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Retinal Layer Location of Increased Retinal Thickness in Eyes with Subclinical and Clinical Macular Edema in Diabetes Type 2

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Key Words
Diabetic retinopathy · Diabetes · Optical coherence tomography · Macular edema

Abstract
Purpose: To identify the retinal layer predominantly affected in eyes with subclinical and clinical macular edema in diabetes type 2. ***Methods:*** A cohort of 194 type 2 diabetic eyes/patients with mild nonproliferative diabetic retinopathy (ETDRS levels 20/35) were examined with Cirrus spectral-domain optical coherence tomography (OCT) at the baseline visit (ClinicalTrials.gov identifier: NCT01145599). Automated segmentation of the retinal layers of the eyes with subclinical and clinical macular edema was compared with a sample of 31 eyes from diabetic patients with normal OCT and an age-matched control group of 58 healthy eyes. ***Results:***

See EVICR.net Study Group at http://www.evicr.net/index.php?id=9.

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From the 194 eyes in the study, 62 had subclinical macular edema and 12 had clinical macular edema. The highest increases in retinal thickness (RT) were found in the inner nuclear layer (INL; 33.6% in subclinical macular edema and 81.8% in clinical macular edema). Increases were also found in the neighboring layers. Thinning of the retina was registered in the retinal nerve fiber, ganglion cells and inner plexiform layers in the diabetic eyes without macular edema. ***Conclusions:*** The increase in RT occurring in diabetic eyes with macular edema is predominantly located in the INL but extends to neighboring retinal layers indicating that it may be due to extracellular fluid accumulation.

Introduction

Diabetic retinopathy is one of the leading causes of blindness in developed countries. It is primarily considered to be a vasculopathy, and on ophthalmoscopy the first alterations identified are microaneurysms, small hemorrhages or lipoprotein exudates. The vision loss is due to the development of two major vision-threatening complications, proliferative retinopathy and center-involving macular edema, the latter representing the cause for visual acuity impairment in diabetics [1].

The precise pathophysiological mechanisms leading to the development of diabetic macular edema are still unclear, though microvascular damage through hyperglycemia is considered to play a pivotal role. Recently, much attention has been given to neural damage suggesting that intracellular neuroglial swelling could condition the development of diabetic macular edema [2, 3].

With optical coherence tomography (OCT), it became possible to image the retina in vivo and to measure retinal edema by measuring retinal thickness (RT) with high accuracy. Given that the retinal layers may be affected differentially by diabetes, it appears prudent to quantify the thickness of each retinal layer within the retina separately in order to identify the relative contribution of each layer to the increase in RT representing the objective diagnosis of retinal edema. Recently, automated algorithms have been developed that have achieved reliable segmentation of retinal spectral-domain OCT (SD-OCT) scans by detecting 7–9 surface boundaries in the retina [4, 5].

We report here the results of the application of an automated segmentation algorithm to the SD images obtained using Cirrus SD-OCT in a large cohort of eyes from a multicenter observational study which included eyes with mild nonproliferative diabetic retinopathy (NPDR).

Methods

Patients

This analysis was performed in the context of a prospective, multicenter, observational study, designed to follow eyes/patients with mild NPDR [level 20 and 35 of the Early Treatment Diabetic Retinopathy Study (ETDRS) classification]. The results of this study and the procedures are described in Ribeiro et al. [6].

A total of 374 patients were recruited between September 2010 and June 2012 in 19 European clinical sites. Male and female patients with diagnosed adult-onset type 2 diabetes, age 35–82 years, mild NPDR (20 and 35 of the ETDRS classification), best corrected visual acuity ≥75 letters (≥20/32) and refraction with spherical equivalent less than ±5 dpt were included in the study.

Informed consent was obtained from each patient after explanation of the nature of the study and before any study procedure. The tenets of the Declaration of Helsinki were followed, and approval was obtained from each institutional review board (ClinicalTrials.gov identifier: NCT01145599).

Optical Coherence Tomography

Only the 194 patients who were examined with Cirrus SD-OCT (Carl Zeiss Meditec, Dublin, Calif., USA) are reported here, because of the availability of a normative database and validated application of an automated segmentation algorithm [7].

To identify eyes/patients with increased RT in the central subfield (clinical and subclinical macular edema) and in the inner and outer rings, the reference values established by DRCR.net were used:

For clinical macular edema (ClinicalTrials.gov identifier: NCT01909791) [8]:

- RT ≥290 μm in women and ≥305 μm in men for Cirrus SD-OCT.

For subclinical macular edema [9–11]:

- RT >260 μm and <290 μm in women and >275 μm and <305 μm in men for Cirrus SD-OCT.

For the inner and outer rings (ClinicalTrials.gov identifier: NCT01331005):

- Normal RT, if there was no more than 1 area above the normal range (normal mean + 2 standard deviations) and no area 15 μm above the normal range.
- Increased RT, if there were at least 2 areas above the normal range and/or 1 area 15 μm above the normal range.

Automated Segmentation Algorithm

For retinal layer segmentation, a graph theory segmentation algorithm proposed in Li et al. [12] and Garvin et al. [5] was implemented to automatically identify 8 retinal interfaces, namely vitreous to inner limiting membrane (ILM), retinal nerve fiber (RNFL) to ganglion cell layers (GCL), inner plexiform (IPL) to inner nuclear layers (INL), INL to outer plexiform layer (OPL), OPL to outer nuclear layer (ONL), ONL to inner segment (IS), outer segment (OS) to retinal pigment epithelium (RPE) and RPE to choroid.

The algorithm was applied on 512 × 128 Macular Cube Protocol examinations centered at the fovea acquired with Cirrus SD-OCT from 86 eyes of 47 healthy volunteers aged from 23 to 55 years (mean ± standard deviation: 38.3 ± 9.1) and from 105 eyes of 105 NPDR patients, ETDRS levels 20 and 35, aged from 39 to 82 years (mean ± standard deviation: 60.6 ± 9.2).

Results from segmentation were validated manually by one grader on B scans passing through the central subfield, 500 μm ra-

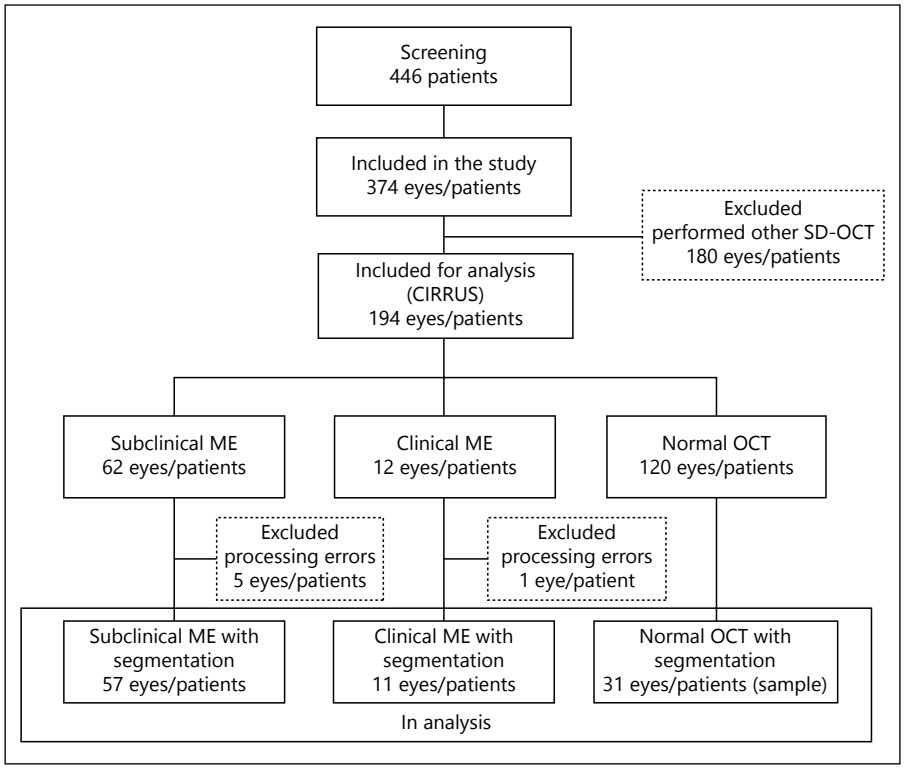

Fig. 1. Consort flow chart. ME = Macular edema.

dius, and every eighth B scan elsewhere. Larger differences between automatic and grader segmentations were found at the OS/RPE interface ranging from 3.97 to 16.61 μm (mean ± standard deviation: 6.92 ± 2.21) and ranging from 1.10 to 20.24 μm (mean ± standard deviation: 5.31 ± 3.39) for healthy subjects and for NPDR patients, respectively.

For healthy subjects, interfaces at the central subfield have larger differences relatively to other ETDRS areas with the exception of the ILM which shows larger differences at the nasal outer areas.

Although differences at the central subfield for NPDR patients are of the same order of magnitude as for the healthy subjects, NPDR patients show larger differences at other areas for the interfaces vitreous/ILM in the inner superior, IPL/INL, INL/OPL, OPL/ONL and RPE/choroid in the outer superior area.

Root mean square errors between automatic and human grader segmentations are close to the device with 5 μm of axial resolution in tissue which makes this segmentation algorithm well suited for detecting individual retinal layer changes in situations where the retinal structure is well preserved, such as mild NPDR, contributing to identify the relative role of the different retinal cells in the retinopathy development.

Data Analysis

Categorical variables are summarized with frequencies and percentages and numerical variables with means and standard deviations.

The nonparametric Kruskal-Wallis test was used to assess differences in retinal layer thickness between study groups and control group, followed by post hoc analysis with Mann-Whitney and Bonferroni adjustment to correct for multiple comparisons.

An age-matched control group was used to compare retinal layer thickness in the study groups [13, 14]. Gender was taken in consideration, as retinal layer thickness measured on Cirrus SD-OCT in healthy eyes showed significant variations by gender [13, 15, 16].

All statistical analyses were performed with STATA version 12.1 (Stata Corp. LP, College Station, Tex., USA), and p values ≤0.05 were considered statistically significant results.

Results

From the 194 eyes/patients (88 left eyes and 106 right eyes) examined with Cirrus SD-OCT at the baseline visit, 62 eyes/patients were classified as subclinical macular edema and 12 eyes/patients as having clinical macular edema. Semiautomated segmentation of the retinal layers on OCT was performed in these two groups and in a sample of 31 diabetic eyes/patients with normal OCT. Due to processing errors of the segmentation tool, 5 eyes/patients classified as subclinical macular edema and 1 eye/patient having clinical macular edema were not included in the final analysis. Therefore a total of 99 eyes/patients (42 left eyes and 64 right eyes) were included in this analysis (fig. 1).

Baseline characteristics of these patients are presented in table 1.

Table 1. Baseline characteristics of the diabetic eyes/patients included in this subanalysis

	Patients included in the analysis (n = 99)		
	normal NPDR (n = 31)	subclinical ME (n = 62)	clinical ME (n = 12)
Gender (female/male)	10 (32.3)/21 (67.7)	23 (37.1)/39 (62.9)	3 (25.0)/9 (75.0)
ETDRS			
Level 20	10 (32.3)	24 (38.7)	2 (16.7)
Level 35	21 (67.7)	38 (61.3)	10 (83.3)
Age, years	59.3±9.5	60.8±9.0	62.9±9.3
HbA_{1c}, %	7.3±1.0	7.9±1.7	7.4±0.6
Systolic blood pressure, mm Hg	131.5±16.3	136.5±17.7	134.8±13.4
Diastolic blood pressure, mm Hg	75.4±13.3	77.1±9.9	73.5±11.4
BMI	29.8±6.6	28.5±4.1	29.0±3.9
BCVA, letters	85.6±4.3	85.5±4.2	85.3±4.0
Microaneurysms, n	2.9±3.3	3.1±5.0	5.0±9.0

Results are expressed as frequency with percentages in parentheses or means ± SD. ME = Macular edema; HbA_{1c} = major fraction of glycosylated hemoglobin; BMI = body mass index; BCVA = best corrected visual acuity.

Table 2. RT values (means ± SD) in the central subfield obtained from the different segmented retinal layers

	Control (n = 58)	NPDR normal OCT (n = 31)	NPDR subclinical ME (n = 57)	NPDR clinical ME (n = 11)	Mean difference from control adjusted by gender					
					NPDR normal OCT increase (n = 31)		NPDR subclinical ME increase (n = 57)		NPDR clinical ME increase (n = 11)	
					diff.	%	diff.	%	diff.	%
RNFL	5.9±3.5	3.8±2.1	5.8±2.7	7±2.8	–3.4	*–58.20*	–1	–17.30	0.8	13.20
GCL + IPL	44±10.2	33.6±4	42.4±6.9	54.3±4.7	–6.9	*–15.60*	1.1	2.50	12.6	*28.60*
INL	17±5.5	15.7±3	21.7±4.3	25.6±3.2	0.4	2.30	5.7	*33.60*	13.9	*81.80*
OPL	21.3±5.3	22.4±7.1	27.7±5.4	31.4±5.5	0.7	3.50	6	*28.20*	9.6	*45.30*
ONL	110.4±8	111.1±10.7	116.8±12.8	126.1±13.3	0.1	0.00	6.1	5.50	10.7	9.70
IS/OS	45.4±2.6	43.6±3.9	42.9±3.6	45.7±3.1	–0.8	–1.80	–0.3	–0.60	–1.1	–2.40
RPE	25.9±2.1	25.6±3.9	24.4±2.1	26.9±2.9	0.4	1.40	–0.3	–1.00	0.6	2.40

ME = Macular edema. The italicized values indicate a statistically significant difference between patients and controls ($p < 0.006$; Bonferroni corrected p value 0.008).

The healthy control group included 58 nondiabetic eyes which were age-matched with the 99 eyes/patients from the study.

Retinal Thickness

In the central subfield, 12 eyes/patients (6.2%; 95% confidence interval, CI: 2.8–9.6%) had an OCT diagnosis of clinical macular edema, according to the DRCR.net standards [8]. Subclinical macular edema was identified in 62 eyes/patients (32.0%; 95% CI: 25.4–38.5%).

In the inner ring, 23 eyes/patients (11.9%) showed increased RT, and in the outer ring increased RT was found in 36 eyes/patients (18.6%).

Identification of Individual Retinal Layer Thickness Changes

When comparing RT values obtained from the different segmented retinal layers (RNFL, GCL + IPL, INL, OPL, ONL, IS/OS, RPE) in eyes/patients with NPDR and subclinical macular edema and NPDR eyes with clinical macular edema with eyes/patients with NPDR and normal overall RT and with age-matched nondiabetic control eyes, selective involvement of specific retinal layers is clearly apparent (table 2 and fig. 2).

In the central subfield, the INL shows the larger increases. These increases are in order of 33.6% in subclinical macular edema and 81.8% in clinical macular edema.

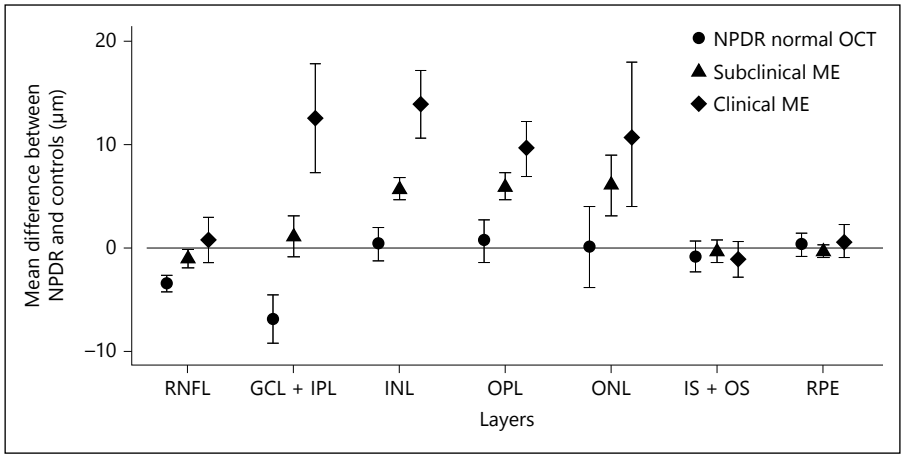

Fig. 2. Mean retinal layer thickness difference (µm) and 95% CI between eyes/patients with normal OCT, subclinical macular edema (ME) and clinical macular edema, and controls in the central subfield.

There is also a marked increase in the OPL in subclinical macular edema (28.2%) and in clinical macular edema (45.3%) with RT increases being registered also in GCL + IPL and in almost all other retinal layers when there is clinical macular edema.

In subclinical and clinical macular edema, the preferential involvement of the INL in RT increase is present but less well defined in the inner and outer ring areas.

There is also thinning of the retina, i.e. decreased RT, in the RNFL and GCL + IPL, in the eyes with NPDR and no evidence of subclinical or clinical macular edema (table 2 and fig. 2). The mean retinal layer thickness difference and 95% CI between eyes from diabetic patients with normal OCT and controls in RNFL were –3.4 µm (95% CI: –4.2 to –2.7) and in GCL + IPL –6.9 µm (95% CI: –9.2 to –4.5).

Discussion

This study is a post hoc analysis of a prospective 1-year, observational, longitudinal study of 374 eyes/patients with diabetes type 2, mild NPDR (ETDRS levels 20 and 35) and good visual acuity, examined at baseline and at 3, 6 and 12 months, in 19 clinical sites across Europe focusing on identification of the retinal layer location of increased RT using automated segmentation of the OCT scans of the eyes/patients examined with Cirrus SD-OCT at baseline.

Center-involved macular edema with indication for treatment based on OCT values, i.e. clinical macular edema, was found in 12 eyes/patients (6.2%). There were also 62 eyes/patients (32.0%) that met the OCT criteria of subclinical macular edema.

There was therefore a total of 74 eyes/patients who showed increased RT at baseline in this cohort of 194 eyes/patients with NPDR (ETDRS 20 and 35).

Automated segmentation of these eyes with increased RT showed that the larger increases in RT in the central subfield are in the INL. These increases are in order of 33.6% in subclinical macular edema and 81.8% in clinical macular edema. The next higher increases in RT were also found predominantly in the neighboring retinal layers, particularly in the OPL.

These findings suggest that retinal edema in the early stages of NPDR in diabetes type 2 is probably due to extracellular fluid accumulation resulting from alteration of the blood-retinal barrier in the deep retinal vascular plexus.

This conclusion is supported by concurring findings. Firstly, the increase in thickness does not involve only one layer but, on the contrary, although the highest RT increase is in the INL, there are also clear increases in the immediately neighboring retinal layers, indicating a gradient in the RT increase which can be better explained by extracellular fluid distribution. This gradient in RT increase around the INL is well demonstrated by figure 2. The RT increase, as it progresses from subclinical to clinical macular edema, involves progressively different layers with different cell types, indicating that no specific cell is preferentially involved. Finally, the preferential involvement of the INL is also well explained by a higher accumulation of extracellular fluid due to the colocalization of the deep retinal vascular net, suspected from histological studies to be preferentially involved in the diabetic retinal microvascular pathology.

The earliest alterations that may be detected clinically in the retina in diabetes are the breakdown of the blood-retinal barrier and alterations in the neurosensory retinal

function. Both these alterations can be detected before ophthalmoscopic signs of diabetic retinopathy become visible, in preclinical retinopathy [17, 18].

Several recent clinical studies have used imaging techniques to demonstrate thinning of the RNFL in diabetes, often in patients with little or no evidence of a vascular phenotype [15, 19]. This thinning of the retina was also found in our study, involving the RNFL and GCL + IPL in eyes with diabetes type 2 that had no edema of the retina demonstrating the presence of neurodegeneration. Thinning, however, disappeared in the presence of clinical macular edema probably masked by the generalized increase in RT spreading to all retinal layers when higher degrees of edema are present.

There is, therefore, apparently a neuropathy, involving primarily the nerve fiber layer and the ganglion cells of the retina that occurs early in the diabetic retinal disease process which is manifested by localized thinning of the inner retinal layers.

In certain patients, however, microvascular damage occurs with alteration of the blood-retinal barrier, manifested by thickening of the INL and neighboring retinal layers, apparently resulting from leakage and increase in the retinal extracellular space around the deep retinal capillary net.

The mechanisms linking neurodegeneration and microvascular abnormalities in the early stages of diabetic retinal disease appear to gain with the results here reported increasing relevance calling for more research in this area.

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Disclosure Statement

None of the authors has any conflict of interest.

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