Ganglion cell analysis versus retinal nerve fiber layer thickness in glaucoma diagnosis
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Received: 12 September 2019
Accepted: 24 October 2019
Published: 20 February 2020

Journal of the Egyptian Ophthalmological Society 2019, 112:122–129

Background
Glaucoma is a progressive optic neuropathy, which can result in irreversible blindness. The main pathologic change in glaucoma is retinal ganglion cell loss. The macula has the greatest density of retinal ganglion cells (∼50%).

The advent of spectral domain-optical coherence tomography (OCT) technology has allowed advanced macular imaging protocols to play an important role in the diagnosis and monitoring of glaucoma. The ganglion cell analysis is obtained by the Cirrus HD-OCT system segments and measures the thickness of the ganglion cell-inner plexiform layer, thereby potentially increasing its diagnostic accuracy compared with conventional peripapillary retinal nerve fiber layer (RNFL) thickness measurement.

Patients and methods
The study was done on 40 eyes of 40 patients, and patients were divided into two groups:

- Group 1 included 20 adult patients more than 18 years who were diagnosed with primary open-angle glaucoma. Group 2 included 20 normal controls. One eye of each individual was prospectively enrolled.

- The OCT was done using 3D-OCT 2000 (Topcon) to evaluate the following: RNFL parameters (average total thickness, superior average thickness, and inferior average thickness) and ganglion cell complex (GCC) parameters (total average thickness, superior average thickness, and inferior average thickness).

Result
There was a positive statistically significant correlation of high probability between the two groups regarding RNFL thickness (P<0.001).

There was a positive statistically significant correlation of high probability between the two groups regarding GCC thickness (P<0.001).

There was a positive statistical significant correlation of high probability between the groups regarding intraocular pressure (P<0.001).

There was a positive statistical significant correlation of high probability between the groups regarding cup-to-disc ratio (P<0.001).

Conclusion
Early diagnosis of glaucoma and early initiation of treatment is extremely important, as further vision loss can be stopped or slowed down. RNFL and GCC measurement with spectral domain-OCT could provide important information for detection and evaluation of glaucoma. There is strong positive correlation between the RNFL thickness and the GCC thickness in the glaucomatous patients.

Keywords:
ganglion cell, glaucoma, optical coherence tomography, retinal nerve fiber

Introduction
Glaucoma is defined as a progressive optic neuropathy, which can result in irreversible blindness. The big challenge facing ophthalmologists is the early diagnosis of the disease and how to prevent its progression [1].

The main pathologic change in glaucoma is retinal ganglion cell (RGC) loss, which results in atrophy of all related inner retinal layers, namely, retinal nerve fiber layer (RNFL), which are axons of ganglion cells (GC); ganglion cell layer (GCL), which has the body of GCs; and inner plexiform layer (IPL), having the dendrites of GCs [1].

There is a good correlation found between RNFL thickness attenuation and visual field loss [2].
The macula was found to have the greatest number of RGCs (~50%) [3].

Objective quantitative analysis of RNFL thickness has become the standard method for the detection of glaucoma since the introduction of optical coherence tomography (OCT) [3].

Macular thickness measurements with the Stratus OCT was not found to be superior to RNFL measurements [2].

The use of spectral domain-optical coherence tomography (SD-OCT) technology has allowed macular imaging to play an important role in the diagnosis and monitoring of glaucoma [4]. The ganglion cell analysis (GCA) is obtained by the Cirrus HD-OCT system (Topcon) segments and measures the thickness of the GC?IPL, so increasing its diagnostic accuracy compared to conventional RNFL thickness measurement [5].

Published studies have reported comparing values of GCA and RNFL thickness for diagnosing glaucoma [5].

If there is a visual field defect, a differentiation between glaucomatous patients and normal is not difficult. The challenge comes in how to differentiate a glaucoma suspect from normal when visual field is normal [6].

If there is a significantly lesser number of RGCs in this group of patients, it may result in earlier intervention in the disease before functional loss happens [7].

Although RNFL thickness measurement has proven to be useful in the diagnosis of glaucoma, as RNFL loss is preceded by RGC death, so measurement of this layer (RGC) helps to detect the disease early [8].

**Inclusion criteria**

Adult patients, above 18 years, already diagnosed with POAG, good visual acuity, with clear media were included.

Control group included adult individuals above 18 years, with normal intraocular pressure (IOP) and normal visual field, having no history of any eye disease.

**Exclusion criteria**

Patients with media opacity interfering with good-quality OCT scans such as corneal opacity, cataracts, etc.; history of intraocular diseases; complicated intraocular surgery; nonglaucomatous secondary causes of elevated IOP (e.g. iridocyclitis and trauma); coexisting retinal disease (e.g. diabetic retinopathy); other diseases affecting visual field (e.g. pituitary lesions); taking medications known to affect visual field sensitivity; or with problems other than glaucoma affecting color vision were excluded from this study.

**Tests**

Each patient will have a comprehensive ophthalmic examination, including best-corrected visual acuity, IOP) measured by Goldmann applanation tonometry, slit-lamp biomicroscopy, and stereoscopic fundus evaluation on the slit lamp using a 90.0 D lens optic discs.

All included individuals were scanned with OCT.

**Optical coherence tomography scanning**

For RNFL thickness measurements, the optic disc 200×200 scan will be used to acquire a cube of side 6 mm, while the patient is fixated so that the optic disc will be near the center of the scan.

For macular GCA, the macular cube 200×200 protocol will be used for GCA.

Optical coherence tomography macular scan and optic nerve head (ONH) scan were performed using the Topcon SD-OCT model 2000 version.

All patients signed an informed consent under the tenets of the Helsinki guidelines.

**Statistical analysis**

The results were analyzed using the SPSS for Windows software, version 10.0, (SPSS Inc., Chicago, Illinois, USA). Descriptive statistics were computed for all the variables measured.
Results

Sex
Our study included 40 eyes of 40 patients divided into two groups:

1. Group 1 included 20 patients with POAG, comprising 11 (55%) males and nine (45%) females.
2. Group 2 included 20 normal controls, comprising 11 (55%) males and nine (45%) females, as shown in Table 1 and Fig. 1.

There was an insignificant correlation between the two groups according to sex, as shown in Fig. 1.

Group 1: patients with primary open-angle glaucoma
Of 20 patients with POAG, 11 were on one line of treatment of antiglaucoma drops and nine were on two line of treatments, as shown in Tables 2 and 3 and Figs 2 and 3.

Age
The mean age of the patients in group 1 was 54.60 ±10.67 years, ranging from 37 to 71 years. Group 2 had mean age of 40.75±13.55 years, ranging from 20 to 67 years (Table 4 and Fig. 4). There was a positive statistically significant correlation of high probability between the group 1 and group 2 regarding age (P=0.001).

Visual acuity
As shown in Table 5 and Fig. 5, group 1 had mean V/A of 0.52±0.26 logMar, ranging from 0.2 to 1, and group

Table 3 Distribution of the studied cases according to duration of the disease

| Duration (years) | n (%) |
|------------------|-------|
| ≤10              | 11 (55.0) |
| >10              | 9 (45.0) |
| Min.–max.        | 3.0–20.0 |
| Means±SD         | 11.15±5.61 |
| Median           | 10.0 |

Max., maximum; min., minimum.

Figure 2

Figure 1

Sex of two studied groups.
2 had mean V/A of 0.39±0.20 logMar, ranging from 0.0 to 0.8.

There was an insignificant correlation of low probability between the both groups regarding best-corrected visual acuity (\(P=0.127\)).

### Intraocular pressure

Group 1 had mean IOP of 18.40±4.08, ranging from 12 to 28 mmHg and group 2 had mean IOP of 13.20 ±1.88, ranging from 10 to 16 mmHg, as seen in Table 6 and Fig. 6. There was a positive statistically significant correlation of high probability between both groups regarding IOP (\(P<0.001\)).

### Cup-to-disc ratio

Table 7 and Fig. 7 show that in group 1 had an average cup-to-disc (C/D) area ratio of 0.63±0.10,

### Table 4 Age of the studied groups

| Age (years) | Group 1 [n (%)] | Group 2 [n (%)] | Test of significance | \(P\) |
|-------------|-----------------|-----------------|-----------------------|------|
| Min.–max.   | 37.0–71.0       | 20.0–67.0       | \(t=3.591^*\)        | 0.001^* |
| Mean±SD     | 54.60±10.67     | 40.75±13.55     |                       |      |
| Median      | 56.50           | 41.0            |                       |      |

Max., maximum; min., minimum; \(P\), \(P\) value for comparing between the two studied groups. *Statistically significant at \(P\) value less than or equal to 0.05.
ranging from 0.5 to 0.8 and group 2 had an average C/D area ratio of 0.46±0.06, ranging from 0.4 to 0.6.

There was a positive statistically significant correlation of high probability between both groups regarding C/D ratio ($P<0.001$).

**Optical coherence tomography findings**

**Retinal nerve fiber layer parameters**

Group 1 had an average RNFL thickness of 25.55±3.97 μm, ranging from 19.0 to 32.0 μm. The superior average thickness was 25.35±6.31 μm, ranging from 18.0 to 38.0 μm, and the inferior average thickness was 26.35±4.58 microns, ranging from 20.0 to 33.0.

Group 2 had an average RNFL thickness of 56.95±9.68 μm, ranging from 44.0 to 74.00 μm. The superior average thickness was 55.95±9.43 μm, ranging from 44.0 to 71.00 μm, and the inferior average thickness was 58.30±10.15 μm, ranging from 44.0 to 77.0 μm, as shown in Table 8 and Fig. 8.

There was a positive statistically significant correlation of high probability between the two groups regarding RNFL thickness ($P<0.001$).

**The ganglion cell complex parameters**

(1) Group 1 had an average ganglion cell complex (GCC) thickness of 69.35±12.78 μm, ranging from 52.0 to 93.0 μm. The superior average thickness of the GCC was 69.70±16.48 μm, ranging from 48.0 to 107.0 μm, and the inferior average thickness was 69.45±10.75 μm, ranging from 57.0 to 95.0 μm.

(2) Group 2 had an average GCC thickness of 111.6±9.37 μm, ranging from 99.0 to 135.0 μm. The superior average thickness of the GCC was 112.8±10.30 μm, ranging from 100.0 to 148.0 μm, and the inferior average thickness was 110.6±8.83 μm, ranging from 99.0 to 132.0 μm, as seen in Table 9 and Fig. 9.

### Table 6 Comparison between the two studied groups according to intraocular pressure

| IOP        | Group 1 | Group 2 | t    | P   |
|------------|---------|---------|------|-----|
| Min.–max.  | 12.0–28.0 | 10.0–16.0 | 5.173 | <0.001* |
| Mean±SD    | 18.40±4.08 | 13.20±1.88 |      |     |
| Median     | 18.0    | 13.0    |      |     |

IOP, intraocular pressure; max., maximum; min., minimum. *There was a positive statistically significant correlation of high probability between the two groups regarding IOP ($P$ less than 0.001).

### Table 7 Comparison between the two studied groups according to cup-to-disc ratio

| C/D ratio | Group 1 | Group 2 | t    | P   |
|-----------|---------|---------|------|-----|
| Min.–max. | 0.50–0.80 | 0.40–0.60 | 6.413 | <0.001* |
| Mean±SD   | 0.63±0.10 | 0.46±0.06 |      |     |
| Median    | 0.60    | 0.45    |      |     |

C/D, cup-to-disc; max., maximum; min., minimum. *There was a positive statistically significant correlation of high probability between the two groups regarding C/D ($P$ less than 0.001).
There was a positive statistically significant correlation of high probability between the two groups regarding GCC thickness ($P < 0.001$).

### Discussion

Glaucoma occurs owing to death of RGCs, and RGCs being concentrated at the macula lead to the use of macular thickness as a useful discriminator for glaucoma. Although the GCA and RNFL are measured at different locations of the retina, as the macula contains 50% of RGCs in the retina, measuring macular GCL appears to be a good approach to assess GC death. The RNFL measured around the disc are axons of GCs which converge on the optic disc before leaving the eye [1].

A study by Inuzuka et al. [9] observed a significant correlation between the macular GCC of the inner or outer sector of the parafovea and in the change of the visual field in each hemifield defect or the apparently normal hemifield. The decrease of the GCC corresponding to the apparently normal hemifield.

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### Table 8 Comparison between the two studied groups according to retinal nerve fiber layer

| RNFL | Group 1 | Group 2 | t     | P    |
|------|---------|---------|-------|------|
| S    | Min.–max. 18.0–38.0 | 44.0–71.0 | 12.057 | <0.001 $^*$ |
|      | Mean±SD 25.35±6.31  | 55.95±9.43 |       |      |
|      | Median 23.50       | 56.0      |       |      |
| I    | Min.–max. 20.0–33.0 | 44.0–77.0 | 12.835 | <0.001 $^*$ |
|      | Mean±SD 26.35±4.58 | 58.30±10.15 |    |      |
|      | Median 27.0        | 58.50     |       |      |
| T    | Min.–max. 19.0–32.0 | 44.0–74.0 | 13.421 | <0.001 $^*$ |
|      | Mean±SD 25.55±3.97 | 56.95±8.68 |     |      |
|      | Median 25.0        | 57.0      |       |      |

Max., maximum; min., minimum; RNFL, retinal nerve fiber layer. $^*$There was a positive statistically significant correlation of high probability between the two groups regarding IOP ($P$ less than 0.001).

There was a positive statistically significant correlation of high probability between the two groups regarding GCC thickness ($P<0.001$).

### Table 9 Comparison between the two studied groups according to ganglion cell complex thickness

| GC | Group 1 | Group 2 | t     | P    |
|----|---------|---------|-------|------|
| S  | Min.–max. 48.0–107.0 | 100.0–138.0 | 9.917 | <0.001 $^*$ |
|    | Mean±SD 69.70±16.48 | 112.8±10.30 |       |      |
|    | Median 64.50         | 111.5     |       |      |
| I  | Min.–max. 57.0–95.0  | 99.0–132.0 | 13.215 | <0.001 $^*$ |
|    | Mean±SD 69.45±10.75 | 110.6±8.83 |       |      |
|    | Median 64.50         | 110.0     |       |      |
| T  | Min.–max. 52.0–93.0  | 99.0–135.0 | 11.912 | <0.001 $^*$ |
|    | Mean±SD 69.35±12.78 | 111.6±9.37 |       |      |
|    | Median 62.0          | 110.0     |       |      |

Max., maximum; min., minimum. $^*$There was a positive statistically significant correlation of high probability between the two groups regarding IOP ($P$ less than 0.001).
correlated with the progression of the severity of the glaucomatous defects.

In a study by Takagi et al. [10], it was found that the thickness of the macular GCC in the normal hemifield of the glaucomatous eyes was significantly less than in normal eyes. In contrast, the total thickness of the macular retinas between the glaucomatous and normal eyes showed no significant difference. In addition, the thicknesses of the macular GCC and RNFL in the normal hemisphere of the glaucomatous eyes significantly correlated with the total deviation in the visual field parameters of the corresponding area.

In a study by Na et al. [11], it was found that perimetrically normal hemifields of glaucomatous eyes had significantly lower macular GCC and pRNFL thicknesses than did the corresponding retinal regions of healthy eyes. SD-OCT may be a diagnostic tool for evaluation of early macular and circumpapillary structural changes in glaucomatous eyes with localized VF defects.

A study by Moreno et al. [12] found that the ability of macular GC–IPL parameters to discriminate normal eyes and eyes with early glaucoma is high and comparable to that of the best pRNFL and ONH parameters.

Sevim et al. [13] in their study found that GCC and RNFL thickness measurements performed by FD-OCT showed high diagnostic ability in detecting glaucoma.

In the study by Oddone et al. [14], with the use of OCT, RNFL parameters are still preferable to macular parameters for diagnosing manifest glaucoma, but the differences are small.

A study of Schulze et al. [15] found that imaging of the GCC using FD-OCT (RTVue-100) has a comparable diagnostic ability to RNFL and ONH measurements in distinguishing between patients with glaucoma and healthy participants. No differences were found between patients with OHT and normal participants regarding ONH, RNFL, and GCC parameters.

Moreover, Medeiros et al. [16] in their study found that glaucomatous eyes with the earliest detectable visual field loss on automated perimetry may already show substantial loss of RGCs. Empirical estimates of RGC counts combining structural and functional tests agreed closely with previous histologic reports on the number of RGCs associated with early visual fields defects.

Our study found that there was a positive statistically significant correlation of high probability between the two groups regarding RNFL thickness ($P<0.001$)

Group 1 had an average RNFL thickness of 25.55±3.97 μm, ranging from 19.0 to 32.0 μm. The superior average thickness was 25.35±6.31 μm, ranging from 18.0 to 38.0 μm. The inferior average thickness was 26.35±4.58 μm, ranging from 20.0 to 33.0 μm.

Group 2 had an average RNFL thickness of 56.95±9.68 μm, ranging from 44.0 to 74.00 μm. The superior average thickness was 55.95±9.43 μm, ranging from 44.0 to 71.00 μm. The inferior average thickness was 58.30±10.15 μm, ranging from 44.0 to 77.0 μm (Table 8).

There was a highly statistically significant correlation between the two groups in GCC thickness ($P<0.001$).

(1) Group 1 had an average GCC thickness of 69.35±12.78 μm, ranging from 52.0 to 93.0 μm. The superior average thickness of the GCC was 69.70±16.48 μm, ranging from 48.0 to 107.0 μm. The inferior average thickness was 69.45±10.75 μm, ranging from 57.0 to 95.0 μm.

(2) Group 2 had an average GCC thickness of 111.6±9.37 μm, ranging from 99.0 to 135.0 μm. The superior average thickness of the GCC was 112.8±10.30 μm, ranging from 100.0 to 148.0 μm. The inferior average thickness was 110.6±8.83 μm, ranging from 99.0 to 132.0 μm (Table 9).

Single baseline measurements of GCC layer in glaucomatous and nonglaucomatous eyes also showed significant difference ($P<0.001$ for superior, inferior, and total GCC values in normal eyes compared with glaucomatous eyes). The total GCC values in glaucomatous eyes compared with normal eyes showed very significant statistical difference.

Conclusion
Diagnosis of glaucoma and early initiation of treatment is extremely important, as further vision loss can be stopped or slowed down. RNFL and GCC measurement with SD-OCT could provide important information for detection and evaluation of glaucoma. There is a strong positive correlation between the RNFL thickness and the GCC thickness in the
glaucomatous patients. Imaging of the GCC has a comparable diagnostic ability to RNFL and ONH measurements in distinguishing between patients with glaucoma and healthy participants.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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