Shedding new light on diabetic retinopathy with optical coherence tomography

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Chapter 8

Progression of retinal neurodegeneration in patients with diabetes mellitus irrespective of presence or progression of retinal vasculopathy

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ABSTRACT

Purpose: This longitudinal study investigated the change of inner retinal layer thickness in patients with diabetes mellitus type 1 and no or minimal diabetic retinopathy, and its association with duration of diabetes, glycemic control, and the presence and progression of retinal vasculopathy.

Methods: Mean layer thickness was calculated for the ganglion cell plus inner plexiform layer (GCL+IPL) and retinal nerve fiber layer (RNFL) at different time points over a 5 year period following automated segmentation of Time Domain Optical Coherence Tomography images. A retinal specialist graded the fundus photographs for retinal vasculopathy.

Results: The GCL+IPL thinned by approximately 0.29 μm per year, and the RNFL by 0.25 μm per year taking into account age, gender, duration of diabetes, HbA1c, and the presence and progression of retinal vasculopathy. Patients with longer duration of diabetes prior to inclusion had thinner neuroretinal layers in the pericentral area at baseline. Neither presence, nor progression of vasculopathy or HbA1c were associated with the progressive thinning of the inner retinal layers.

Conclusion: Diabetic retinal neurodegeneration in diabetes type 1 is progressive over time and is related to duration of diabetes, but occurs independently of glycemic control and presence or progression of visible retinal vasculopathy.
INTRODUCTION

Diabetic retinopathy (DR) is a microvascular disease defined by the presence of retinal ischemia and vascular leakage, as indicated by the typical lesions of micro-aneurysms, capillary non-perfusion, neovascularization, hemorrhages, macular edema, and lipoprotein exudates. Many studies have shown that DR can be accompanied by neurodegenerative changes including neural apoptosis, loss of ganglion cell bodies, glial reactivity and reduction in thickness of the inner retinal layers in the earliest stages of DR, in both animal models and in humans. This diabetic retinal neuropathy corresponds to early retinal functional deficits that have been found in patients with diabetes, including electroretinogram abnormalities, loss of dark adaptation and contrast sensitivity, color vision disturbances, abnormal microperimetry and frequency doubling technology (FDT) visual field testing, even in the absence of DR.

In the retina, glia and neurons closely interact with the retinal vasculature to maintain the homeostasis necessary for normal neuroretinal function. However, the pathophysiological relationship between vascular diabetic retinopathy and diabetic retinal neuropathy is unknown. Diabetic retinal neuropathy could be a secondary effect of vascular damage, itself resulting from hyperglycemia, leading to increased permeability and occlusion of the retinal microvasculature and subsequent neuronal loss. In this scenario diabetic vasculopathy would preceed the retinal neuropathy. An alternative hypothesis is that diabetes primarily affects the neuroretina, and that this secondarily compromises vascular integrity by an unknown mechanism, in which case diabetic vasculopathy is preceded by diabetic retinal neuropathy. Currently, neither of these hypotheses has been refuted, and it is also possible that these pathological changes occur independently, as two separate sequelae of the diabetic state both interfering with visual function.

The introduction of optical coherence tomography (OCT) has allowed measurements of the thickness of the different retinal layers with high accuracy in vivo. Fully automated algorithms have been developed for the segmentation of retinal time-domain and spectral-domain OCT scans that are capable of detecting seven to 11 surface boundaries in the retina. We and others have shown in cross-sectional studies that decreased retinal thickness in diabetic patients with no or minimal DR is decreased in previous cross-sectional studies, due to decreased thickness of the inner retinal layers, specifically the ganglion cell layer. This inner retinal thinning was related to diabetes duration, but not to the presence of DR.

The purpose of the present prospective longitudinal study is to investigate the progression patterns of thinning of ganglion cell and nerve fiber layers thickness in diabetes over time, correcting for the effects of normal aging, and to determine the possible relationship between this inner retinal change and diabetes parameters, and the presence or progression of retinal vasculopathy.
MATERIALS AND METHODS

Patients
Forty-five patients, recruited from the outpatient clinic of the department of Internal Medicine at the Academic Medical Center at the University of Amsterdam, the Netherlands between July 2004 and June 2005, participated in this ongoing longitudinal observational cohort study. These patients also participated in our previously reported observational cross-sectional studies that demonstrated significant loss of thickness of the GCL and corresponding loss of RNFL thickness in type 1 diabetic patients with minimal DR when compared to normal non-diabetic controls.\textsuperscript{20, 21} The study adhered to the tenets of the Declaration of Helsinki. Investigative Review Board approval was obtained at both the AMC and the University of Iowa and all participants provided written informed consent.

Patients with type 1 diabetes mellitus (DM) were included if they had no DR or minimal DR as determined by a retinal specialist through slit-lamp stereo biomicroscopy and fundus photographs (TRC-50IX; Topcon Corporation, Tokyo, Japan). Minimal DR was defined as the presence of at least one micro-aneurysm and/or hemorrhage in the central retina in the absence of peripheral lesions, conform stage 2 of the International Clinical Diabetic Retinopathy Disease Severity Scale.\textsuperscript{39} Patients with any sign of diabetic macular edema were excluded. Other exclusion criteria were refractive errors over S+5 or under S-8 diopters, visual acuity below 20/25, significant media opacities, previous ocular surgery, and a previous diagnosis of glaucoma, uveitis, or other retinal disease. All patients underwent complete ophthalmologic examination at baseline and thereafter approximately every 12 months. Visual acuity was measured using an Early Treatment Diabetic Retinopathy Study chart at 4 meters. After pupil dilation with 0.5% phenylephrine hydrochloride and 1.0% tropicamide, both eyes were examined with stereoscopic slit-lamp biomicroscopy and a handheld lens (SuperField; Volk Optical, Inc., Mentor, OH). Subjects were imaged with StratusOCT (StratusOCT, Model 3000, Carl Zeiss Meditec, Dublin, CA, USA, software version 4.0.1). The fast macular thickness OCT scan protocol was performed. This scan protocol obtains six cross-sectional B-scans, 6 mm in length, at equally spaced angular orientations (30°) in a radial spoke pattern centered on the fovea. At each visit fundus photographs (IMAGEnet i-base, version 3.5.4; Topcon Europe Medical, Capelle a/d/IJssel, the Netherlands) were taken and a retinal specialist graded the diabetic vasculopathy (grade 0= 0 microaneurysms; grade 1= 0-5 microaneurysms; grade 2= 5-10 microaneurysms; grade 3= 10-20 microaneurysms; grade 4= >20 microaneurysms). This grading described the presence and progression of vascular lesions. Blood pressure and HbA1c were measured at each visit.

Automated segmentation and thickness measurement of the separate layers
The 6 retinal layers that can be identified using automated segmentation of the six cross-sectional B-scans were interpreted as follows (from inner to outer surface): retinal nerve
fibre layer (RNFL), ganglion cell layer (GCL) + inner plexiform layer (IPL), inner nuclear layer, outer plexiform layer, outer nuclear layer + inner segments (photoreceptors), outer segments (photoreceptors). In this paper we focussed on the RNFL and the GCL+IPL because in our previous studies in DM patients a significant thinning of these inner retinal layers was found compared to healthy control subjects. After segmentation, two retinal areas of interest were defined as follows: the pericentral area, a donut shaped ring centered on the fovea with an inner diameter of 1 mm and an outer diameter of 3 mm; and the peripheral area, with an inner diameter of 3 mm and outer diameter of 6 mm.

Statistical analysis
Statistical analysis was performed using SPSS software version 20.0 (SPSS Inc., Chicago, IL, USA) and SAS software version 9.2 (SAS Institute Inc., Cary, North Carolina). Analysis of variance (ANOVA) was used to assess differences between the 4 follow up visits in mean HbA1c, mean blood pressure and mean best corrected visual acuity (BCVA). In a previous study it was demonstrated that there was no statistical difference in measurements between both eyes, and one eye was at random chosen for the present analysis. Changes over time in the thickness of the inner retinal layers were assessed using a linear mixed regression model with a first-order autoregressive covariance structure, to take into account that repeated measurements on the same subject over time are highly correlated. The first-order autoregressive covariance structure takes into account that observations on the same subject that are closer in time are more highly correlated than measurements at times further apart. The correlation between two measurements within the same subject decreases exponentially as the length of time between the measurements increases. The mixed-effects model is also robust to missing data and can handle uneven spacing of the repeated measurements. The model was adjusted for age, gender, duration of diabetes, HbA1c, DR at inclusion and progression of DR, which were included as fixed-effects in the model. Age, HbA1c and progression of DR were included in the model as time updated co-variates. The primary research objective was to test if there was progressive thinning of the pericentral and peripheral RNFL and/or GCL+IPL. To limit the risk for type I errors because of multiple testing, a Bonferroni correction was applied: P-values below 0.0125 were considered to be statistically significant. All reported p-values are 2-sided.

RESULTS
In total, 45 patients with type 1 diabetes mellitus, who had already been described in a previous cross-sectional study, were included. At the time of inclusion 21 patients had no DR and 24 patients showed minimal DR. During follow up, 18 patients showed progressive vasculopathy. The mean HbA1c at baseline was 8.2 (SD 1.2%). There were no statistically significant differences in mean HbA1c, blood pressure and BCVA between the 4 study visits. The median follow up time was 73.0 months (IQR 57.5 – 80). The minimal follow-up
time was 37 months, the maximum was 79 months. Some patients skipped intermediary visits (n=6), and some were lost to follow-up (n=10) because of migration.

The linear mixed model showed that the inner retinal layers decrease in thickness over time while adjusting for gender, age at inclusion, duration of diabetes, HbA1c, DR at inclusion and DR progression. The changes in RNFL and the GCL+IPL thickness over time are shown in Table 2. We observed thinning of the pericentral and peripheral RNFL and in the pericentral GCL+IPL over time. The macular GCL+IPL thins approximately 0.29 μm per year, and the peripheral and macular RNFL thickness by 0.25 μm per year. Although the GCL+IPL layer in the peripheral ring shows a trend to become thicker over time, this change was not statistically significant.

Table 1. Baseline characteristics of the study group at inclusion.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Diabetic patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients*</td>
<td>45</td>
</tr>
<tr>
<td>Age (years)</td>
<td>31 ± 10</td>
</tr>
<tr>
<td>Male gender, n</td>
<td>17 (37.9%)</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>16.4 ± 7.6</td>
</tr>
<tr>
<td>No. of patients with minimal DR</td>
<td>24 (53.3%)</td>
</tr>
<tr>
<td>BCVA (logMAR)</td>
<td>-0.05 ± 0.07</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.2 ± 1.2</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>123 ± 14</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>76 ± 10</td>
</tr>
</tbody>
</table>

Values are the mean ± standard deviation or number of patients. DR, diabetic retinopathy; BCVA, best corrected visual acuity; HbA1c, glycosylated hemoglobin.

Patients with longer duration of diabetes prior to inclusion had thinner RNFL and thinner GCL layers in the pericentral area at inclusion. The thinner layers observed with each year of longer diabetes duration are in agreement (both in direction and magnitude) with the decrease per year during the observation period. Each year of longer duration of diabetes prior to inclusion was associated with a 0.21 and 0.19 μm thinner RNFL in the peripheral and pericentral macula, and during follow up the peripheral and pericentral RNFL thickness decreased with 0.25 and 0.25 μm per year. Each year of longer duration of diabetes prior to inclusion was associated with a 0.44 μm thinner GCL-IPL in the pericentral macula and during follow up the GCL-IPL thickness decreased with 0.29 μm per year.
Progressive retinal neurodegeneration in type 1 diabetic patients

Table 2. Four linear mixed regression models: change over time of the retinal nerve fiber layer thickness and the ganglion cell plus inner plexiform layer thickness in type 1 diabetes patients with no or minimal diabetic retinopathy (DR). In the table the change in thickness is shown per 1 unit change of each independent variable.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Model 1: RNFL (peripheral) *</th>
<th>Model 2: RNFL (pericentral) *</th>
<th>Model 3: GCL+IPL (peripheral) *</th>
<th>Model 4: GCL+IPL (pericentral) *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>38.72 (32.68 to 44.76)</td>
<td>15.05 (10.05 to 20.05)</td>
<td>71.21 (65.40 to 77.03)</td>
<td>104.18 (93.58 to 114.78)</td>
</tr>
<tr>
<td></td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Female gender</td>
<td>-0.27 (-1.84 to 1.31)</td>
<td>-1.79 (-3.10 to -0.48)</td>
<td>0.11 (-1.50 to 1.71)</td>
<td>-4.22 (-7.49 to -0.95)</td>
</tr>
<tr>
<td></td>
<td>0.739</td>
<td>0.008</td>
<td>0.896</td>
<td>0.012</td>
</tr>
<tr>
<td>Age at inclusion (year)</td>
<td>-0.02 (-0.13 to 0.08)</td>
<td>0.08 (-0.02 to 0.15)</td>
<td>-0.06 (-0.15 to 0.06)</td>
<td>0.04 (-0.19 to 0.27)</td>
</tr>
<tr>
<td></td>
<td>0.612</td>
<td>0.135</td>
<td>0.374</td>
<td>0.786</td>
</tr>
<tr>
<td>Duration prior DM (year)</td>
<td>-0.21 (-0.34 to -0.06)</td>
<td>-0.19 (-0.30 to -0.08)</td>
<td>-0.11 (-0.27 to 0.04)</td>
<td>-0.44 (-0.74 to -0.13)</td>
</tr>
<tr>
<td></td>
<td>0.005</td>
<td>0.002</td>
<td>0.120</td>
<td>0.004</td>
</tr>
<tr>
<td>Minimal DR at inclusion</td>
<td>-0.19 (-2.09 to 1.69)</td>
<td>1.03 (-0.55 to 2.60)</td>
<td>0.17 (-1.73 to 2.05)</td>
<td>1.71 (-1.98 to 5.38)</td>
</tr>
<tr>
<td></td>
<td>0.838</td>
<td>0.203</td>
<td>0.864</td>
<td>0.361</td>
</tr>
<tr>
<td>Follow-up (year)</td>
<td>-0.25 (-0.42 to -0.08)</td>
<td>-0.25 (-0.40 to -0.11)</td>
<td>0.19 (0.04 to 0.36)</td>
<td>-0.29 (-0.49 to -0.08)</td>
</tr>
<tr>
<td></td>
<td>0.004</td>
<td>&lt;.001</td>
<td>0.021</td>
<td>0.006</td>
</tr>
<tr>
<td>DR score (grade)</td>
<td>0.06 (-0.59 to 0.70)</td>
<td>0.06 (-0.48 to 0.61)</td>
<td>-0.32 (-0.93 to 0.29)</td>
<td>-0.63 (-1.48 to 0.23)</td>
</tr>
<tr>
<td></td>
<td>0.855</td>
<td>0.815</td>
<td>0.283</td>
<td>0.147</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>-0.40 (-0.80 to -0.00)</td>
<td>0.13 (-0.19 to 0.46)</td>
<td>0.17 (-0.19 to 0.51)</td>
<td>0.06 (-0.42 to 0.53)</td>
</tr>
<tr>
<td></td>
<td>0.048</td>
<td>0.432</td>
<td>0.366</td>
<td>0.811</td>
</tr>
</tbody>
</table>

* fixed-effect parameter estimate (95% confidence interval), t statistic two-tailed p-value. DR, diabetic retinopathy; HbA1c, glycylated hemoglobin; RNFL retinal nerve fiber layer; GCL, ganglion cell layer; IPL, inner plexiform layer.

DISCUSSION

Several previous reports have shown that the inner retinal layers in the macula of diabetic patients with minimal DR are thinner compared to normal non-diabetic. The results of our present study now demonstrate that the thinning of the inner retinal layers – specifically RNFL, GCL, and IPL - in the macula is progressive over time and that duration of diabetes appears to be a key determinant. In contrast, this retinal neurodegeneration was not associated with presence or progression of visible retinal vasculopathy, or magnitude of hyperglycaemia and therefore these processes seem to occur independently in the context of diabetes.

Although the negative relationship between duration of diabetes and inner retinal layer thickness seems statistically robust, the hypothesis that retinal neurodegeneration is the direct result of hyperglycaemia is disputable. The results of this present study provide no evidence for a strong correlation between HbA1c levels and the thinning of the
peripheral RNFL or GCL. The question is which other mechanisms than hyperglycemia in diabetes could be the cause of the observed retinal neurodegeneration. The molecular mechanisms involved in retinal neurodegeneration in diabetes have previously been proposed to be complex and may include a combination of ocular factors such as increased oxidative stress, loss of neuroprotective factors, increased inflammation, glutamate excitotoxicity, and systemic factors including hyperglycemia, dyslipidemia, and insulin deficiency.\textsuperscript{19}

When studying changes in retinal layer thickness, it is essential to distinguish disease processes from normal aging-related changes. Various previous papers described age-related thinning of inner retinal layers in the macula in normal subjects. Ooto \textit{et al.} found age-related losses in the thicknesses of the RNFL, GCL and IPL over the whole macula of 0.05, 0.07 and 0.05 μm/year, respectively.\textsuperscript{41} Demirkaya \textit{et al.} postulated that a healthy individual will lose 0.133 μm/year of peripapillary RNFL, 0.103 μm/year of pericentral macular GCL and 0.046 μm/year of peripheral macular IPL.\textsuperscript{42} Kim \textit{et al.} showed that the mean inner retinal layer thickness (RNFL, GCL and IPL) decreased by approximately 0.159 μm/year.\textsuperscript{43} Mwanza \textit{et al.} found a 0.102% decrease in thickness per calendar year (approximately 0.07 μm/year).\textsuperscript{44} The data of these previous studies and our study are difficult to compare because the methods differed in OCT devices, scanning area and segmentation algorithms. However, the rate of thinning of the macular RNFL and GCL in our group of diabetic patients did exceed the thinning observed in the normal population in these previous studies. By extrapolation, the results of the present study suggest a decline in GCL-IPL thickness in the pericentral macula of 2.9 μm per decade (0.29 μm per year) in type 1 diabetes patients, and for the RNFL thickness a decline of 2.5 μm per decade (0.25 μm per year). In our patient population, the linear mixed regression model showed that age was not significantly associated with macular RNFL and GCL thickness, which might be because our patient population is relatively young with a mean age of 31 years at the time of inclusion, and the limited age range. Mwanza \textit{et al.} showed that in their patient population the GCL-IPL thickness was stable between 18 and 49 years and then decreased progressively. They found that the correlation of average GCL-IPL thickness with age was curvilinear, with a steeper drop beyond the age of 60 years.\textsuperscript{44}

Our results indicate a strong relationship between the inner retinal layer thicknesses and gender, although we did not find evidence that the rate of thinning is different for men and women. Both the GCL-IPL and the RNFL are significantly thinner in the macula of female subjects compared to men, but the rate of thinning over time was not significantly different between men and women. This is in line with earlier studies showing that retinal thickness is significantly thinner in female subjects.\textsuperscript{41,45} Koh \textit{et al.}, described that specifically the GCL-IPL thickness is significantly lower in females compared to men.\textsuperscript{46} Other studies however have found no relationship between inner retinal layer thickness and gender.\textsuperscript{43,47} The present findings indicate that gender must be considered while interpreting macular retinal thickness data.
The present study has several limitations. The grading of the severity and progression of DR was done by a single reader through ophthalmologic examination including indirect fundoscopy, slit-lamp stereo biomicroscopy, and 3-field fundus photography, instead of the gold standard, i.e. 7 field stereoscopic fundus photography assessment by independent trained graders. Time domain OCT is limited because only 6 scans are obtained that need to be interpolated over relatively large areas. With the advent of spectral domain OCT, regional differences in layer thickness can be determined more reliably. Although the faster scanning time and increased depth resolution of the spectral domain OCT allow for a substantial improvement of retinal thickness mapping resolution, with less movement artefacts, there is no reason to believe that the outcome of this study would have been different using the spectral domain OCT.

In summary, this study shows for the first time that thinning of the inner neuroretinal layers in the macula is progressive over time and exceeds the expected effects of normal aging, and that this diabetic retinal neurodegeneration is associated with duration of diabetes, but occurs independently of presence or progression of visible retinal vasculopathy, and glycemic control.
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