflow phenomena. This included one eye (16.67%) with slight NPDR, three (50.00%) with moderate NPDR, five (62.50%) with severe NPDR, and nine (81.82%) with PDR. Inverted inflow

phenomena had a positive relationship with DR severity ($r=0.460$, $P=0.009$).

Changes in SFCT and CA indicated DC

In Group Two, both the SFCT (364 μm [250–400 μm]) and CA (906,246 μm^2 [571,500–996,030 μm^2]) in SMD eyes with DME was significantly greater than in other eyes ($P<0.05$; Table 1). The CA in PRP-treated cases (947,448 μm^2 [786,294–996,030 μm^2]) was larger than that in non-PRP treated cases (777,474 μm^2 [255,240–970,200 μm^2]; $Z=-2.435$, $P=0.015$). In contrast, the SFCT was only marginally significantly different ($Z=-1.968$, $P=0.049$) between PRP-treated cases (365 μm [276–400 μm]) and non-PRP treated cases (288 μm [142–397 μm]).

Discussion

Phosphatase enzyme histochemistry can quantify the loss of choriocapillaris in DC[16]. This has resulted in new insights into the causes of occlusions. Neuronal nitric oxide synthase in the parasympathetic perivascular nerve fiber of choroids can also result in diabetes-induced neuronal disorders. Thus, DC encompasses both diabetic neuropathy and microangiopathy[17].

Ischemia and delays in filling choriocapillaris can lead to early hypofluorescent spots[3,18,19,20]. In this study, the late phase choroidal non-perfusion regions detected by ICGA were significantly associated with DR severity. These regions are indicative of choroidal vascular occlusions. This is consistent with a previous study that has suggested that the association between late large hyperfluorescent spots and glycosylated hemoglobin levels could be an indicator for intrachoroidal microvascular abnormalities[21].

Another finding of this study was a positive trend between inverted inflow phenomena and DR severity. The occlusion of choroidal vessels may disturb the filling speeds of both the retinal and choroidal inflow systems. In diabetes mellitus patients, higher glucose plasma levels increased the resistance index (RI) of ocular/choroidal blood flow[22]. Similarly, measures of blood velocity in the short posterior ciliary, central retinal, and ophthalmic arteries of diabetics have suggested that the RI increased in choroidal vessels and/or the diameter of ophthalmic arteries decreased[23]. In contrast, retinal vascular resistance was normal in patients without retinopathy. To the best of our knowledge, RI represents arterial compliance[24]. Thus, it can be inferred that, in our study, choroidal vascular resistance aggravated ocular/retinal ischemia and contributed to inverted inflow phenomena. Moreover, some choroidal circulatory disorders may occur before DR. For example, choroidal blood flow deficit can be an early pathological change in DR[25].

The present study also establishes a new model for quantitatively assessing choroids by EDI SD-OCT. This model uses CA as a supply parameter for SFCT to describe choroidal vascular abnormalities. We were able to apply this model to eyes with NPDR, DME, as well as SMD.

Evidence from several studies suggests that choroidal factors may induce SMD in DR. First, the damage seen in outer

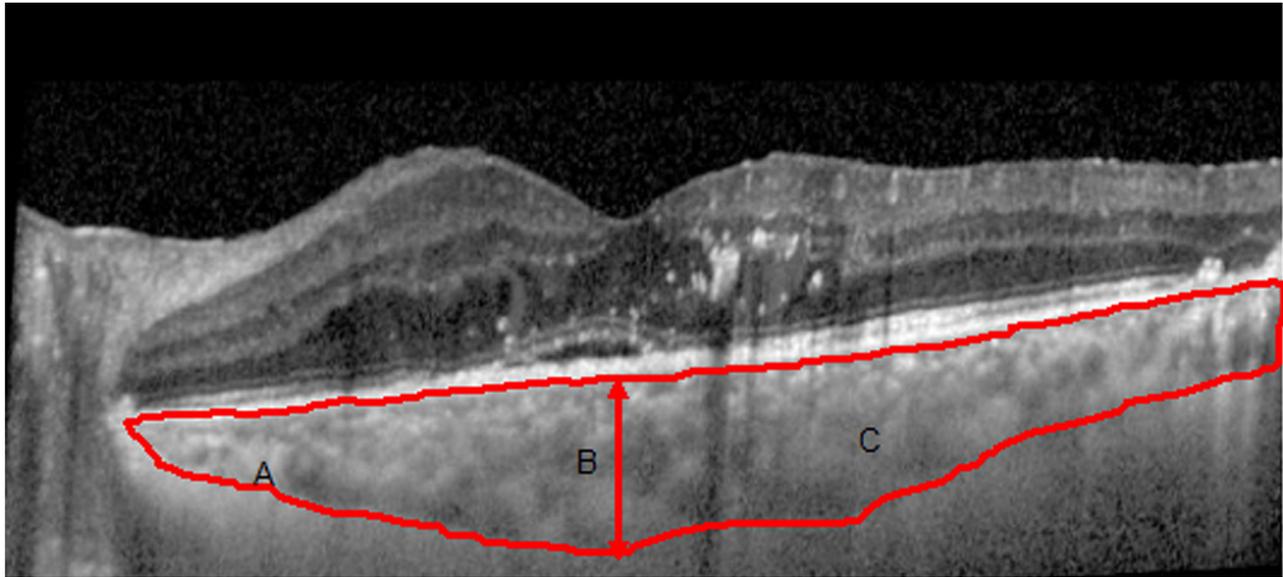


Figure 2. EDI SD-OCT of SMD cases. (A) inner scleral border line; (B) subfoveal choroidal thickness; (C) choroidal area (red boundaries).

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blood retinal barrier has been associated with choroidal ischemia[26]. Second, some cases of DME with SMD also have macular ischemia, and choroids are primarily responsible for macular oxygen and metabolism[27]. Third, choriocapillary obstruction has been histopathologically observed in some cases of DM[3,28,29]. Finally, in CCSCR patients, the SFCT markedly increased, indicating enhanced choroidal permeability and elevated hydrostatic press, which results in SMD[6].

Larger CAs were also observed in the PRP vs. non-PRP sub-group. Mean choroidal blood flow has been shown to increase after PRP in NPDR and non-severe PDR patients[30]. This may be a possible explanation for the observations made in this study. This study also had limitations, however. For example, we did not measure the choroidal volume to analyze DC at this time. In addition, the number of patients with DC who were quantitatively assessed was slightly low. Thus, more research is needed.

In summary, early hypofluorescent spots, late phase choroidal non-perfusion regions, inverted inflow phenomena, higher SFCT, and larger CA are qualitative and quantitative indexes for DC. Moreover, late choroidal non-perfusion regions are a risk factor for DC with DR, and inverted inflow phenomenon was positively associated with DR severity. To

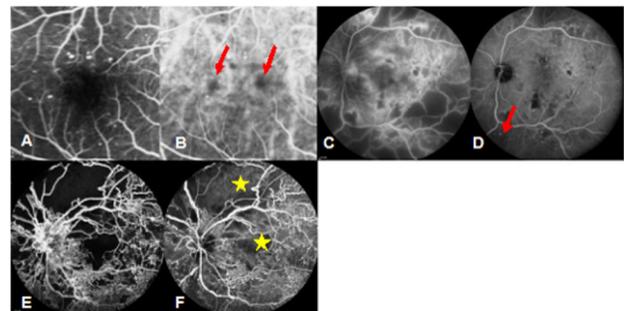


Figure 3. Choroidal abnormalities. Early and late hypofluorescent spots were observed by ICGA (B and D, red arrow) but not by FFA (A and C). Late choroidal non-perfusion regions were also observed by ICGA (F, yellow stars), which was consistent with the dark background fluorescence of FFA (E).

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Table 1. Subfoveal choroidal thickness and choroidal area (SMD and non-SMD sub-group).

Sub-group	Number of eyes	Subfoveal choroidal thickness (μm) ^a	choroidal area (μm^2) ^b
SMD	9	364 (250-400)	906,246 (571,500-996,030)
Non-SMD	9	276 (142-351)	798,066 (255,240-906,876)

^a SMD compared with non-SMD: $z=-2.668$, $P=0.008<0.05$. ^b SMD compared with non-SMD: $z=-2.310$, $P=0.021<0.05$.

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our knowledge, this study not only certified the use of changes in choroidal thickness as a measure of DC but also suggested and applied CA for the first time. Measuring CA is an innovative strategy for qualitative and quantitative choroidal studies. Thus, our study suggests that the combined use of ICGA and EDI SD-OCT, as a supplement to FFA, is a better choice for DR patients.

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Author Contributions

Conceived and designed the experiments: RH LC. Performed the experiments: RH LML. Analyzed the data: RH XLW. Wrote the manuscript: RH XLW LML LC.

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