# Evaluation of Macular, Ganglion Cell Complex and Retinal Nerve Fibre Layer Thickness Changes in Preperimetric Glaucomatous Eyes

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## ABSTRACT

**Purpose:** To investigate macular thickness (MT), ganglion cell complex (GCC), and retinal nerve fiber layer (RNFL) thickness changes in preperimetric glaucoma patients.

**Materials and Methods:** In the present study 105 eyes of 75 patients were investigated. Of these patients, 56 eyes of 45 patients who were diagnosed as preperimetric glaucoma are taken as a study group and 49 eyes of 30 cases without any ocular pathology are taken as the control group. The mean values of central MT, parafoveal MT, perifoveal MT, parafoveal GCC thickness, and perifoveal GCC thickness evaluations using optical coherence tomography (OCT) were compared between the two groups. Peripapillary RNFL measurements; the mean values of the total, superior hemi-central, inferior hemi-central, superior, inferior, nasal, and temporal quadrants, and twelve sectors were compared between the two groups.

**Results:** In the study group, perifoveal GCC thickness was significantly thinner in the superonasal, superotemporal, inferonasal, and inferotemporal quadrants compared to the control group. Peripapillary RNFL thickness was significantly thinner in the study group in total, superior hemi-central, inferior hemi-central, superior, inferior, nasal, temporal, and hour 1,2,3,4,5,7,8,9 quadrants. No significant difference was detected between the two groups in other measurements ($p>0.05$).

**Conclusion:** However both macular and peripapillary cellular losses have been initiated in cases with preperimetric glaucoma, this loss can not be detected by standard automated perimetry. As a result, perifoveal GCC and peripapillary RNFL measurements by OCT in patients with preperimetric glaucoma are very helpful for diagnosing suspicious cases.


**Keywords:** Preperimetric glaucoma, Macula thickness, Optical coherence tomography, Ganglion cell complex, Retina nerve fiber layer.

## INTRODUCTION

Glaucoma is an optic neuropathy that causes progressive and irreversible blindness.[1] The primary risk factor is increased intraocular pressure (IOP) as well as multiple risk factors. In addition to having an IOP above 21 mm Hg, progressive excavation of the optic nerve head and glaucomatous visual field defects are clinical signs of the disease.[2]

Although standard automated perimetry has been shown as the gold standard diagnostic method in demonstrating glaucomatous damage and following the progression, visual field damage occurs only when 30-50% of the retinal ganglion cells are missing.[3,4] Preperimetric glaucoma is described as the presence of damage to the characteristic glaucomatous optic disc and retinal nerve fiber layer (RNFL) with no visual field damage in a conventional achromatic automatic perimeter.[5]

Because glaucomatous damage is progressive, chronic, and irreversible, it is very important to diagnose glaucoma in preperimetric stage and to follow up the progression.[6] Even if the visual field test results are normal, clinicians should complete assessments with diagnostic tests based on quantitative imaging techniques of the optic nerve or RNFL.[7]

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The purpose of this study was to investigate changes in macula thickness (MT) ganglion cell complex (GCC) thickness and RNFL thickness by using optical coherence tomography (OCT) between preperimetric glaucoma cases and normal subjects.

## MATERIALS AND METHODS

### Study population

This retrospective, comparative case series was conducted with 56 eyes of 45 patients diagnosed as preperimetric glaucoma in the glaucoma department of a university teaching hospital between May 2013 and May 2016. The control group consisted of 49 eyes of 30 age and sex-matched healthy individuals who applied to the ophthalmology outpatient clinic for a routine eye examination. Preperimetric glaucoma was defined as high IOP (IOP > 21 mmHg), and suspected glaucomatous optic nerve head changes (increased vertical cup to disc ratio, neuroretinal rim thinning or notching, disc hemorrhages) and/or RNFL damage without visual field defects. To confirm the diagnosis of high IOP, the patients were measured twice on the same day, and patients with IOP >21 mmHg in 4 consecutive measurements at least 2 weeks later were diagnosed as high IOP. All IOP values were evaluated by adjusting for Ehler's formula [corrected IOP=uncorrected IOP-(central corneal thickness-545) x (5/70)]. A measurement result with a RNFL thickness value below the RNFL thickness values determined for age in the internal database of OCT, regardless of size, was considered as peripapillary RNFL damage. No macular OCT measurement results were used in the diagnosis of preperimetric glaucoma. After the patients were diagnosed with preperimetric glaucoma, the macular measurement results were subsequently analyzed for statistical analysis. Informed patient consents were obtained from all of the participants. The procedures of the study were approved by the institutional review board of the hospital and adhered to the tenets of the Declaration of Helsinki. The study protocol was approved by the local ethics committee (approval code: TÜTF-BAEK 2016/171).

Patients who had a history of previous ocular surgery (except cataract surgery), ≥5.00D spherical or ≥3.00D cylindrical refractive error, ≤8/10 best-corrected visual acuity (BCVA), degenerative fundus findings and congenital optic nerve anomalies, accompanying ocular comorbidities (age-related macular degeneration, diabetic retinopathy, retinal vascular disease), patients with secondary glaucoma (exfoliative, neovascular, pigmentary, traumatic, uveitic, congenital), corneal or lens-based media opacities which prevent OCT image quality, and unreliable visual field tests were excluded from the study. Patients with specific visual field defects that could be attributed to glaucoma were also excluded.

The best-corrected visual acuity values of all participants were obtained by Snellen's visual acuity chart. Anterior segment slit-lamp biomicroscopic examination and gonioscopic examination (Goldmann Three Mirror Lens, Ocular, WA, USA) were performed. Goldmann applanation tonometry (AT 900, Haag-Streit Diagnostics, Koeniz, CH) was used for detecting IOP. Central corneal thickness was measured by corneal pachymetry (Pachymeter SP-3000, Tomey Corporation, Nagoya, JP). A detailed ophthalmoscopic examination was performed using a non-contact lens (+90D SuperField Volk Lens, OH, USA) after pupil dilatation by 1% tropicamide eye drop.

### Visual field test

Using standard automated perimetry (Humphrey Field Analyser; Carl Zeiss Meditec, Inc. Dublin, CA) with the 30-2 Swedish interactive threshold algorithm, visual field testing was performed on patients. The eyes were considered to have glaucomatous visual field loss if the glaucoma hemifield test results were outside the normal limits. A reliable visual field test was defined as false-positive and false-negative error rates of less than 15% respectively and fixation loss of less than 20%. The mean deviation (MD), pattern standard deviation (PSD), and short-term fluctuation (SF) values of the study group were obtained from the visual field tests.

### Optical coherence tomography parameters

Optical coherence tomography images were taken from both eyes using the "macula map" and "disc map" protocols with the spectral-domain OCT (OCT RS-3000 Lite, NIDEK Corporation, Tokyo, Japan). Optic disc analysis, MT, GCC thickness, and peripapillary RNFL thickness were measured using OCT. Parameters for the statistical analysis of the MT were evaluated in the following areas: central 1 mm of the macular area (fovea), 1-3 mm of the parafoveal macular area (superior, inferior, nasal, temporal quadrants), and 3-6 mm of the perifoveal macular area (superior, inferior, nasal, temporal quadrants). Parameters for the statistical analysis of the GCC thickness were evaluated in the following areas: 1-3 mm of the parafoveal macular area (superonasal, superotemporal, inferonasal, inferotemporal quadrants) and 3-6 mm of the perifoveal macular area (superonasal, superotemporal, inferonasal, inferotemporal quadrants). Optic disc analysis was included total disc area, cup area, horizontal and vertical cup/disc ratio. The disc map protocol was used to evaluate optic disc analysis and peripapillary RNFL. Parameters for the statistical analysis of the peripapillary RNFL thickness were evaluated in the following areas: total, superior hemi-

central, inferior hemi-central, superior, nasal, inferior, and temporal quadrants. RNFL thickness was also evaluated on twelve sectors (sector number 1 started from the superonasal area).

Outputs of the actual visual field, OCT macula map, and peripapillary RNFL thickness tests are shown in figure1 and figure 2.

### Statistical analysis

In this study, we utilized the "SPSS (Statistical Package for Social Sciences) 20.0 for Windows" program for statistical analysis. The Kolmogorov-Smirnov test was used to determine the suitability of the data with normal distribution. For parametric variables independent samples t-test, and for non-parametric variables, Mann-Whitney U test was used. A p-value less than 0.05 was approved statistically significant.

## RESULTS

There were 56 eyes of 45 patients (female 18, male 27) in preperimetric glaucoma group, and 49 eyes of 30 cases in the control group (female 14, male 16). The average ages of preperimetric glaucoma and control groups were 64.4±11.9 (between 32 to 86) and 65±10.2 (between 50 to 86) years, respectively. There were no significant differences between the two groups in terms of age and sex (respectively; p=0.835 and p=1.000).

In visual field tests of the study group; the mean MD value was -3.6±0.3 (between -10.2 to -0.8), the mean PSD value was 2.2±0.08 (between 1.3 to 4.6), and the mean SF value was 1.3±0.06 (between 0.6 to 3.3). The mean fixation losses of the study group were 3.7±0.7 (between 0 to 16). The false-negative and false-positive mean reliability indexes of the study group were 1.8±0.4 (between 0 to 12) and 1.3±0.4 (between 0 to 12) respectively.

There was no statistically significant difference between the two groups (p=0.641) in terms of mean optic disc area (study group: 2.3±0.07 and control group: 2.4±0.06). The horizontal cup/disc ratio was 0.6±0.2 (between 0.3 to 0.8) in the study group and 0.3±0.1 (between 0.3 to 0.5) in the control group. The mean vertical cup/disc ratio was 0.6±0.01 (between 0.2 to 0.8) in the study group and 0.3±0.01 (between 0.2 to 0.4) in the control group. The mean cup area of the optic disc was 0.9±0.06 (between 0.05 to 1.8) in the study group and 0.3±0.02 (between 0.1 to 0.5) in the control group. All horizontal-vertical cup/disc ratio and optic disc cup area values of the study group were statistically significantly higher than the control group (respectively; p<0.001, p<0.001, and p<0.001).

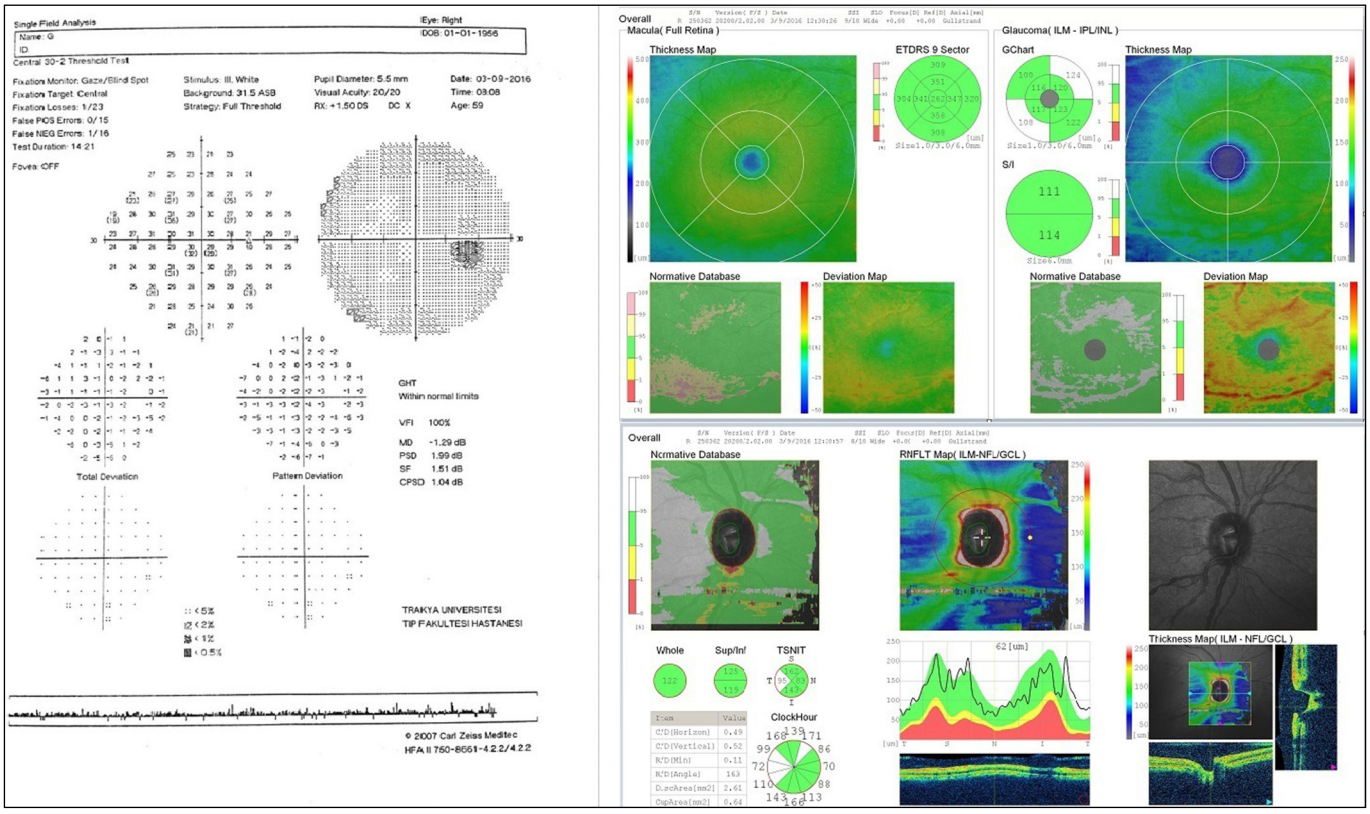


**Figure 1:** *Output of actual visual field, optical coherence tomography macula map and peripapillary retinal nerve fiber layer thickness test protocol of a patient.*

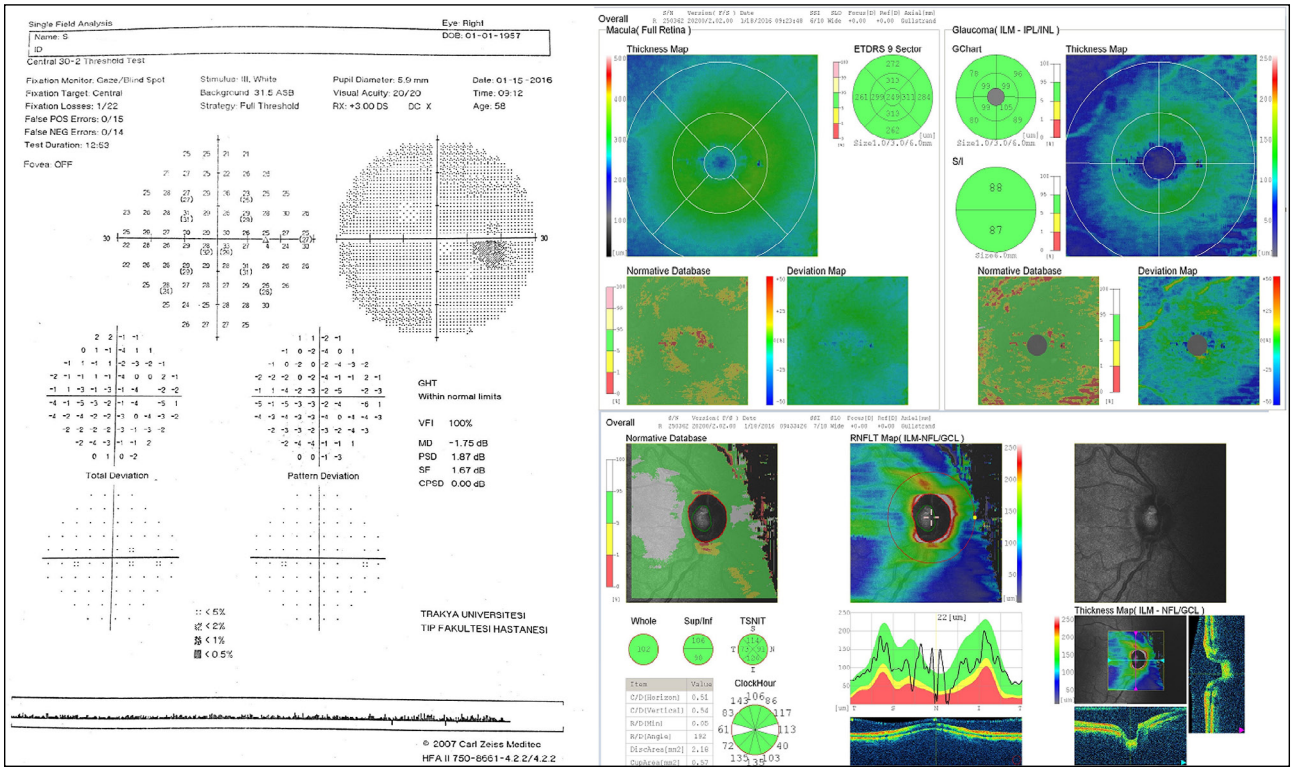


**Figure 2:** *Output of actual visual field, optical coherence tomography macula map and peripapillary retinal nerve fiber layer thickness test protocol of a patient.*

The mean values of central MT, parafoveal MT, and perifoveal MT were not significantly different between the two groups (p>0.05) (Table 1). Also, parafoveal GCC thickness was not significantly different between the two groups (p>0.05) (Table 1). In the study group, perifoveal GCC thickness was found to be significantly thinner in all quadrants than in the control group (Table 1).

**Table 1:** *Comparison of macula thickness and ganglion cell complex thickness between study and control groups.*

| | Patients (n=56 eyes) | Control (n=49 eyes) | p value |
|---|---|---|---|
| **Central MT** | 263.93±18.58 | 260.49±19.92 | 0.362* |
| **Parafoveal MT** | | | |
| temporal | 325.02±15.89 | 322.45±15.96 | 0.486** |
| nasal | 338.89±16.62 | 333.78±16.63 | 0.169** |
| superior | 338.48±16.62 | 333.96±15.88 | 0.202** |
| inferior | 331.93±26.86 | 332.22±16.17 | 0.518** |
| **Perifoveal MT** | | | |
| temporal | 279.54±18.68 | 283.41±14.81 | 0.247* |
| nasal | 319.45±82.40 | 308.92±14.49 | 0.985** |
| superior | 294.30±15.86 | 297.35±14.86 | 0.315* |
| inferior | 282.54±17.38 | 288.55±14.34 | 0.058* |
| **Parafoveal GCC** | | | |
| superonasal | 117.07±16.13 | 115.69±9.46 | 0.688** |
| inferonasal | 114.63±10.06 | 116.61±9.84 | 0.310* |
| superotemporal | 110.27±9.03 | 110.80±9.01 | 0.765* |
| inferotemporal | 110.13±11.33 | 112.41±9.90 | 0.382** |
| **Perifoveal GCC** | | | |
| superonasal | 108.55±11.94 | 113.39±8.42 | **0.008**** |
| inferonasal | 105.54±11.37 | 112.90±8.96 | **<0.001*** |
| superotemporal | 88.89±9.71 | 92.90±6.94 | **0.004**** |
| inferotemporal | 90.11±17.58 | 95.18±6.46 | **0.001**** |

**MT:** Macula thickness; **GCC:** Ganglion cell complex; *independent T-test; **Mann-Whitney U test.

In the study group, peripapillary RNFL thickness was found to be significantly thinner than the control group. Comparison of peripapillary RNFL thicknesses of 12 sectors of study and control group; in the study group, 1, 2, 3, 4, 5. 7, 8, 9, 11 numbered sectors were significantly thinner, while there were no significant differences in the other sectors (p>0.05) (Table 2).

## DISCUSSION

Glaucoma is a disease that affects axons and bodies in retinal ganglion cells. Retinal ganglion cell axons, retinal ganglion cell bodies, and retinal ganglion cell dendrites are thought to be present in the retinal nerve fiber layer, ganglion cell layer, and inner plexiform layer, respectively.[8] Retinal ganglion cell loss and thinning in RNFL are the main reasons for the development of glaucomatous visual field defects.[9-14] Therefore, identifying structural changes is very crucial for the early diagnosis of glaucoma.[12-14] Optical coherence tomography shows peripapillary RNFL thickness, optic disc head analysis, and GCC thickness with reproducible, noninvasive, and comparable with related age-normal data.[15-19]

The data obtained with OCT is very important in determining treatment and follow-up plans for preperimetric glaucoma. 50% of all retinal ganglion cells are located in the macular area.[20] For this reason, examining retinal ganglion cell loss in the macular region seems to be the most rational way to make an early diagnosis of glaucoma. Arintawati et al.[21] reported that GCC and RNFL thickness measurements made with OCT allow preperimetric and perimetric glaucoma eyes to be separated from healthy eyes. In many previous studies,[22-24] peripapillary RNFL parameters with OCT were evaluated in preperimetric glaucoma. Since Zeimer et al.[25] reported that MT measurements could be used in detecting early glaucomatous damage and glaucoma progression, MT measurements have been accepted as a prominent parameter in the early diagnosis of glaucoma. Many studies have shown that MT measurements allow the detection of glaucomatous damage.[8,26-32] Some of the studies in the literature have also reported that MT measurements do not have a higher predictive value than peripapillary RNFL thickness measurements.[25-28,33,34] Leung et al.[26] reported that the peripapillary RNFL parameters were more valuable than the MT measurements for detecting early diagnosis of glaucoma. Wollstein et al.[35] reported that in their studies evaluating the progression of patients with suspected glaucoma and glaucoma for 5 years, 22% of patients showed thinning of RNFL with OCT, despite no visual field progression. Guedes et al.[36] showed that both MT and RNFL thickness reduced significantly in glaucomatous eyes.

Kim et al.[7] reported that the superior and inferior quadrant GCC thicknesses were thinner in preperimetric glaucoma than the control group. In another similar study[37] found that the superior and inferior quadrant GCC thicknesses were thinner in preperimetric glaucoma than the control group. In our study, there were differences in the GCC measurement

**Table 2:** *Comparison of retinal nerve fiber layer thickness between study and control groups.*

| RNFL | Patients (n=56 eyes) | Control (n=49 eyes) | p value |
|---|---|---|---|
| total | 95.02±13.45 | 104.59±12.28 | **<0.001*** |
| superior hemi-central | 98.50±19.14 | 107.06±14.18 | **0.001**** |
| inferior hemi-central | 94.80±18.33 | 102.27±12.30 | **0.002**** |
| superior | 118.09±20.47 | 128.71±21.26 | **0.011*** |
| inferior | 121.29±22.14 | 134.18±19.38 | **0.002*** |
| nazal | 75.29±16.78 | 81.59±12.49 | **0.032**** |
| temporal | 62.80±9.37 | 70.69±12.76 | **0.001*** |
| Sector number 1 | 105.89±26.82 | 130.94±30.83 | **<0.001**** |
| Sector number 2 | 90.18±23.14 | 134.29±27.08 | **<0.001**** |
| Sector number 3 | 59.82±15.97 | 86.47±19.01 | **<0.001**** |
| Sector number 4 | 72.27±20.09 | 54.59±13.97 | **<0.001**** |
| Sector number 5 | 103.86±29.05 | 66.90±16.22 | **<0.001**** |
| Sector number 6 | 131.73±29.74 | 139.00±32.99 | 0.242** |
| Sector number 7 | 121.55±32.80 | 146.33±26.20 | **<0.001**** |
| Sector number 8 | 62.66±15.47 | 109.78±31.02 | **<0.001**** |
| Sector number 9 | 48.63±8.79 | 78.65±21.01 | **<0.001*** |
| Sector number 10 | 74.04±12.87 | 69.53±14.62 | 0.097** |
| Sector number 11 | 125.09±26.03 | 93.55±17.95 | **<0.001*** |
| Sector number 12 | 117.50±33.63 | 116.92±24.13 | 0.655** |

**RNFL:** Retina nerve fiber layer; *independent T-test; **Mann-Whitney U test.

method, and unlike these studies, we only found significant thinning of the perifoveal area. The currently used OCT macular scanning pattern is measuring the central 6 mm area. However, glaucomatous damage affects more peripheral regions. We also obtained significant results in perifoveal GCC and peripapillary RNFL measurements, although we could not detect a significant difference in total macular quadrants and parafoveal GCC measurements in our study. The nerve fibers coming from the superior, inferior, and nasal quadrants of the optic nerve are present in a very small amount in the central 6 mm area. Most of the nerve fibers in this central area are composed of nerve fibers coming from the maculopapillary band. In particular, the nerve fibers in the parafoveal region are absent from the nerve fibers outside the maculopapular band. Neuroretinal rim loss begins primarily from the superotemporal and superonasal quadrants of the optic nerve in glaucomatous damage. The nerve fibers on the temporal quadrant are subsequently affected.[38] Therefore, in the measurements of macular GCC thickness, the more the measurement region is limited to the central region, the more difficult it will be to detect the nerve fibers coming from the superotemporal and inferotemporal regions of the optic disc that begin earlier in the loss. For this reason, in our study, macular GCC thickness was not statistically significant in the parafoveal 3 mm region, but macular GCC thickness was significantly decreased in the perifoveal 6 mm area. In this way, we believe that the measurement of macular GCC thickness to be performed in a larger area will be more effective in the early diagnosis of glaucomatous damage compared to the 6 mm measurements of the central region.

As a result, it is not enough to evaluate patients with suspected glaucoma with only perimetry. Although both macular and peripapillary cellular losses have been initiated, this loss can not be detected by standard automated perimetry in preperimetric glaucoma cases. Peripapillary RNFL measurement and perifoveal GCC thickness measurement are to be performed together because it is necessary for the early diagnosis of preperimetric and glaucoma suspect cases.

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