Retinal Nerve Fiber Layer and Ganglion Cell Complex Thicknesses Are Reduced in Children With Type 1 Diabetes With No Evidence of Vascular Retinopathy

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PURPOSE. To determine whether type 1 diabetes (T1DM) in children with a mean age of 12.21 ± 3.04 years affects the retinal nerve fiber layer (RNFL) and ganglion cell complex (GCC) when compared to age- and sex-matched healthy children.

METHODS. Forty-six children with T1DM with no diabetic retinopathy (DR) and 50 normal age- and sex-matched controls underwent full clinical ophthalmic and spectral-domain optical coherence tomography (SD-OCT) examination. Using RTVue Fourier-Domain OCT (version 6.11.0.12) average, superior, and inferior RNFL and GCC thicknesses (in μm) were measured. Mean values of patients and the control group were compared.

RESULTS. In children with T1DM with no DR, the mean average RNFL thickness was 110.9 μm ± 10.46, and the mean GCC thickness was 95.59 μm ± 5.13; both were significantly thinner than the control group (115.62 μm and 99.30 μm, respectively). The retinal nerve fiber layer and GCC thickness showed no correlation to either age of onset, duration of the disease, or glycosylated hemoglobin (HbA1c). A positive correlation was found between the daily insulin dose and the average RNFL thickness (r = 0.378, P = 0.01). The average GCC in children with dyslipidemia was thinner than those with normal lipid profile (91.29 ± 6.46 μm, 97.11 ± 3.59 μm, respectively) with a P value of 0.011.

CONCLUSIONS. Thinning of the RNFL and GCC in children with T1DM with no DR compared to healthy controls suggests that neurodegenerative changes occur in the absence of vascular changes. It also shows that neurodegeneration is not related to either disease duration, onset, or control.

Keywords: type 1 diabetes mellitus, children, optical coherence tomography, retinal nerve fiber layer, ganglion cell complex, neuro-degeneration, vasculopathy

Diabetes mellitus is an endemic disease with rapidly increasing prevalence around the world.1 Diabetic retinopathy (DR) is considered one of the most frequent and serious complication and a leading cause of blindness in developed countries despite the advances in medical and surgical management. This necessitates early diagnosis, improved monitoring, and proper management of the ocular disease in order to improve patient care and to reduce the incidence of blindness. The clinical onset of vascular changes in DR including microaneurysms, hemorrhages, and/or exudates is clinically detected either by slit-lamp biomicroscopy, indirect ophthalmoscopy, or fundus photography.2–4 This has been used to determine the severity of the retinal disease, and has regarded DR as a primary microvascular disease. However, other studies have shown that there is neuronal cell loss especially retinal ganglion cells, apoptosis of glial and neural cells in the retina, decreased thickness of inner retinal layers, and retinal dysfunc tion, such as multifocal ERG defects, in early stages before microvascular disease can be clinically detected.5–21

Some have proposed that the microvascular pathology causes the retinal neuropathy, and others have suggested a primary neurologic damage from chronic hyperglycemia or both mechanisms acting together.22–25

This open debate has led researchers to investigate more thoroughly the neurologic changes that occur early in the retina before the occurrence of any clinically detected vascular changes. They studied the relation of these changes to the type, onset, control, and duration of diabetes to determine whether they are secondary to the vascular damage or to the hyperglycemic load or are primarily neurologic.

Spectral-domain optical coherence tomography (SD-OCT) is a noninvasive technique that has now become an important tool for accurate measurements of retinal thickness with high resolution and enhanced definition of the different retinal layers.24 Previous OCT studies have shown different results regarding retinal thickness changes in diabetes patients, as some have reported an increase,25–28 while others have shown a decrease in the thickness of the central retina.29–32 More recent studies report that there is a selective thinning of the inner retina, which occurs during the very early stages of DR. This supports the consensus of the presence of a neurodegenerative process in the pathophysiology of DR.32,33
The purpose of this study was to measure the peripapillary retinal nerve fiber layer (RNFL) thickness and the ganglion cell complex (GCC) in the perimacular area in children with type 1 diabetes (T1DM) with no DR and compare them to normal age- and sex-matched controls. Tackling a different age group (5–18 years) compared to previous clinical studies was our main aim, in addition to trying to find possible relationship among the OCT values and age of onset, duration, and control of diabetes as indicated by the levels of glycosylated hemoglobin (HbA1c), as well as a relationship with the age of patients at the time of the study.

**Materials and Methods**

**Patients**

The study protocol of this case-control cross-sectional study was approved by Cairo University Hospital Research Committee. The study and data collection conformed to all local laws and were compliant with the principles of the Declaration of Helsinki.

Children with T1DM were recruited from the outpatients’ clinic of the Diabetes, Endocrine, & Metabolism Pediatric Unit (DEMPU) of the Kasr Alainy Hospitals in the period from January 2015 to December 2015. Our inclusion criteria were patients in the age group (5–18 years), diagnosed with T1DM for a minimum duration of 5 years with normal fundus examination and having no recent change in their visual acuity. Children having glaucoma, previous ocular trauma, ocular surgery, refractive errors, >5 diopter sphere, best-corrected visual acuity (BCVA) < 20/25, or any other existing ocular pathology were excluded from the study.

A healthy control group (n = 50) of normal children was matched for sex and age. They did not have any history of ocular disease or relevant systemic disease, nor family history of glaucoma nor any relevant systemic disease. They were coming to the pediatric ophthalmology clinic for minor complaints and/or refractive error correction.

All patients underwent physical examination, by a pediatric endocrinologist. Age, sex, and onset and duration of T1DM were recorded. The following parameters were measured: HbA1c, total cholesterol (TC), triglycerides (TG), serum creatinine, urine creatinine, thyroid stimulating hormone (TSH), and free thyroxin (T4). Height, weight, body mass index, as well as the daily insulin dose taken by these children and their blood pressure were also recorded.

Patients underwent a full ophthalmic examination. Slit-lamp examination for the anterior segment as well as IOP measurements were taken. Visual acuity was measured using an Early Treatment Diabetic Retinopathy Study (ETDRS) chart at 4 m. Best-corrected visual acuity was recorded as Snellen equivalent. Fundus examination by a retina specialist was carried out after pupil dilation with 0.5% phenylephrine hydrochloride and 0.1% tropicamide, with indirect ophthalmoscope as well as by slit-lamp biomicroscopy. The clinical grading of DR used by the retina specialist was based on the ETDRS grading scheme. This scheme classified DR into mild and moderate nonproliferative DR (background DR), severe nonproliferative retinopathy (pre-proliferative DR) and non-high-risk and high-risk proliferative DR (proliferative DR). Absence of any of these findings by clinical examination was considered as a normal fundus.

**OCT Measurements**

Subsequently, all the subjects were examined using SD-OCT (RTVue Fourier-Domain OCT, v 6.11.0.12, Optovue, Inc., USA).

The optic nerve head (ONH) analysis measures the disc area, the rim area, and the cup-to-disc ratio. Retinal nerve fiber layer thickness was calculated from the images of 6 circular and 12 linear scans along a 3.45-mm diameter circle around the optic disc, whereas GCC thickness, defined as the distance from the internal limiting membrane to the outer boundary of the inner plexiform layer, was calculated from a 7 × 7 mm grid of the macula 1-mm temporal to the fovea. Eyes were divided in two sectors, superior and inferior. Retinal nerve fiber layer and GCC were expressed as the average thickness (in µm) of both sectors (AvgRNFL; AvgGCC), as well as of the superior (SupRNFL; SupGCC) and inferior (Inf RNFL; Inf GCC) sector.

In addition, the RTVue SD-OCT device is equipped with a software that allowed the analysis of diffuse and focal GCC defects by calculating global loss volume (GLV) and focal loss volume (FLV), respectively. The former, which corresponds to the overall thinning of the topography of the GCC, is computed as the sum of the negative fractional deviation in the entire area, whereas the latter, which represents the “potholes in the tomography of the GCC,” is computed as the total sum of statistically significant GCC volume loss divided by the GCC map area.

**Statistical Analysis**

Data were coded and entered using the statistical package SPSS version 22. Data were summarized using mean and standard deviation for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Comparisons between groups were done using unpaired t test. For comparing categorical data, χ² test was performed. Exact test was used instead when the expected frequency is less than 5. Correlations between quantitative variables were done using Pearson correlation coefficient. P values less than 0.05 were considered as statistically significant.

**Results**

A total of 150 children with T1DM were examined in the pediatric endocrinology clinic in the duration from January 2015 to December 2015. Forty-six of which met our inclusion criteria. There was no significant difference in age and sex in both patients and control groups. Patients were in average glycemic control (mean HbA1c, 8.77; SD, 1.73, 4%). Dyslipidemia was present in 12 patients (26.1%), whereas the rest had normal lipid profile. Nephropathy was present in 18 children (39.1%), and 14 children (30.4%) were hypertensive and on angiotensin converting enzyme inhibitors.

Eighteen children (39.1%) gave a positive family history of DM. Demographics and clinical data of the diabetics and the normal controls were shown in Tables 1 and 2.

All eyes included in the analysis had a BCVA of at least 20/25 and the intraocular pressure was within the normal range. The spherical equivalent refraction (SER) in the patients group ranged from −2.5 to +1.0 and that of the control group ranged from −2.0 to +1.5 with no statistically significant difference.
between both groups. Leung et al.35 in 2006 concluded that the RNFL thickness decreased with the axial length and negative spherical equivalent of the eye; however, this was found in highly myopic eyes with SER > 6.35 This was not applicable to either our patients or our control group, so no correction for the axial lengths on the SD-OCT was done. In our study we found that the mean average RNFL and the mean average GCC thicknesses in diabetics were significantly lower than the mean average RNFL and mean average GCC thickness in the control group (Figs. 1, 2). Also the average superior and inferior RNFL thickness, as well as the superior and inferior GCC, were all found to be significantly thinner in T1DM patients than in control eyes as shown in Table 3.

No correlation could be found between the values of the RNFL and the GCC on one hand and the age of onset of DM or the duration of DM on the other. Also no correlation was found between either the average RNFL or the average GCC and HbA1c.

A statistically significant moderate positive correlation between the average RNFL thickness (r = 0.37, P = 0.01), superior (r = 0.33, P = 0.025) and inferior RNFL thicknesses (r = 0.327, P = 0.026), and the amount of daily insulin intake was found. A similar correlation was found between the superior average GCC and amount of daily insulin dose (r = 0.296, P = 0.046).

We also found that children with insulin-dependent diabetes mellitus (IDDM) who had hyperlipidemia had a statistically significant lower average GCC (91.29 μm) and superior GCC thickness (91.38 μm) than those who were not hyperlipidemic (97.11 μm, 96.5 μm; P = 0.011, P = 0.032).

The inferior average RNFL was also found to be thicker in the patients who had nephropathy (115.29 μm) than those who did not (106.63 μm) with a P value of 0.009.

**DISCUSSION**

Molecular mechanisms involved in retinal neurodegeneration in diabetes are complex. A combination of ocular factors is involved as increased oxidative stress, loss of neuroprotective factors, increased inflammation, glutamate excitotoxicity, in addition to other systemic factors including hyperglycemia, dyslipidemia, and insulin deficiency.36-37

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Children With IDDM, N = 46</th>
<th>Reference Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c</td>
<td>8.77 ± 1.73</td>
<td>&lt;7.5 good control</td>
</tr>
<tr>
<td>Height, cm</td>
<td>145 ± 14.46</td>
<td>NA</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>44.72 ± 17.26</td>
<td>NA</td>
</tr>
<tr>
<td>BMI</td>
<td>20.27 ± 4.69</td>
<td>NA</td>
</tr>
<tr>
<td>Insulin dose, IU/kg/d</td>
<td>1.27 ± 0.38</td>
<td>NA</td>
</tr>
<tr>
<td>FT4</td>
<td>1.34 ± 0.26</td>
<td>0.8-2 ng%</td>
</tr>
<tr>
<td>TSH</td>
<td>1.88 ± 0.79</td>
<td>Up to 5 IU/mL</td>
</tr>
<tr>
<td>TG</td>
<td>88.35 ± 35.32</td>
<td>&lt;150 mg/dL</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>162.87 ± 35.21</td>
<td>&lt;200 mg/dL</td>
</tr>
<tr>
<td>LDL</td>
<td>90.42 ± 24.97</td>
<td>&lt;100 mg/dL</td>
</tr>
<tr>
<td>HDL</td>
<td>47.41 ± 15.7</td>
<td>&gt; 40 mg/dL in males and &gt;50 in females</td>
</tr>
<tr>
<td>A/C ratio</td>
<td>32.40 ± 34.5</td>
<td>&lt;30</td>
</tr>
</tbody>
</table>

FT4, free thyroxine; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; A/C ratio, albumin/creatinine ratio.
The exact relationship between vascular DR and diabetic retinal neuropathy is not yet known. It is also possible that these pathologic changes occur independently as two separate sequelae of the diabetic state. Previous studies indicated that neuro-retinal degeneration is one of the earliest detectable retinal abnormalities in patients with DM, and possibly even preceding vasculopathy.38–40

Earlier studies were limited to older age groups and changes were reported in patients with no or minimal DR.22,29,33 However, in our study patients were all in the pediatric age group with no DR, and our results emphasized the presence of primary pathology in inner retinal layers, particularly the GCC and confirmed its occurrence very early in the disease course even before any clinically detected fundus changes.

This study demonstrated that in children with T1DM there was a significant reduction in the average GCC thickness at the macula and in the average RNFL thickness in the peripapillary region in which hyperlipidemia seemed to have an aggravating effect.

The daily dose of insulin demonstrated a possible neuroprotective role as patients with higher daily insulin dose had a significantly thicker RNFL and GCC compared to others on a lower dose.41

Our study also showed that children with nephropathy had relatively thicker inferior RNFL thickness compared to the rest of the patients, which may reflect the presence of tissue edema, secondary to hypoalbuminemia, in dependent parts. However, this cannot rule out the possibility of mild edema in the inner retina caused by subclinical retinal vascular permeability.

While previous studies have emphasized the role of DM duration in retinal changes,22 we could not detect such finding. In addition, we could not find a statistically significant correlation between inner layer thickness changes and HbA1c.

In a recent study by Sohn et al.,42 the authors elicited a

**Figure 2.** RTVue-100 (Optovue, Inc., Fremont, CA, USA) OCT measurements of the right eye revealed both the peripapillary RNFL thickness and GCC significance maps reporting normal findings in one of the control group. OD, right eye; IN, inferonasal sector; IT, inferotemporal sector; SN, superonasal sector; ST, superotemporal sector; TL, lower temporal sector; TU, upper temporal sector.

**Table 3.** OCT Parameters in the Children With T1DM and the Control Group

<table>
<thead>
<tr>
<th>OCT Parameters</th>
<th>Patients Mean</th>
<th>SD</th>
<th>Control Mean</th>
<th>SD</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average RNFL, μm</td>
<td>110.99</td>
<td>10.46</td>
<td>115.62</td>
<td>6.91</td>
<td>0.013</td>
</tr>
<tr>
<td>Sup. Avg RNFL, μm</td>
<td>111.96</td>
<td>12.81</td>
<td>128.18</td>
<td>20.43</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Inf. Avg RNFL, μm</td>
<td>110.02</td>
<td>11.28</td>
<td>123.08</td>
<td>15.66</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Avg. GCC, μm</td>
<td>95.59</td>
<td>5.13</td>
<td>99.30</td>
<td>3.87</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Sup. GCC, μm</td>
<td>95.16</td>
<td>5.39</td>
<td>98.63</td>
<td>3.94</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Inf. GCC, μm</td>
<td>96.48</td>
<td>6.03</td>
<td>100.02</td>
<td>4.94</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FLV %</td>
<td>0.72</td>
<td>0.82</td>
<td>0.37</td>
<td>0.35</td>
<td>0.009</td>
</tr>
<tr>
<td>GLV %</td>
<td>2.21</td>
<td>2.97</td>
<td>1.63</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

RNFL, retinal nerve fibre layer; Sup. Avg, superior average; Inf. Avg, inferior average; Sup. GCC, superior ganglion cell complex; Inf. GCC, inferior ganglion cell complex.
progressive change in inner retinal OCT parameters over a 4-year follow-up period that was independent of glycated hemoglobin, age, and sex.44

Two other longitudinal studies demonstrating progression of neurodegeneration show no association between progressive neurodegenerative process and progression of vascular DR. Van Dijk et al.43 showed that the thinning of inner retinal layers in the macula in diabetes type 1 is progressive over time and is related to disease duration but occurs independently of retinal vasculopathy.45

However, contradicting this, another study performed by Lasta et al.44 indicated that the retinal vascular response to flicker stimulation is reduced before the reduction in pattern ERG in patients with T1DM without DR.

This seemed to indicate that in early diabetes, the abnormal retinal response may not be a consequence of reduced neuronal activity. However, they used pattern ERG instead of multifocal ERG, and the latter is superior in measuring early retinal neurodegeneration in diabetic patients.

Our study had some limitations; for the current study design and the number of patients to detect a difference of 5 μm in the average RNFL between the two groups assuming an alpha error of 0.05 and a standard deviation of 10 μm in the average RNFL, the power of the study is 0.659. In order to have a study power of 0.8 for the same parameters, the number of patients to be recruited should be 64 in each group. This relatively small sample size could be attributed to the strict inclusion criteria we put including a minimum duration of 5 years of diabetes, BCVA < 20/25 and absence of ocular trauma, ocular surgery, or any other coexisting ocular pathology, in addition to excluding any uncooperative child and/or any unreliable test results. However, to detect a difference of 5 μm in the average GCC between the two groups assuming an alpha error of 0.05 and a standard deviation of 5 μm in the average GCC, the power of the study is 0.997. Calculation of the sample size before performing the study was quite difficult as we were targeting a different age group.

We also lack the follow-up of these children to see the possibility of progression of the neurodegenerative changes and their relation to the possible development of vascular changes. In addition, we did not exclude the possibility of preclinical vascular changes, not visible with funduscopy or fundus photography. Such subtle capillary dropouts can be visualized by fundus fluorescein angiography (FFA).

In our study, we assumed that both neurodegenerative and vascular changes occurred independent of each other; as the neurodegenerative changes occurred in the absence of the vascular changes. However, once both processes are established, they will definitely modify each other. This is mainly because the retina behaves as a neurovascular unit in which neurons, glial and microglial cells, and blood vessels are prepared to adapt to varying conditions.45

When neurodegeneration precedes and possibly causes or accelerates the microvascular changes in DR, then retinal neuroprotection could possibly prevent or inhibit these microvascular changes and prevent vision loss.

In conclusion, our study is the first to report early neurodegenerative changes in children having T1DM with normal fundus examination. Lopes et al.46 in 2002 reported the first quantitative report of a loss of RNFL in humans with T1DM based on measurements in vivo. They also reported asymmetric RNFL loss in patients with T1DM with no ophthalmoscopically detectable retinopathy;46 however, this study was done on a different age group ranging from 18 to 45 years and was done on a smaller sample size: 12 patients and 12 participants in the control group. Measurements were obtained with the nerve fibre analyser (NFA/GDx, Laser Diagnostics Technologies, San Diego, CA, USA), which consisted of a confocal scanning laser ophthalmoscope (SLO) with an integrated polarisation modulator, but in our study we used SD-OCT to get accurate measurements of the RNFL and the GCC.

Our main findings suggest that retinal diabetic neuropathy precedes clinically detected microvascular changes. As the neurodegenerative process involves the RNFL and the GCC, irrespective of the age of onset, duration and control of the disease, we recommend that children having IDDM for more than 5 years have at least once/year OCT assessment of their RNFL and GCC to be able to detect rate of progression of the neuro-degenerative process. Working on the development of neuroprotective drugs that target the retina might help delay or prevent the possible development of the diabetic vascular complications.

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References


