• Clinical Research •

Retinal ganglion cell complex changes using spectral domain optical coherence tomography in diabetic patients without retinopathy

Ahmed I. Hegazy, Rasha H. Zedan, Tamer A. Macky, Soheir M. Esmat

Department of Ophthalmology, Cairo University, Kasr Alainy Faculty of Medicine Ringgold Standard Institution, Manial, Cairo 11223, Egypt

Correspondence to: Tamer A. Macky. 29th, 13th street Apt. 11, Maadi, Cairo 11431, Egypt. tamermacky@gmail.com

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Abstract

• AIM: To assess the ganglion cell complex (GCC) thickness in diabetic eyes without retinopathy.

• METHODS: Two groups included 45 diabetic eyes without retinopathy and 21 non diabetic eyes. All subjects underwent full medical and ophthalmological history, full ophthalmological examination, measuring GCC thickness and central foveal thickness (CFT) using the RTVue® spectral domain-optical coherence tomography (SD-OCT), and HbA1C level.

• RESULTS: GCC focal loss volume (FLV%) was significantly more in diabetic eyes (22.2% below normal) than normal eyes (*P*=0.024). No statistically significant difference was found between the diabetic group and the control group regarding GCC global loss volume (GLV%) (*P*=0.160). CFT was positively correlated to the average, superior and inferior GCC (*P*=0.001, 0.000 and 0.001 respectively) and negatively correlated to GLV% and FLV% (*P*=0.002 and 0.031 respectively) in diabetic eyes. C/D ratio in diabetic eyes was negatively correlated to average, superior and inferior GCC (*P*=0.015, 0.007 and 0.017 respectively). The FLV% was negatively correlated to the refraction and level of HbA1c (*P*=0.019 and 0.013 respectively) and positively correlated to the best corrected visual acuity (BCVA) in logMAR in diabetic group (*P*=0.004).

• CONCLUSION: Significant GCC thinning in diabetes predates retinal vasculopathy, which is mainly focal rather than diffuse. It has no preference to either the superior or inferior halves of the macula. Increase of myopic error is significantly accompanied with increased focal GCC loss. GCC loss is accompanied with increased C/D ratio in diabetic eyes.

• KEYWORDS: retina; ganglion cell complex; diabetes; optical coherence tomography

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INTRODUCTION

Neuroretinal damage in diabetes mellitus (DM) produces functional abnormalities such as the loss of chromatic discrimination, contrast sensitivity and dark adaptation. These alterations can be detected by means of electrophysiological studies in diabetes with diabetic duration of less than two years, *i.e.* before microvascular lesions can be detected in ophthalmologic examination[1-4]. Neurodegeneration seems to be a generalized process that occurs throughout the macula and is not confined to local abnormalities, in the cases with visible signs of retinopathy[5]. The debate is still open as to whether diabetic retinal neuropathy is the effect of vascular diabetic retinopathy or is primarily caused by direct neurologic damage from chronic hyperglycemia. The hypothesis that diabetes causes retinal neuropathy independent of retinopathy is intriguing and potentially links retinal neuropathy to other diabetic neuropathies[5].

Neuroretinal degeneration initiates and/or activates several metabolic and signalling pathways which participates in the microangiopathic process as well as in the disruption of the blood-retinal barrier which is a crucial element in the pathogenesis of diabetic retinopathy[3]. Retinal ganglion cells are the earliest affected cells and have the highest rate of apoptosis. However, an elevated rate of apoptosis has been also observed in the outer nuclear layer (photoreceptors) and in the retinal pigment epithelium (RPE)[6-7]. The use of spectral domain optical coherence tomography (SD-OCT) makes it possible to measure the thickness of individual layers at higher resolution and indicates that the thinning of the inner retina in the macula is primarily due to loss of ganglion cells[5].

SUBJECTS AND METHODS

Approval for the study was obtained from the hospital's Ethical Committee, and followed the tenets of the Declaration of Helsinki. All patients received a thorough explanation of the study design and aims, and were provided with written

informed consent. This was a prospective case-control study conducted from January to July 2014 at Kasr Alainy Hospital, Cairo University. The subjects were divided into two groups: group 1 consists of diabetic patients free from diabetic retinopathy (45 eyes of 24 consecutive patients); group 2 consists of non-diabetic subjects, free from any ocular pathology (21 eyes of 11 consecutive subjects).

Exclusion criteria included diabetic patients with diabetic retinopathy, patients having other ocular diseases as glaucoma or uveitis and eyes with history of previous ocular surgeries, trauma, intraocular injections or photocoagulation and patients with high refractive errors (myopia >-6.00 diopters or hypermetropia >+4.00 diopters).

All subjects were subjected to full medical and ophthalmological history, refraction, best corrected visual acuity (BCVA) (using the E-chart then converted to logMAR), slit-lamp examination [including measuring the intraocular pressure (IOP) by Goldman applanation tonometer], dilated fundus examination by binocular indirect slit-lamp biomicroscopy including estimating the C/D ratio clinically by one examiner blinded from the subject's group and then measuring of the retinal ganglion cell complex (GCC) thickness (which consists of the retinal nerve fiber layer, the ganglion cell layer and the inner plexiform layer) and the central foveal thickness (CFT) using the RTVue® SD-OCT (Optovue, Inc.) at the LASER Unit, Kasr Alainy Hospital. In addition; diabetic patients were subjected to fundus fluorescein angiography to exclude diabetic changes that could be missed on clinical examination and measuring level of HbA1c in blood at Kasr Alainy Hospital Chemical Pathology Unit.

Mapping of the GCC using the RTVue® GCC scan consists of 15 vertical line scans covering a 7 mm square region. The GCC scan centers at 1 mm temporal to fovea center for better coverage of the temporal region. The GCC thickness values are analyzed and compared to an extensive age-matched normative database. If the patient's values are outside the normal range, the measurement is color-coded appropriately. The deviation map shows the percent loss from normal as determined by the normative database. The significance map shows regions where the change from normal reaches statistical significance (Figure 1A).

Focal loss volume (FLV) and global loss volume (GLV) are two parameters that provide quantitative measures for the amount of significant GCC loss. GLV measures the average amount of GCC loss over the entire GCC map. FLV measures the average amount of focal loss over the entire GCC map, it is the total sum of significant GCC loss (in volume) divided by the map area. As such it provides a percent of significant tissue loss for volume. FLV detects focal loss using a pattern deviation map to correct for overall absolute changes[8].

Statistical Analysis Comparison of numerical variables between the study groups was done using Student's *t*-test for independent samples in normally distributed data and Mann Whitney *U* test for independent samples in non-normal data. For comparing categorical data, Chi-square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. Within group comparison between superior and inferior GCC was done using McNemar test. Agreement was tested using kappa statistic. Correlation between various variables was done using Spearman rank correlation equation. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

RESULTS

Age ranges from 23 to 70y in group 1, with mean age of 50.96y, and ranges from 24 to 66y in group 2, with mean age of 49.6y. Out of the 35 subjects, 22 (62.9%) were female and 13 (37.1%) were male. In group 1, 14 (58.3%) were female and 10 (41.6%) were male. In group 2, 8 (72.7%) were female and 3 (27.2%) were male. In group 1, 19 (79.1%) patients have type 2 diabetes, while 5 (20.8%) patients have type 1 diabetes. Duration of diabetes in group 1 ranges from 2 to 22y, with mean duration of 9y.

The refraction of group 1 ranges from -5.75 to +2.75 diopters, with mean -0.68±2.37 diopters, and of group 2 ranges from -5.50 to +1.00 diopters, with mean -1.71±1.67 diopters (*P*=0.053). The BCVA in logMAR in group 1 eyes ranges from 0 to 1.0, with mean 0.28±0.25, and in group 2 eyes ranges from 0 to 0.7 , with mean 0.26±0.19 (*P*=0.851). The IOP in group 1 eyes ranges from 11 to 21 mm Hg, with mean 14.49± 2.48 mm Hg, and in group 2 eyes ranges from 10 to 19 mm Hg, with mean 14.43±2.06 mm Hg (*P*=0.776). The C/D ratio in group 1 ranges from 0.1 to 0.4, with mean 0.21±0.09, and in group 2 ranges from 0.1 to 0.3, with mean 0.23±0.06 (*P*=0.407). The CFT in group 1 ranges from 150 to 309 microns, with mean thickness of 236.6±32.50 microns, and in group 2 ranges from 216 to 300 microns, with mean thickness of 247.19±22.33 microns (*P*=0.156). The average GCC thickness in group 1 eyes ranges from 69.38 to 113.99 microns, with mean thickness of 94.65±9.05 microns. Out of the 45 eyes of group 1, average GCC thickness was within normal in 39 eyes (86.7%), borderline in 3 eyes (6.7%) and below normal limits in 3 eyes (6.7%). The average GCC thickness in group 2 eyes ranges from 84.96 to 112.24 microns, with mean thickness of 96.25±6.57 microns. All of the 21 eyes of group 2 have within normal GCC average thickness. There was no statistically significant difference in the average GCC thickness between both groups (*P*=0.214).

The loss in average GCC thickness was significantly more in the eyes of patients with type 1 DM (out of 10 eyes, 2 eyes are

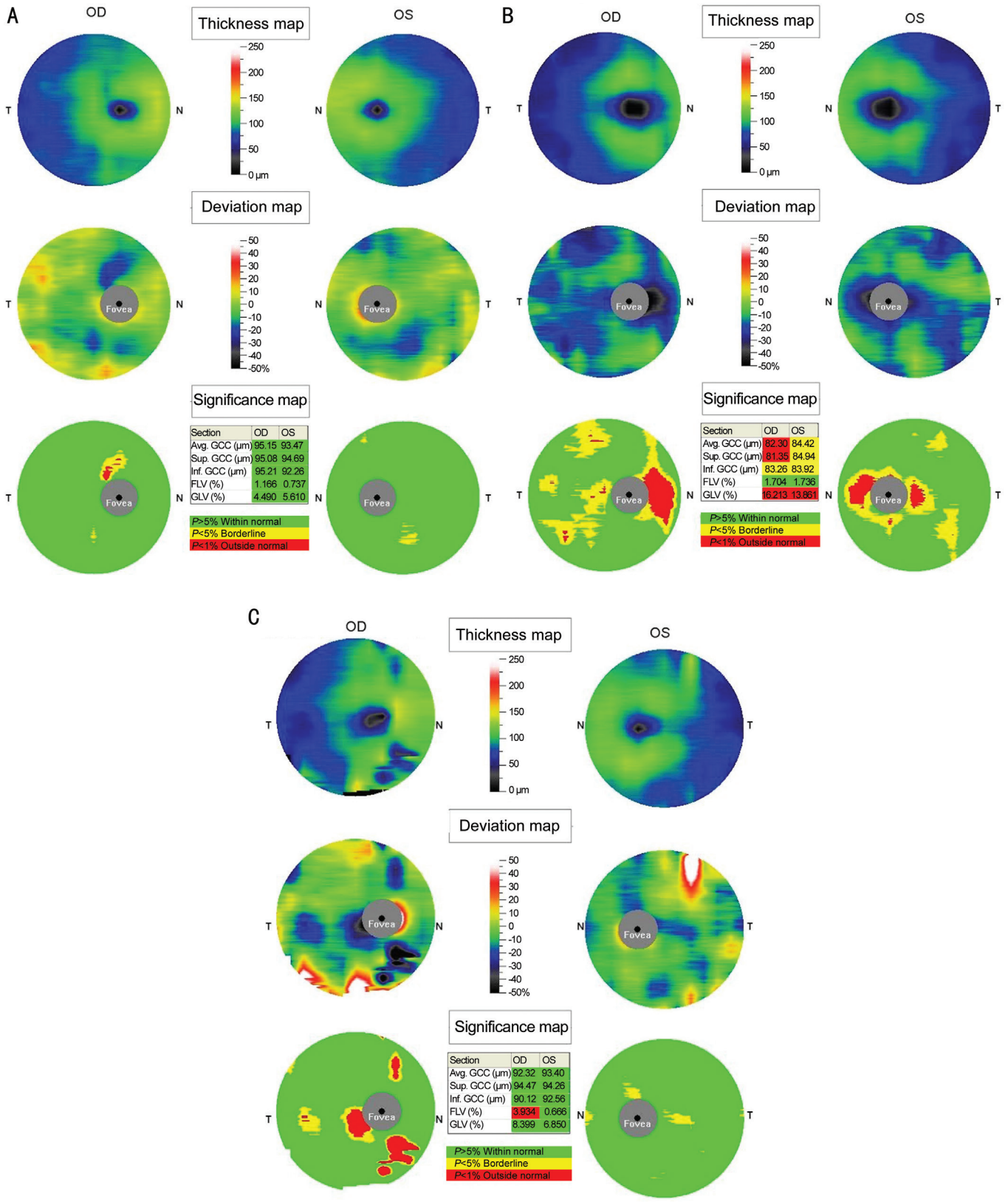

Figure 1 The ganglion cell complex analysis printouts A: This is a normal GCC analysis showing the thickness, deviation and significance maps with the table of GCC parameters; B: An example of group 1 patient with GLV% outside normal limits bilaterally; C: A group 1 patient with FLV% outside the normal limits in the right eye.

borderline and 3 eyes are below normal limits) than type 2 DM (out of 35 eyes only 1 eye is borderline) (P=0.000). Average GCC thickness in group 1 eyes had a significant positive correlation with CFT (P=0.001) and a significant negative correlation with both C/D ratio and FLV% (P=0.015 and 0.004 respectively) (Table 1). Out of the 45 eyes of group 1, 38 eyes have within normal superior GCC thickness (84.4%), 4 eyes are borderline (8.9%), and 3 eyes are below normal limits (6.7%). All the 21 eyes in group 2 had within normal superior GCC thickness.

Table 1 Correlation between loss in average GCC thickness and patients' variables in group 1

Variables	$\bar{x} \pm s$	Correlation with average GCC thickness
DM duration (a)	9.16±6.16	*P*=0.285
HbA1c (%)	8.67±1.90	*P*=0.493
BCVA (logMAR)	0.28±0.25	*P*=0.442
IOP (mm Hg)	14.49±2.48	*P*=0.383
C/D ratio	0.21±0.09	*P*=0.015
Refraction (diopter)	-0.68±2.37	*P*=0.603
FLV%	1.76±2.11	*P*=0.004
GLV%	6.76±5.88	*P*=0.000
CFT (microns)	236.6±32.50	*P*=0.001

DM: Diabetes mellitus; BCVA: Best corrected visual acuity; IOP: Intraocular pressure; FLV: Focal loss volume; GLV: Global loss volume; CFT: Central foveal thickness.

There was no statistically significant difference in superior GCC thickness between both groups of eyes (*P*=0.161). In group 1, there was a significant difference in superior GCC thickness between the eyes with type 1 DM (out of 10 eyes, 2 eyes are borderline and 3 eyes are below normal limits) and type 2 DM (out of 35 eyes, only 2 eyes are borderline) (*P*=0.000).

The superior GCC thickness in group 1 eyes had significant negative correlation with C/D ratio, FLV% and GLV% (*P*=0.007, 0.001 and 0.000 respectively), and a significant positive correlation with average GCC thickness and CFT (*P*=0.000 for both). Out of the 45 eyes of group 1, 39 eyes have within normal inferior GCC thickness (86.7%), 4 eyes are borderline (8.9%), and 2 eyes are below normal limits (4.4%). All the 21 eyes of group 2 have within normal inferior GCC thickness. There was no statistically significant difference in inferior GCC thickness between both groups of eyes (*P*=0.214). In group 1 eyes, there was a significant difference in inferior GCC thickness between the eyes with type 1 DM (out of 10 eyes, 3 eyes are borderline and 2 eyes are below normal limits) and type 2 DM (out of 35 eyes, only 1 eye are borderline) (*P*=0.000). The inferior GCC thickness in group 1 had a significant negative correlation with C/D ratio, FLV%, and GLV% (*P*=0.017, 0.004 and 0.000 respectively). And a significant positive correlation with average GCC thickness and CFT (*P*=0.000 and 0.001).

The GLV% in group 1 ranges from 0.00 to 24.47, with mean 6.76±5.88. Out of the 45 eyes of group 1, 36 (80%) eyes have within normal GLV%, 2 (4.4%) eyes are borderline, and 7 (15.6%) eyes are outside normal limits (Figure 1B). The GLV% in group 2 ranges from 0.05 to 11.881, with mean 4.32±3.33. Out of the 21 eyes of group 2, 20 (95.2%) eyes have within normal GLV%, only 1 (4.8%) eye is borderline, and there were no eyes outside the normal limits. There was no statistically significant difference between the GLV% of both groups of eyes (*P*=0.160). There was a significant GLV% difference between the eyes of patients with type 1 DM (out of 10 eyes, 5 eyes are outside normal limits) and eyes of patients with type 2 DM (out of 35 eyes, 2 eyes are borderline and 2 eyes are outside normal limits) (*P*=0.003). The GLV% in group 1 has a significant positive correlation with both C/D ratio (*P*=0.006, correlation coefficient=0.312) and FLV% (*P*=0.000, correlation coefficient=0.685), and a significant negative correlation with CFT (*P*=0.002, correlation coefficient=-0.443) (Table 2).

Table 2 Correlation between GLV% and patients' variables in group 1

Variables	$\bar{x} \pm s$	Correlation with GLV%
Duration of DM (a)	9.16±6.16	*P*=0.151
HbA1c (%)	8.67±1.90	*P*=0.172
BCVA (logMAR)	0.28±0.25	*P*=0.186
IOP (mm Hg)	14.49±2.48	*P*=0.181
C/D ratio	0.21±0.09	*P*=0.006
Refraction (diopters)	-0.68±2.37	*P*=0.326
FLV%	1.76±2.11	*P*=0.000
GCC (microns)	94.65±9.05	*P*=0.000
CFT (microns)	236.6±32.50	*P*=0.002

DM: Diabetes mellitus; BCVA: Best corrected visual acuity; IOP: Intraocular pressure; FLV: Focal loss volume; GCC: Ganglion cell complex; CFT: Central foveal thickness.

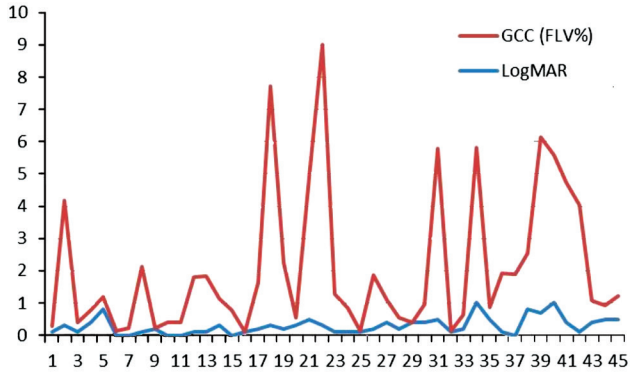

Figure 2 Positive correlation between FLV% and BCVA (logMAR) in group 1.

The FLV% in group 1 ranges from 0 to 8.72, with mean 1.76±2.11. Out of the 45 eyes of group 1, 35 (77.8%) eyes have normal FLV%, and 10 (22.2%) eyes are outside normal limits (Figure 1C). The FLV% in group 2 ranges from 0 to 2.54, with mean 0.77±0.75. All the 21 eyes of group 2 have normal FLV%. The difference in FLV% between both groups of eyes was statistically significant (*P*=0.024). The FLV% in group 1 eyes has a significant positive correlation with GLV% (*P*=0.000, correlation coefficient=0.685) and BCVA (*P*=0.004, correlation coefficient=0.318) (Figure 2), and significant negative correlations with average GCC thickness (*P*=0.010, correlation coefficient=-0.473), refraction (*P*=0.019, correlation coefficient=-0.239) and HbA1c (*P*=0.013, correlation coefficient=-0.402) (Table 3).

Table 3 Correlation between FLV% and other variables in group 1

Variables	$\bar{x} \pm s$	Correlation with FLV%
Duration of DM (a)	9.16±6.16	*P*=0.715
Type of DM	-	*P*=0.513
HbA1c (%)	8.67±1.90	*P*=0.013
BCVA (logMAR)	0.28±0.25	*P*=0.004
Refraction (diopters)	-0.68±2.37	*P*=0.019
IOP (mm Hg)	14.49±2.48	*P*=0.163
C/D ratio	0.21±0.09	*P*=0.191

DM: Diabetes mellitus; BCVA: Best corrected visual acuity; IOP: Intraocular pressure.

DISCUSSION

Previous studies showed that functional (decreased contrast sensitivity and color vision) and structural impairment may precede the earliest clinical manifestations of diabetic retinal vasculopathy, and DM causes nonglaucomatous optic neuropathy secondary to retinal nerve fiber layer (RNFL) damage[9]. Chihara *et al*[10] in 1993 detected nerve fiber layer defects in 20% of diabetics without microaneurysms, and 57% with microaneurysms using red-free photography. Lopes de Faria *et al*[11] in 2002 found thinning of nerve fiber layer in superior retina of diabetic patients using scanning laser polarimetry.

A study by Asefzadeh *et al*[12] in 2008 showed that, in diabetic patients with no or mild diabetic retinopathy, the macular and foveal thickness is significantly thinner with longer duration of DM. In 2010, Lima *et al*[13] found that the GCC was thinner in a study group of type 2 diabetic patients when compared to a non-diabetic control group using the SD-OCT. van Dijk *et al*[5] in 2010 used the SD-OCT to determine which retinal layers are most affected by diabetes. It was found that there was a selective ganglion cell layer thinning in the pericentral area and corresponding loss of RNFL thickness in the peripheral macula in those patients compared with control subjects. These results support the concept that diabetes has an early neurodegenerative effect on the retina, which occurs even though the vascular component of diabetic retinopathy is minimal.

In the same study[5] , there was a significant correlation between the ganglion cell layer (GCL) thickness in the pericentral area and the RNFL thickness in the peripheral area of the macula. The duration of DM was correlated significantly and inversely with GCL thickness. In the multiple linear regression analysis including (age, sex, HbA1c, diabetes duration, and diabetic retinopathy status); diabetic retinopathy status was the most important explanatory variable. In 2011, Gonul *et al*[14] evaluated the RNFL thickness with OCT in type 1 DM patients with and without retinopathy. The RNFL thickness was found less compared to control subjects and this was more prominent in patients with established retinopathy. Also Shahidi *et al*[15] in 2012 found that an inferior quadrant RNFL thinning is associated with peripheral neuropathy in patients with type 2 diabetes, and is more pronounced in those at higher risk of foot ulceration. In the study by Salvi *et al*[16] in 2016, the GCC was significantly affected in patients with type 2 diabetes. The OCT parameters did not differ significantly according to the diabetic retinopathy grade. But RNFL thickness was lower and GLV and FLV were higher in patients versus those without diabetic peripheral neuropathy and concluded that SD-OCT might represent a useful tool to detect peripheral neuropathy, but not retinopathy in those patients[16].

Another study was done by van Dijk *et al*[17] in 2012 in type 2 DM patients with no or minimal diabetic retinopathy, to determine whether diabetes type 2 causes thinning of retinal layers as a sign of neurodegeneration. Results showed thinning of the RNFL, GCL, and inner plexiform layer (IPL) of the pericentral area of the macula compared to controls. Toprak *et al*[18] in 2012 used the Heidelberg retina tomography II (HRT II) to analyze the optic disc topography in type 2 diabetic patients. Mean RNFL thickness was found to be significantly lower in diabetic group of patients when compared with the control group.

In our study, we have assessed the thickness of the retinal GCC in a group of eyes of diabetic patients, and comparing it to the GCC thickness in a control group of eyes of normal subjects, using the SD-OCT technology in the form of the RTVue® machine. There was no statistically significant difference in either age, sex, BCVA in logMAR, IOP, C/D ratio or CFT between the study and control groups. Our GCC analysis included describing the loss in terms of either global or focal, in addition to the real thickness. Lima *et al*[13] investigated only the loss in the real GCC thickness without any parameters to determine the focal loss. It was found in our study that the FLV% was significantly more in the diabetic eyes than normal eyes. These results show that DM causes a significant focal loss of retinal ganglion cells in the macular area prior to the appearance of vasculopathy. These results support those of van Dijk *et al*[5,17]. Both types of DM were included in our study, while in the study of van Dijk *et al*[5] only type 1 patients were included, and in the studies of Lima *et al*[13] and van Dijk *et al*[17] only type 2 patients were included. Comparing the GCC results of both types of DM revealed that the GLV is significantly less with type 2 DM than with type 1 (while they both share a significant focal loss when compared to controls). However, number of type 1 DM eyes included in the study was much less than type 2 DM eyes (10 eyes type 1 versus 35 eyes type 2). The sample of type 1 DM eyes is too small to study the effect of this type of diabetes particularly. Further GCC thickness studying in larger groups of diabetic eyes is still needed to compare effect of both types of diabetes. There

was no statistically significant difference between the FLV% of type 1 DM eyes and that of type 2 DM eyes.

Previously, Sima and Kamiya[19] in 2006 studied differences between type 1 and type 2 diabetic polyneuropathy and found that progressive axonal atrophy and loss and paranodal degenerative changes are more in type 1. These differences can be related to the differences in insulin action and signal transduction affecting the expression of neurotrophic factors and their receptors in type 1 diabetes. Downstream effects on neuroskeletal and adhesive proteins, their post-translational modifications, and nociceptive peptides underlie the more severe resultant pathology in type 1 DM[19].

In the results of Toprak *et al*[18], it was found that mean RNFL thickness is significantly lower in patients with HbA1c level ≥7%. Verma *et al*[20] in 2012 used microperimetry to detect neuronal damage in type 2 DM eyes without retinopathy and found significantly lower mean retinal sensitivity with HbA1c <7% as compared to those with HbA1c ≥7%. van Dijk *et al*[5,17] found no statistically significant correlation between the level of HbA1c level and GCL thickness.

OCT by van Dijk *et al*[5,17] was done using the spectral domain 3-D OCT-1000 machine (Topcon Corp.), while Lima *et al*[13] used the spectral domain RTVue® machine (Optovue, Inc.) which is the same machine used in our study. The former measured the thickness of different retinal layers individually (RNFL, GCL, IPL, INL, OPL, ONL+IS, OS, RPE), while the latter measured the GCC thickness with the complex (RNFL+GCL+IPL).

Another study by Demir *et al*[21] in 2014 showed that there is a nonsignificant loss of RNFL and GCC in patients with type 2 diabetes. Zhu *et al*[22] evaluated the changes in retinal thickness and visual function in type 2 diabetic patients without clinical evidence of diabetic retinopathy. Superior macular GCC thicknesses were significantly decreased in diabetic cases, and no significant peripapillary RNFL thickness changes were observed. These results indicate that GCC thickness could be observed in diabetic subjects before the onset of any significant diabetic retinopathy and that GCC reduction occur much earlier than peripapillary RNFL thinning in diabetic patients without retinopathy.

Clinical application of these findings at this stage would be too early and difficult, as further research is needed to answer more questions. We need to do more longitudinal studies to know how the diabetic retinopathy for these patients will unfold in the future and what are the risks of developing diabetic maculopathy or high risk proliferative disease. Also we need to know if any interventions, like tighter blood sugar or blood pressure control at these earlier stages, could halt the development of retinopathy.

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Conflicts of Interest: Hegazy AI, None; **Zedan RH,** None; **Macky TA,** None; **Esmat SM,** None.

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