



## Macular Thickness Changes in Normal and Glaucomatous Eyes

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**Received:** April 23, 2021

**Published:** May 26, 2021

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### Abstract

**Purpose:** To evaluate macular nerve fiber layer thickness (NFL) in glaucoma patients by optical coherence tomography, to correlate between total macular thickness, macular NFL thickness and peripapillary NFL thickness and to correlate between macular thickness and peripapillary NFL thickness and visual field changes.

**Participants:** Total "60" subjects (110 eyes) were studied including: 30 normal subjects (60 eyes) and 30 glaucoma patients (50 eyes).

**Methods:** Optical coherence tomography (OCT) was used to measure macular NFL thickness, total macular thickness and peripapillary NFL thickness in each diagnostic group.

**Main Outcome Measures:** Measurements of macular NFL thickness, total macular thickness, peripapillary NFL thickness in total mean, (4) quadrants and (12) clock hours.

**Results:** A significant difference in macular NFL thickness between normal and glaucoma patients was observed. Mean macular NFL thickness demonstrated a stronger correlation with visual function than mean total macular thickness ( $R = 0.7, P = 0.02$  versus  $R = 0.45, P = 0.05$ ). Inner ring, outer ring, mean macular thickness were found to be significantly different between normal and glaucoma patients.

**Conclusion:** Thinning of both macular NFL and peripapillary NFL in glaucoma suggested that retinal ganglion cells of both the macular and peripheral retina are involved in the degenerative process of glaucoma.

Macular NFL is a better surrogate marker for glaucomatous damage than total macular thickness. Peripapillary NFL may be the most useful clinical marker in diagnosing and monitoring glaucomatous damages among all three measurements.

**Keywords:** Macular Nerve Fiber Layer Thickness; Glaucoma; Optical Coherence Tomography

### Introduction

Glaucoma is characterized by gradual loss of retinal ganglion cells (RGCS) and thinning of the nerve fiber layer. While the etiology of this damage is uncertain, the loss itself is well documented. Numerous studies have shown that glaucomatous damage to the

retinal nerve fiber layer (NFL) precedes functional loss by as much as 5 years [1,2].

The NFL is composed primarily of RGCS axons, neuroglia and astrocytes. Loss of retinal ganglion cells and their axons is known to occur in the posterior pole, where these cells may constitute

30% to 35% of the retinal thickness in the macular region [3]. Studies of chronic experimental glaucoma in monkey eyes have shown substantial loss of retinal ganglion cells in the zone surrounding the fovea [4,5].

The anatomic macula measuring approximately 6 mm is recognized histologically by the presence of xanthophyll pigment and multi-layered ganglion cells. There are up to (7) layers of ganglion cell bodies in the central retina or macula and as few as (1) cell layer in the peripheral retina. Approximately 50% of retinal ganglion cells are located in the macular region (4 to 5) mm from the center of the fovea with the peak density occurring 750 to 1100  $\mu$ m from the fovea [6,7].

Since ganglion cell bodies have 10 - 20 times the diameter of their axon, it has been suggested that macular imaging might enhance initial detection of glaucomatous damage [8].

Theoretically, the retinal NFL thickness is more specific than total retinal thickness in representing the proportional loss of retinal ganglion cells. Previous studies focused mostly on the peripapillary region [9,10]. Which is the core area for evaluation of retinal NFL and little is known about the role of macular NFL in glaucoma. As retinal nerve fiber bundles arising from individual retinal ganglion cells converge toward the optic disc, retinal NFL over the macula is much thinner than that around the peripapillary region, thus rendering this layer difficult to delineate and to quantify.

Optical coherence tomography (OCT) is the optical equivalent of ultrasonography with high *in vivo* resolution. OCT is one of the imaging technologies commercially available for measuring total retinal thickness and retinal RNL thickness [11]. OCT of the macular area has also been found reproducible [12].

### Purpose of the Study

In this study the purpose is to evaluate the clinical significance of macular NFL thickness in glaucoma, to identify the most favorable surrogate marker for glaucomatous damage among all measurements of macular NFL thickness, total macular thickness and peripapillary NFL thickness and to correlate between macular NFL thickness and visual function.

### Subjects and Methods

A total of "60" subjects (110 eyes) were examined between April 2008 to October 2008 at Mansoura Ophthalmic Center. All

subjects underwent a thorough ophthalmologic examination. This consisted of the following: medical history (including ocular and family histories), visual acuity, refraction, intra-ocular pressure measurement with Goldman tonometry, dilated slit lamp examination with stereoscopic biomicroscopy of optic nerve head (ONH) and nerve fiber layer NFL and indirect ophthalmoscopy, Humphrey 24-2 visual field testing and OCT testing of both the macula and peripapillary NFL. The inclusion criteria included best corrected visual acuity of at least 20/40. Refractive error were between +3.0 and -6.00 Diopters. Patients with any kind of retinal pathology, retinal laser procedure, retinal surgery, opaque media neurological diseases or a history of diabetes were excluded. Standard visual field testing was obtained with static automated white-on-white threshold perimetry (Humphrey field Analyser 640 Carl Zeiss Co., San Leandro, Calif) A visual field was defined as reliable when fixation losses were < 20% and false-positive and false-negative rate were < 25%. Subjects would not be included in the study if subjects could not complete a reliable visual field test within 3 attempts.

Normal subjects consisted of healthy individuals with no history of intraocular pressure > 21mmHg, normal optic nerve head appearance on the basis of stereoscopic examination under slit lamp and normal Humphrey visual field result. Normal optic nerve head appearance was defined as symmetric cup-to-disc ratio of < 0.5 with uniform neuroretinal rim.

A normal visual field was one with less than "3" non edge contiguous points identified significant ( $P < 0.05$ ) on the same side of the horizontal meridian in the pattern deviation plot and was graded as within normal limits in the glaucoma hemifield test.

Glaucomatous neuropathy was defined as having loss or thinning of neuroretinal rim, notching or excavation or asymmetric cup-to-disc ratio > 0.2 with an associated visual field defect in the corresponding location. A glaucomatous visual field defect exhibited a typical arcuate or paracentral scotoma and/or nasal step on their visual field test, with clusters of three or adjacent points depressed more than (5) dB or two or more adjacent points depressed more than (10) dB.

### Optical coherence tomography measurements

OCT was performed with (Topcon, 3D OCT, 1000, USA). OCT is an optical technique for high resolution measurements and cross-sectional imaging of the human retina which is based on low coher-

ence interferometer. OCT measurements are performed using fiber optically integrated Michelson interferometer with short coherence length superluminescent diode source. Near-infrared illumination (840 nm) is used to minimize subject discomfort. Before the scan, the pupil of each subject was determined. If the pupil size was < 4mm, the pupil was dilated with 1% tropicamide and 2.5% phenylephrine. Subjects underwent (2) Scanning protocols including:

1. Peripapillary NFL thickness scan with three sequential circular scans of 3.4mm diameter over the optic nerve head.
2. Macular thickness scan with six linear scans spaced (30°) with each other over the macula for measurements of macular NFL thickness and total macular thickness.

Internal Fixation was chosen (subject fixated with the eye being studied) because of better reproducibility than external fixation (Subject fixated with fellow eye). Peripapillary NFL thickness were reported as averages over each quadrant. (superior, inferior, nasal, temporal), as averages for each clock hour. Macular scans (macular thickness map) was divided into nine sections, and it was displayed as three concentric circles including: central circle, an inner ring and an outer ring, with each ring divided into four quadrants (superior, inferior, nasal, temporal). The central circle, inner ring and outer ring had diameters of (1) mm, (3) mm and (6) mm, respectively. The central 1 mm circular region represents the fovea area. The NFL with its high reflectivity signal can be visualized as the first layer in red on the scan. Its thickness is determined by the difference in distance between the vitreoretinal interface and a posterior border. The location of the vitreoretinal interface and the retinal pigment epithelium defined the inner and outer boundaries respectively of the retina. These two boundaries were associated with the sharpest edge in each OCT scan because of the high contrast in optical reflectivity between the relatively non reflective vitreous and reflective neurosensory retina and between the minimally reflective photoreceptor outer segments and the highly reflective retinal pigment epithelium choriocapillaris.

A good quality scan was defined as one with signal to noise ratio of more than (45) dB and well delineated NFL in the scan image. Subjects with scan not meeting the criteria of a good quality scan after (3) attempts were excluded.

**Statistical analysis**

The subject information was entered into computer database and was analysed using SPSS (statistical package for social science).

Because most subjects in the study had two eyes, linear mixed regression models was used to compute all means. Chi square (X<sup>2</sup>) test of significance was used for comparison between the groups. Kruskal Wallis test was used for comparison between groups Spearman's correlation coefficient was used to calculate correlation between variables. P < 0.05 was considered to be statistically significant, R < 0.5 indicate weak correlation, R ≥ 0.5 indicate good correlation).

**Results**

A total of (110) eyes from 60 subjects were included in the study. As regard to age, gender and refractive error, there were no significant difference between the groups (Table 1).

	Normal	Glaucoma
Age: Mean ± SD	40 ± 15	38 ± 13
Range	(35 - 55)	(37 - 54)
Sex		
Female	15 (50%)	16 (53%)
Male	15 (50%)	14 (47%)

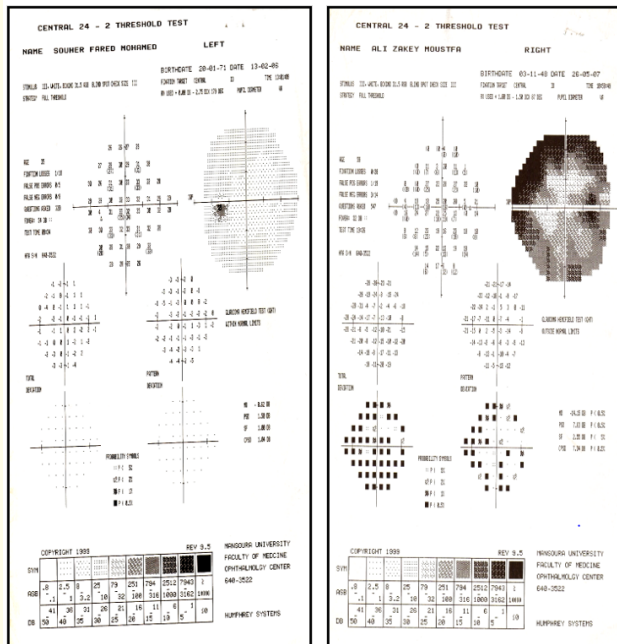
**Table 1:** Demographic characteristics.

For the mean deviation and pattern standard deviation in the visual field results, significant differences were found between glaucoma and normal groups (p = 0.000) (Table 2 and figure 1).

	Normal	Glaucomatous subjects
Mean deviation: mean ± SD	1.17 ± 0.3	8.9 ± 7.7
Range	(0.0 - 1.5)	(2 - 15)
Corrected pattern Standard Deviation		
Mean ± SD	0.27 ± 0.2	4.2 ± 2.7
Range	(0,0 - 0.5)	(2.1 - 9)
P	0.000	0.000

**Table 2:** Visual field among groups.

Peripapillary NFL in all sectors and the mean NFL were significantly different between normal subjects and glaucoma patients (Table 3).



**Figure 1:** Visual field of a normal eye and visual field of a glaucomatous eye.

	Normal	Glaucoma	p
Superior NFL	113.2 ± 23	77.7 ± 25.5	0.001
	(91 - 144)	(29 - 85)	
Inferior NFL	110 ± 20	80.4 ± 30	0.000
	(98 - 130)	(33 - 83)	
Nasal NFL	70 ± 15	45.5 ± 19	0.001
	(66 - 103)	(15 - 46)	
Temporal NFL	63 ± 10	42.3 ± 20	0.001
	(49 - 88)	(13 - 45)	
Mean NFL	90 ± 7.2	70 ± 40	0.002
	(90 - 150)	(30 - 77)	

**Table 3:** Peripapillary NFL thickness among groups.

Inferior peripapillary NFL demonstrated the best performance for discriminating glaucoma subjects from normal subjects.

A distinctive thinning in the NFL at the macula was seen in the glaucoma group. The results of the total mean and 4-quadrants

analyses of macular NFL thickness and total macular thickness were tabulated and compared (Table 4, 5 and figure 2). A decreasing trend was observed for both macular NFL thickness and total macular thickness between normal and glaucoma patients. The result had significant differences between normal and glaucoma except the temporal macular NFL thickness and foveal macular thickness.

	Normal	Glaucoma	P
Central Ring (Foveal macular thickness)	239 ± 15	235 ± 20	0.9
	(190 - 250)	(195 - 245)	
Inner ring (mean macular thickness)	269 ± 20	230 ± 15	0,001
	(270 - 290)	(220 - 255)	
Outer ring (mean macular thickness)	245 ± 15	230 ± 12	0.002
	(240 - 260)	(211 - 235)	
Inferior macular thickness (1 - 3 mm)	271 ± 25	240 ± 30	0.001
	(270 - 303)	(205 - 245)	
Nasal macular thickness (1 - 3 mm)	275 ± 20	250 ± 30	
	(270 - 305)	(200 - 260)	
Temporal macular thickness (1 - 3 mm)	260 ± 15	241 ± 25	0.001
	(259 - 289)	(209 ± 241)	
Superior macular thickness (1 - 3 mm)	270 ± 30	230 ± 20	0.002
	(269 ± 300)	(190 - 240)	
Superior macular thickness (1 - 6)	249 ± 24	228 ± 25	0.002
	(240 - 265)	(190 - 230)	
Inferior macular thickness (1 - 6 mm)	245 ± 20	226 ± 20	0.001
	(237 ± 260)	(180 - 239)	
Nasal macular thickness (1 - 6 mm)	255 ± 10	220 ± 15	0.001
	(253 - 272)	(178 - 240)	
Temporal macular thickness (1 - 6 mm)	234 ± 15	200 ± 16	0.002
	(233 - 245)	(170 - 220)	

**Table 4:** Total macular thickness among groups in microns.

The mean macular NFL thickness in glaucoma and normal subjects were (19.1 µm), (23,4 µm) respectively. The mean total macular thickness (1 - 3 mm) in normal subject was (269 µm and in glaucoma patient was (230 µm).

The reduction of all macular parameters correlated significantly with visual Field mean deviation. Mean macular NFL thickness

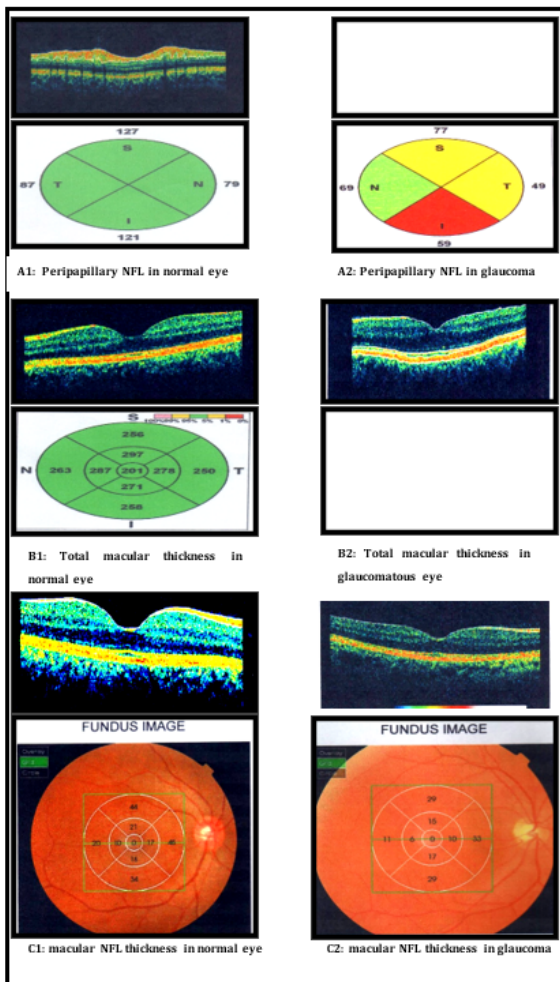


Figure 2: OCT in normal and glaucomatous subjects.

	Normal	Glaucoma	p
Mean macular NFL (1 - 3 mm)	23.4 ± 4.4 (19 - 25)	19.1 ± 7.1 (8 - 18)	0.002
Superior macular NFL (1 - 3 mm)	20 ± 2.1 (19 - 26)	17.2 ± 5.02 (10 - 18)	0.01
Inferior macular NFL (1 - 3 mm)	18.4 ± 2.0 (16 - 26)	14 ± 4.0 (5 - 14)	0.001
Nasal macular NFL (1 - 3 mm)	16.3 ± 3.0 (12 - 20)	11 ± 3.3 (8.5 - 11.5)	0.001
Temporal macular NFL (1 - 3 mm)	10.2 ± 2.0 (9 - 14)	9.0 ± 1.0 (7 - 14)	0.5
Superior macular NFL (1 - 6 mm)	31.2 ± 2.1 (29 - 44)	20 ± 7.0 (12 - 26)	0.000
Inferior macular NFL (1 - 6 mm)	33.2 ± 2.5 (27 - 39)	25 ± 6.0 (14 - 25.5)	0.000
Nasal macular NFL (1 - 6 mm)	43.5 ± 3.2 (37 - 56)	30 ± 8.5 (15 - 32)	0.001
Temporal macular NFL (1 - 6 mm)	15.3 ± 1.5 (12.3 - 20)	14 ± 2.5 (5 - 10)	0.5

Table 5: macular NFL thickness among groups.

Visual Field	Mean macular NFL thickness	Mean total macular thickness (1 - 3 mm)
Mean deviation	R = 0.7 P = 0.02	R = 0.45 P = 0.05

Table 6: Correlation between visual field and macular parameters in glaucoma patients.

had a significantly stronger correlation than mean macular thickness (1 - 3 mm) (Table 6).

Overall macular NFL thickness demonstrated comparable discriminating power for diagnosis glaucoma subjects compared with total macular thickness.

The mean peripapillary NFL thickness had significant stronger correlation with visual field mean deviation (Table 7).

Peripapillary NFL thickness	Visual Field (mean deviation)	
	R	P
Superior NFL	0.6	0.05
Inferior NFL	0.8	0.02
Nasal NFL	0.45	0.001
Temporal NFL	0.5	0.02
Mean NFL	0.66	0.002

Table 7: Correlation between visual field and peripapillary NFL thickness in glaucoma.

There was correlation between macular thickness, macular NFL and peripapillary NFL (Table 8).

	Mean macular NFL	Total macular thickness (mean)
Mean Peripapillary NFL	R = 0.88	R = 0.49
	P = 0.002	P = 0.01

**Table 8:** Correlation between peripapillary NFL, macular NFL and total macular thickness.

### Discussion

Glaucoma is a complex multi factorial disorder characterized by a typical pattern of optic nerve damage and visual field loss that is usually but not always associated with elevated intraocular pressure. Accepted parameters for monitoring glaucoma include descriptions and photography of optical disc appearance, measurement of intra ocular pressure and periodic threshold perimetry. Advances in posterior segment imaging technology provide a means for generating structural data useful in monitoring eyes with glaucomatous optic nerve damage [13-15].

Numerous experimental and clinical studies have demonstrated that the retinal NFL becomes atrophic in glaucoma [16,17]. Damages to the retinal NFL and the optic disc frequently precede visual field loss. Most modern imaging devices, including confocal scanning laser ophthalmoscopy, scanning laser polarimetry and optical coherence tomography are non-invasive techniques designed to target on the optic nerve head for scanning. The advantages of OCT have been recognized as being unaffected by other birefringence ocular structures during measurement, requiring no reference plane and having high axial and transverse resolving power. The use of OCT in evaluation of retinal NFL thickness has been demonstrated to be reproducible and reliable in both normal and glaucomatous eyes [18].

A standard protocol that uses a circular scan of diameter 3.4 mm centered at the optic nerve head is adopted in measuring peripapillary NFL thickness by OCT. Kanamori, *et al.* demonstrated that OCT has the ability to detect early glaucomatous changes by measuring peripapillary NFL thickness particularly the inferior quadrant. A significant relationship existed between the mean deviation of visual field and the peripapillary NFL thickness in all parameters except the nasal area [19].

Zangwill, *et al.* and Soliman, *et al.* reported similar correlation between peripapillary NFL thickness and visual field defects [20,21].

Similarly, Leung, *et al.* found that the mean and inferior quadrants of peripapillary NFL showed the best performance for detection of glaucoma and the strongest correlation with visual function. The largest reductions in peripapillary NFL thickness were found at superotemporal and inferotemporal sectors [28].

Also, EL-Beltagi, *et al.* found that localized retinal NFL thinning as measured by OCT was related to localized visual field defects topographically [22]. OCT measurement of peripapillary NFL thickness also, discriminated very well between glaucoma and normal but was less sensitive for glaucoma suspect. The inferior quadrant was the only parameter that could show a statistically significant difference between normal subjects and glaucoma suspects [10]. It is well known that optic nerve defects associated with glaucoma often occur initially at the inferior pole and that visual field defects associated with glaucoma frequently manifest first in the superior visual field corresponding to the inferior pole defects [26].

Similarly, in this study there was a reduction in peripapillary NFL in all quadrants especially in the superior and inferior quadrants. These locations reflect the frequency distribution of the most commonly observed visual field defect patterns found in glaucoma. There was correlation between peripapillary NFL thickness and visual field.

The macular thickness measurements represent a neglected structural end point for glaucoma. Although glaucoma is an optic nerve disorder, the fundamental defining abnormality is localized at the level of the retinal ganglion cell. Macular thickness measurements represent a surrogate indicator of retinal ganglion cell thickness.

The loss of ganglion cells and nerve fiber thickness were observed involving the posterior pole in glaucoma [3]. The macula has the highest density of ganglion cells with a peak at 750 to 1100 μm from the foveal center [7]. Ganglion cell loss in glaucoma may result in a decrease in macular cellularity and macular thickness. Lederer, *et al.* reported a significant reduction in macular volume, as measured by OCT with standard macular thickness scan in early and advanced glaucoma but not in glaucoma suspect [23].

Greenfield, *et al.* also demonstrated that macular thickness was significantly thinner in glaucoma than in normal eyes. The changes correlated closely with the peripapillary NFL thickness [8].

Tanito, *et al.* also found a reduction of perifoveal retinal thickness with the retinal thickness analyzer [24].

In addition, Guedes, *et al.* found that the outer ring macular thickness provided better correlation than did inner ring thickness in comparing normal with glaucomatous subjects [10]. Similarly, in this study, there was reduction in macular thickness in glaucomatous patients compared with normal subjects.

Despite the hypothetical advantages of macular thickness assessment in glaucoma in this study, whereas macular thickness changes was significantly associated with glaucoma, NFL thickness showed a still stronger relationship with the disease. This finding may be due to under sampling the tissue at risk, because, only approximately (50%) of the RGC are presented in the macula, yet nearly (100%) of RGC are assessed in a peripapillary OCT NFL scan. Because glaucoma is a diffuse disease, the ability to measure the damage done by glaucoma in the entire eye may give peripapillary NFL assessment a distinct advantage over macular thickness evaluation in detecting glaucoma. Furthermore, the absolute thickness changes are greater when measuring nearly all of the RGCS, even if just their axons, than when measuring just the subpopulation in the macula despite the greater size of the RGC soma than that of its axon. In addition, the nature of OCT macular map leaves large areas of the macula unsampled, Another major advantage of NFL over macular thickness assessment is the confounding of macular thickness measures by non-glaucomatous macular disease. Entities such as diabetes and macular degeneration, for examples, directly affect macular thickness and could obscure or exaggerate the abnormalities seen with glaucoma. These are not significant issues in peripapillary NFL assessment. This is not to say that macular thickness may not be a useful parameter in the evaluation of glaucoma. It's significantly associated with disease.

Macular NFL may provide a more direct assessment of the retinal ganglion cells compared with measurement of the total macular thickness in which the ganglion cell bodies and axons constitute only 30% to 50% of total thickness [25].

In this study, the macular NFL thickness scan was performed by adjusting the internal fixation light on the present location over the

central fovea (which is the same location for internal fixation during macular thickness scan). The limitation of linear macular thickness scan is that scan depends on the Fixation capability of examined subjects. The desired location of the scans can be ensured by monitoring the foveal position and the saccadic movement through the built- in real-time fundus camera in OCT. The location of the fovea and internal foveal fixation light can be visualized in the camera during the scanning process. Any saccadic foveal deviation can be detected simultaneously on the OCT scan image panel and fundus camera panel. In addition, during performing linear macular thickness scan, one can ensure the fovea in the center of the scan by visualizing the central foveal depression located in the middle of the linear OCT scan image.

Another limitation of macular NFL thickness scan is that the minimal measurable macular NFL thickness is restricted by resolving power of the OCT. In cases of advanced glaucoma, the reflectivity signal of the macular NFL which appeared red in the false color - coding OCT image, were much attenuated, thus rendering the analysis software unable to delineate the boundaries of the NFL layer and report bad data during the analysis (these cases were excluded from the study). These images represented a significant loss of NFL. Excluding these cases would narrow the macular NFL measurement difference between normal and glaucoma groups, thus lowering the discriminating performance of macular NFL thickness for glaucoma detection.

In studying the normal retinal nerve fiber anatomy, it has been shown that foveal fibers contribute a large proportion of the temporal aspect of the optic nerve head whereas fibers from areas temporal to the fovea are displaced to more superior and inferior topography, the peaks of macular NFL correspond to the superior and inferior arcuate fiber and the nasal side of the fovea constitutes the foveal fiber, the papillomacular bundles which project toward the temporal side of the optic disc [27].

Leung, *et al.* found that macular NFL thickness was significantly reduced in all clock hours and quadrants except the temporal quadrants. Preferential losses of nerve bundles were most evident over the inferior and superior arcuate fibers. For macular thickness measurement, the mean macular thickness (1 - 6 mm) and the mean and 4- quadrant macular thickness (1 - 3 mm) also showed significant reduction in the glaucoma group. The foveal retinal thickness at the central (1 mm) region contains the minimal number of ganglion cells, thus resulting in no significant change [28].

In addition, in this study, there was reduction in macular NFL thickness in all quadrants except temporally. The lack of a significant reduction of temporal macular nerve fiber thickness in glaucoma may be explained by the retinal nerve fibers being displaced in more superior and inferior location [27]. It is also possible that the resolution of OCT with scanning resolution up to 2  $\mu\text{m}$  in the near future may overcome this limitation and improve the diagnostic sensitivity in glaucoma [29,30].

Wollstein, *et al.* found correlation between macular thickness and peripapillary NFL ( $r = 0,52$  for overall mean,  $r = 0.55$  for inferior macular with inferior peripapillary and  $r = 0.44$  for superior macular with superior peripapillary) [31].

Leung, *et al.* found that macular NFL matched with peripapillary NFL [28].

Also, in this study, there was a correlation between macular NFL and total macular thickness and peripapillary NFL.

Because macular thickness corresponds well with peripapillary NFL thickness, macular scanning can provide a confirmation of abnormalities detected by peripapillary OCT scans, especially in the subtle cases, particularly those with minimal or no perimetric findings. Macular and peripapillary scans may reinforce each other in confirming the presence of early abnormalities.

## Conclusion

In conclusion, Macular NFL is a better surrogate marker for glaucomatous damage than total macular thickness in terms of a stronger correlation with visual function, However, it still may not be as sensitive as peripapillary NFL measurement. As the axonal fibers arising from individual retinal ganglion cells coming from all retinal ganglion cell populations converge towards the optic nerve head, it is conceivable that any loss of retinal NFL is most readily detected at the peripapillary region. The peripapillary NFL may be the most useful clinical marker in diagnosing and monitoring glaucomatous damages among all three measurements.

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**Volume 4 Issue 6 June 2021**

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