Title: comparison of retinal nerve fiber layer and macular thickness for discriminating primary open-angle glaucoma and normal-tension glaucoma using optical coherence tomography

Running Head: comparison of retinal nerve fiber layer and macular thickness

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Purpose: The aim of this study was to evaluate the discrimination capabilities of macular and peripapillary retinal nerve fiber layer (pRNFL) thickness parameters as measured using spectral domain optical coherence tomography (SD-OCT) between primary open-angle glaucoma (POAG) and normal-tension glaucoma (NTG).

Methods: A total of 90 subjects were enrolled: 30 healthy subjects, 30 subjects with POAG, and 30 subjects with NTG, consecutively. Retinal nerve fiber layer thickness, macular thickness and volume measurements were obtained with circular and radial SD-OCT scans. All parameters were compared between groups using an analysis of variance test. Areas under the receiver operating characteristic (AROC) curves with sensitivities at specificities greater than or equal to 90 per cent were generated to compare discrimination capabilities of various parameters between POAG and NTG.

Results:

The macular thickness and volume measurements were the highest in the normal subjects, followed by NTG and POAG ($p<0.05$). Average retinal nerve fiber layer thickness had perfect discrimination for normal- POAG(AROC, 1.000; sensitivity, 100%) and near perfect discrimination for normal- NTG (AROC, 0.979; sensitivity 93%) as well as NTG-POAG pairs (AROC, 0.900; sensitivity 60%). Inferior outer macular thickness (IOMT) and total volume were the best macular thickness and volume parameters having similar AROC's and sensitivities between normal and POAG (IOMT, AROC, 0.987; sensitivity, 92% and total volume, AROC, 0.997; sensitivity, 97%), normal and NTG (IOMT, AROC,0.862 , sensitivity 47% and total volume, AROC,0.898 , sensitivity 67%) and also between NTG and POAG (IOMT, AROC,0.910 , sensitivity 53% and total volume, AROC,0.922 , sensitivity 77%). In each comparison group, there was no statistically significant difference in AROC's between average retinal nerve fiber layer and Inferior outer macular thickness, as well as total volume.

Conclusions: The macular parameters offer comparable performance to pRNFL parameters for the discrimination of NTG and POAG. Average retinal nerve fiber layer thickness, total macular volume and inferior outer macular thickness were the best SD-OCT parameters with superior discriminating capabilities.

Keywords: Macular thickness, Macular volume, Normal tension glaucoma, Primary open angle glaucoma, Retinal nerve fiber layer thickness

Glaucoma is a progressive optic neuropathy characterized by the gradual degeneration of retinal ganglion cells (RGCs) and their axons that lead to nerve fiber layer loss, optic disc cupping and consecutive glaucomatous visual field changes.[1] The disease is usually characterized by slow loss of neuronal tissue for which rise in intraocular pressure (IOP) is considered as a primary risk factor. However, both glaucomatous RGC loss and optic nerve atrophy can occur independently of IOP.

Normal tension glaucoma (NTG) and primary open-angle glaucoma (POAG) represent a continuum of open-angle glaucomas in which the mechanism of optic neuropathy shifts from IOP-dependent factors in POAG to additional to pressure-independent factors in NTG, with considerable overlap of causative factors.[2] Therefore, NTG has been considered a subset of POAG, sharing many similar characteristics.[3] Normal IOP is not the only difference between NTG and POAG patients, however, and other differentiating features are present notably the rate of progression and hematovascular differences. Both POAG and NTG have a progressive nature, but NTG progresses relatively slowly over time.[4] Increased frequency of disc hemorrhage,[5,6] localized retinal nerve fiber layer (RNFL) defects closer to the center of the macula,[7] higher peak 24-hour IOP[8,9] and vascular or perfusion abnormalities[10,11] have been suggested as contributing factors for NTG. It therefore becomes imperative to note the differences between NTG and POAG eyes using objective measures such as spectral domain optical coherence tomography (SD-OCT), which is rapildy emerging as a standard of care in managing glaucoma.

OCT, first described by Huang and colleagues, is a new technique for real time, quantitative, objective, high resolution measurements and cross section imaging of the retina from which RNFL and macular parameters are calculated.[12] Studies have consistently shown that both peripapillary retinal nerve fiber layer (pRNFL) and macular thickness are lower in glaucomatous eyes.[13-16] Macular thickness evaluation has received increasing attention after Zeimer and colleagues[17] hypothesized that quantitative detection of glaucomatous damage at the posterior pole by retinal thickness mapping may provide a

method for detecting and monitoring early glaucomatous damage. Reports have suggested that macular thickness assessment could be a valuable surrogate measure for evaluating glaucomatous structural changes, because such damage occurs in retinal ganglion cells (RGCs), which are multilayered and more dense in the macular region.**Error! Bookmark not defined.**,**Error! Bookmark not defined.** However, there have been contrasting reports on the discriminating power and reproducibility of macular parameters when compared to pRNFL parameters in glaucoma.[18-20] Furthermore, although a decrease in RNFL and macular thickness have been reported in glaucomatous eyes, there is a paucity of data on the discriminating capability of these parameters between NTG and POAG. We have previously established normative data on RNFL thickness and also highlighted the relation between RNFL thickness and visual field sensitivity along with the significant potential of intraeye macular thickness asymmetry for the discrimination of NTG and POAG in our earlier studies.[16,21,22] In this study, we aimed to establish the distinction in these parameters between NTG and POAG in order to provide a new dimension to the diagnosis and management of these entities. This could further contribute to early detection, prior to the observance of functional defects on the subjective tests. The aim of this study was to compare the retinal nerve fiber layer and macular parameters as measured by SD-OCT between NTG and POAG. In addition, we investigated the diagnositic performance of RNFL and macular parameters between these variants of open-angle glaucoma.

Material and methods

Participants

Ninety eyes of 90 subjects were included in this prospective cross-sectional study. Thirty participants, matched for age, were included in each of three groups: normal subjects, NTG and POAG. One eye was randomly selected for inclusion in the study when both eyes were eligible. The study was conducted in accordance with the ethical standards stated in the Declaration of Helsinki and was

approved by the Research Ethics Committee of the Department of Ophthalmology, Institute of Medicine, Nepal. Informed written consent was obtained from all the participants before enrollment in the study. Participants were consecutively recruited from the outpatient department (healthy controls) and glaucoma clinic (glaucoma patients) of B. P. Koirala Lions Center for Ophthalmic Studies (BPKLCOS), Institute of Medicine, Tribhuvan University Teaching Hospital.

All participants underwent a comprehensive ophthalmic examination including visual acuity, refraction, intraocular pressure measurement using the Goldmann tonometry, dilated fundus examination with stereoscopic biomicroscopy of optic nerve head using a slit-lamp and binocular indirect ophthalmoscopy, ultrasonic pachymetry (Axis II PR, Quantel Medical), standard white on white automated perimetry (SAP) (Octopus 301 Haag- Streit, Interzeag International- AG, Schlieren, Switzerland) and SD-OCT imaging (SD-OCT Spectralis HRA+OCT, Heidelberg Engineering, Germany). The inclusion criteria were 35 years of age or older; patients diagnosed as normal tension glaucoma (NTG) or primary open angle glaucoma (POAG); open angles; good-quality scans obtained in peripapillary RNFL thickness evaluation by OCT defined as signal-to-noise ratio of greater than 35; reliable SAP performed within 1 month of OCT imaging; and refractive error within 5 dioptre of sphere and 3 dioptre of cylinder. Criteria for exclusion of a patient from the study were best corrected visual acuity on the Snellen chart worse than 20/60, any ocular conditions including corneal and vitro-retinal diseases which could interfere with obtaining reliable visual fields (VFs) or good quality retina scans, or significant parapapillary atrophy that caused blind spot enlargement on the visual field tests and interfered with VF readings. Patients who could not undergo reliable VF test with three attempts and those with any other ophthalmic or neurologic conditions that could result in SAP defects were excluded as well.

Glaucomatous eyes were defined as those with confirmed glaucomatous visual field defect on 2 reliable visual field examinations and by the appearance of a glaucomatous optic disc on slit-lamp biomicroscopy defined as cup-to-disc ratio > 0.7, intereye eye asymmetry in cup to disc ratio > 0.2, or

neuroretinal rim notching, focal thinning, disc hemorrhage, or vertical elongation of the optic cup. Glaucomatous eyes were categorized into 2 subgroups according to the IOP level. The NTG group was defined as those with untreated peak IOP 21mm Hg or lower on 3 measurements taken at different times on separate visits during clinical follow-up. The POAG group included those with a history of IOP exceeding 21mm Hg based on 3 measurements on different days. The control group was comprised of age and sex matched normal subjects selected from patients referred for routine ophthalmic examination and hospital staffs who had no ocular disease, and no previous intraocular or laser surgery other than uncomplicated cataract surgery. The subjects in the healthy control group had a normal anterior segment, open angles, and normal posterior segment findings, as well as a normal optic nerve head appearance in their ophthalmic examinations. IOP measurements were lower than 21 mmHg without any medication and SAP visual field result were normal.

Standard Automated Perimetry

SAP was performed using the normal strategy on OCTOPUS 301 after dark adapting the participant for 3 to 5 minutes prior to the test. All VF tests were reliable, defined as less than 33% fixation loss and less than 20% positive and negative catch trials. Glaucomatous VF defect was defined in accordance with the criteria set by Hodapp, Paris and Anderson[23], as mean deviation greater than +2.0 dB or loss variance greater than 6.0 dB^2 (equivalent to being triggered at the 5% level on the Humphrey Field Analyzer)[24] or both; at least 25% of points depressed below the 5% level and 15% of points depressed below the 1% level and with or without points within central 5° and one or both hemifields with sensitivity 15dB or less.[14] The global indices obtained in the second examination were included in the study to minimize the impact of learning particularly in the group with healthy eyes who were naive to visual field testing. SAP was performed by the same operator for all participants.

Spectral Domain Optical Coherence Tomography

All patients were scanned using the commercially available SD-OCT Spectralis HRA+OCT (Heidelberg Engineering). Details of this device can be obtained elsewhere.[25] Briefly this instrument uses a wavelength of 820 nm in the near infrared spectrum in the scanning laser ophthalmoscopy (SLO) mode. The light source of the SD-OCT is a super luminescent diode with a wavelength of 870 nm. Infrared images and OCT scans (40,000 A-Scans/sec) of the dual laser scanning systems are acquired simultaneously. Sixteen consecutive circular B-scans (3.4-mm diameter, 768 A-scans) centered at the optic disc were automatically averaged to reduce speckle noise (http://www.heidelbergengineering.com, Germany). An online tracking system compensated for eye movements. The Spectralis software version 3.2.1 allows separate measurements of the total retinal thickness and the RNFL thickness. The RNFL borders were clearly identified and marked automatically by the segmentation software. The retinal vessels within the RNFL were considered to be part of the RNFL. To show the distribution of RNFL thickness around the optic disc, thickness data of the circular scans were averaged for 4 sectors (45-degree each). A single user performed at least two retinal scans in all the cases and the scans with best image quality were considered. A posterior pole high speed 12º diameter volume scan was used to obtain the macular thickness and volumes and results were obtained by dividing into 9 areas containing 3 circles each by retinal map analysis system. Each circle was formed by a 1 mm diameter central circle, an inner ring of 3 mm diameter and an outer ring of 6 mm diameter. The thickness and volume of the macula were measured by dividing the inner ring and outer ring into 4 areas: superior, inferior, nasal and temporal quadrants. Presence of uniform signal intensity, strong reflectance signal from the RNFL, and the retinal pigment epithelium resulting in clear demarcation of both layers without the absence of any part of image constituted a good quality scan. The retinal scans were performed within one month of clinical examination.

Statistical methods

All the variables studied followed a normal distribution based on the Wilk-Shapiro test and graphical Q-Q plots. An analysis of variance (ANOVA) was used with Games- Howell (for unequal variances) and Tukey Honestly Significant Difference (for equal variances) corrections were performed to compare quantitative variables among the different groups. The area under the receiver operating characteristic (AROC) curve was calculated to assess the ability of each testing parameters to differentiate the groups. An AROC of 1.0 represents perfect discrimination, whereas an AROC of 0.5 represents chance discrimination. Sensitivity and specificity of each test parameter was determined and sensitivity at at specificity of ≥ 90% is reported for all the parameters. Statistical analyses were done using SPSS v.17 software for Windows and Med Calc, v. 12.3.0 was used to compare the AROC's.

Results

A total of 90 eyes from 90 subjects were included in the study. Table 1 shows the demographic characteristics of the participants. No significant differences were observed for age [$F(2, 87) = 2.420$, $p = .095$], gender, [$\chi 2 (90) = 0.360$, $p = .835$], BCVA, [$F(2, 87) = 1.139$, $p = .325$] and refractive error, $F(2, 87) = 1.855$, $p = .163$]. The visual field indices were significantly different among the three diagnostic groups; this was observed for MD [$F(2, 87) = 49.233$, $p < .001$] and LV [$F(2, 87) = 37.217$, $p < .001$]. Post hoc adjustments for multiple comparisons using the Games-Howell adjustment method revealed a statistically significant difference in MD between normal-POAG, normal-NTG and POAG-NTG comparison groups ($p<.001$).

Table 2 shows that the mean (95% CI) RNFL thickness was highest in the healthy control group, 109.8 µm (106.7-112.9 µm) compared to the NTG group, 85.43 µm (81.78-89.09 µm) and POAG, 64.30 µm (58.90-69.70 µm) [$F(2,87) = 105.5$, $p<0.001$]. RNFL thickness in various quadrants was analyzed to assess the quadrants in which the diagnostic study groups differed significantly. Table 2 shows the summary of mean (95% CI) RNFL thickness in superior, nasal, inferior and temporal quadrants in normal, NTG and POAG. Post-hoc tests using Tukey HSD and Games-Howell adjustments revealed significant

differences in quadratic RNFL thickness across all comparison groups in which all the quandrantic as well as mean RNFL thickness differed significantly (Table 2).

The results of the SD-OCT macular thickness and volumetric parameters are shown in Tables 3 and 4, respectively. There was a significant difference between groups for all macular thickness and volume parameters when analysis of variance was performed. When applying post-hoc adjustment for multiple comparisons using Tukey HSD, all the parameters distinguished various study groups' pairs significantly except center and 1mm thickness for POAG-NTG (p = .975 and p = .820 respectively), 1mm thickness for normal- NTG (p = .346), 1mm volume for POAG-NTG (p = .730), normal-POAG (p = 0.74) and normal-NTG (p = .495), and inferior inner macular volume for normal-NTG (p = .138) (Tables 3 and 4).

To identify the diagnostic ability for NTG and POAG, AROCs were calculated for each of the RNFL thickness (Figure 1), macular thickness and volume parameters and the sensitivities at a high specificity of >90% was determined. Average RNFL was the best parameter based on AROC and sensitivity at a specificity >90% for discriminating normal from NTG and POAG (AROC, 0.979; sensitivity 93% for normal-NTG and perfect discrimination for normal-POAG). Both Inferior RNFL thickness (AROC, 0.917; sensitivity 63%) and average RNFL (AROC, 0.900; sensitivity 60%) thickness were excellent in discriminating POAG and NTG. Among other parameters, superior and inferior RNFL thickness had higher sensitivities and larger AROCs than nasal and temporal RNFL thickness (Table 5). The best macular parameters were inferior inner macular thickness (IIMT) (AROC, 0.937; sensitivity, 83%), inferior outer macular thickness (IOMT) (AROC, 0.987; sensitivity, 92%), inferior inner macular volume (IIMV) (AROC, 0.935; sensitivity, 83%), inferior outer macular volume (IOMV) (AROC, 0.987; sensitivity, 93%) and total volume (AROC, 0.997, sensitivity 97%) for normal and POAG comparison group. Similarly, total volume (AROC,0.898 , sensitivity 67%), IOMT(AROC,0.862 , sensitivity 47%) and IOMV(AROC,0.867 , sensitivity 47%) had better discriminating power for the normal- NTG group. Regarding NTG-POAG comparison group, total volume

(AROC, 0.922; sensitivity 77%) and IOMT (AROC, 0.910; sensitivity 53%) were the parameters with excellent discriminating ability (Tables 6 and 7).

We also assessed whether best RNFL and macular parameters had similar discriminating ability for normal and glaucoma groups as well as NTG-POAG pair. The best parameter each from RNFL thickness, macular thickness and macular volume was considered for comparison. Although average RNFL thickness had perfect discrimination (AROC=1.000; sensitivity=100%) for normal-POAG and near perfect discrimination for normal-NTG (AROC=0.979; sensitivity=93%) as well as NTG-POAG (AROC=0.900; sensitivity=60%), the best macular thickness and volume parameters had similar AROCs and sensitivities between normal and POAG (IOMT, AROC=0.987; sensitivity=92% and total volume, AROC=0.997; sensitivity=97%), normal and NTG (IOMT, AROC=0.862, sensitivity=47% and total volume, AROC=0.898; sensitivity=67%) and also between NTG and POAG (IOMT, AROC=0.910; sensitivity=53% and total volume, AROC=0.922; sensitivity=77%). In normal-POAG, normal-NTG and NTG-POAG comparison groups, there was no statistically significant difference in AROCs between average RNFL and IOMT (p = .178,.128 and .852 respectively) and between average RNFL and total volume (p = .432,.236 and .662 repectively) (Figure 2).

In our study, there was a significant difference in severity between the NTG and POAG groups, with the POAG group having worse mean MD values compared to the NTG group. It is therefore possible that the differences we observed in the structural parameters reflect differences in severity. To address this limitation, we matched the NTG (n=22) and POAG (n=22) groups on MD and re-ran all analyses. The only difference was that no significant difference was found in the nasal sector for RNFL between NTG and POAG. The results of this sub-analysis confirm that our findings are not due to differences in severity between the groups.

Discussion

The study was designed to evaluate the capability of SD-OCT parameters (RNFL and macular parameters) to discriminate between NTG and POAG. We also intended to determine whether macular thickness compared favorably with RNFL thickness in its association with disease by isolating the best parameters and found that both demonstrated comparable performance for the detection of glaucomatous group from normal. Finally, all retinal nerve fiber layer and macular parameters were compared between NTG and POAG to see if any disparities exist. A significant difference was noted in almost all of these protocols between NTG and POAG.

Detectable RNFL loss can precede measurable ONH and VF damage and has been observed in 60% of eyes approximately six years before any detectable VF defects in glaucoma.[26] However other studies have found that functional visual field loss may preceed detectable structural loss of optic nerves.[27] Glaucoma management is largely based on the results of visual field tests. However, with the advent of a variety of new technologies for the objective, non-invasive, measurement of structural changes secondary to RGC damage, this model is shifting towards preference for identifying structural changes, which have been thought to aid in early diagnosis of glaucoma. Over the past decade, SD-OCT has emerged as a more popular diagnostic tool, due to its remarkable advantages in obtaining observer independent diagnosis and follow-up of glaucoma.[28]

According to Zeimer et al's hypothesis,[Error! Bookmark not defined.] quantitative detection of glaucomatous damage at the posterior pole by retinal thickness mapping may provide a unique method for the detection and monitoring of early glaucomatous tissue loss. The ganglion cell layer is more than one cell thick in the macular region and since the photoreceptor layer is not believed to decrease in thickness in glaucoma, the loss of retinal thickness is attributed mainly to the ganglion cell and RNFL. The ganglion cells and NFL contribute 30% to 35% of the retina thickness in the macula, where the ganglion cells are known to be most concentrated.[29] It therefore seems logical to expect that glaucoma detection

would be most readily accomplished through macular thickness assessment, because the RGC soma is 15 µm or more in size, and its axon is only 1 to 2 µm in size.

Several studies have previously reported differences in retinal nerve fiber layer thickness between normal and glaucomatous groups.[30,31] These studies, however, did not include NTG as a diagnostic group. A reduction in macular thickness and retinal nerve fiber layer thickness was noted in both NTG and POAG in separate studies.[32,33 15] Seong and colleagues[20] demonstrated that in early-stage NTG, macular GCC thickness showed a strong correlation with RNFL thickness. There is, however, a lack of literature regarding the comparison of these parameters between NTG and POAG. Therefore, the aim of this study was to examine if there is any difference in macular thickness and pRNFL thickness between NTG and POAG.

In our study, the average pRNFL thickness decreased significantly across the three diagnostic groups: normal, NTG and POAG. Retinal nerve fiber layer thicknesses in all retinal quadrants were lower in NTG and POAG eyes compared to the healthy control eyes with the eyes with POAG having thinnest RNFL. It has been reported that the OCT pRNFL parameters do not differ significantly between high tension glaucoma and NTG in previous studies.[33,34] Our study did not concur with these prior reports. Several reasons could explain the differences in study outcomes. First, both the NTG and POAG study groups in our study were in the early stages of glaucomatous optic neuropathy (MD greater than 2dB). It is possible that difference between the NTG and POAG groups can be measured only in the early stages of the disease. Second, in our study, although there was a difference in severity between NTG and POAG, the results of the sub-analysis obtained after matching these groups on MD establish that our findings cannot be attributed to differences in severity between the groups.Finally, the inherent differences in level of IOP and thus the primary insult mechanism between the groups may dictate the amount of damage of RNFL. Although current study was sufficiently powered to find statistical significance, it has relatively small sample size which may have influenced the study outcome.

We also noted a considerable decrease in the macular thickness and decreased macular volume in NTG and POAG compared to healthy eyes, with POAG having lowest thickness and volume of macula compared to the other groups. There was a significant difference between groups for all the macular thickness parameters. However, when post-hoc comparisons were performed, most parameters were not significantly different between study groups. The exceptions were 1 mm thickness and 1mm volume for all comparison group pairs; center thickness for NTG-POAG pair and inferior inner macular volume for normal- NTG pair. It has been reported that the diagnostic ability of macular structures is limited in case of smaller scans and when the peripheral macula is more severely affected in glaucoma.[35] The fact that we found no significant difference in center and 1 mm parameters can be attributed to this reason. Our findings were consistent with the findings of Nakatani and colleagues[36], who reported considerable difference in macular thickness parameters between the normal and glaucoma group. Guedes and colleagues31 also reported significant differences between normal and advanced glaucomatous groups using the standard macular thickness scan centered at the fovea. The difference with our study is that we have explored the differences between the healthy eyes and normal tension glaucoma, in addition to POAG and found significant differences across these diagnostic groups. Firat and colleagues[37] in their study, found no significant difference in macular thickness between NTG and POAG although they reported differences in pRNFL thickness and GCC thickness. The difference in outcome between studies points to the heterogeneous course of the disease and to the fact that the measurement of more sophisticated parameters (e.g. GCC) may be better suited to identify early damage compared to less refined measurements (e.g. macular thickness). However, further downstream in the disease, all regions are affected.

We calculated AROCs for each of the RNFL thickness (Figure 1), macular thickness and volume parameters and determined the sensitivities at a high specificity of >90% to identify the diagnostic ability for NTG and POAG,. For the normal- NTG comparison group, the best macular thickness and volume

parameters were total volume, IOMT and IOMV. Similarly, IIMT, IOMT, IIMV, IOMV and total volume had better discriminating power for normal and POAG group. These findings concur with the observations that the inferior regions of the optic disc are more susceptible to the glaucomatous damage since the inferior arcuate fibers following the course of nerve fiber topology will eventually converge toward the inferior aspect of the optic disc.[38] Previous studies reported similar findings of greater susceptibility of the inferior macular regions to glaucomatous damage with TD-OCT as well.[39]

The high discriminating power of macular thickness and volume for the normal-NTG and normal-POAG group can be explained by the fact that ganglion cells and RNFL layer constitute 30% to 35% of the macular retinal thickness and glaucomatous damage results in the loss of RGC's and macular nerve fiber layer. The macular parameters measured with SD-OCT may be a good alternative to pRNFL thickness assessment for the quantification of severe glaucomatous damage This may be particularly true in circumstances where pRNFL measurements may be unreliable, especially in eyes with unusually small or large optic discs or in those with peripapillary atrophy or tilted discs.

We explored whether best pRNFL and macular parameters had similar discriminating ability for normal- POAG as well as normal-NTG group pair. One best parameter each from pRNFL thickness, macular thickness and macular volume was considered for comparison. Although average pRNFL had perfect discrimination in POAG and near-perfect discrimination in NTG, the best macular thickness and volume parameters had similar AROC's and sensitivities. Furthermore, there was no statistically significant difference in AROC's between average pRNFL and inferior outer macular thickness (normal-POAG, p = .178; normal-NTG, p=.128 and NTG-POAG, p=.852) and between average pRNFL and total volume (normal-POAG, p = .432; normal-NTG, p=.236 and NTG-POAG, p=.662). This is in agreement with the findings by Nakatani, et al. using SD-OCT `which` showed comparable performance of macular parameters for the diagnosis of early glaucoma to RNFL parameters.**Error! Bookmark not defined.**

However, the study also established the results among early glaucoma group including pre-perimetric glaucoma only.

Guedes and colleagues[31] reported that pRNFL thickness had a better discrimination power (AROC: 0.94) compared with macular thickness (AROC: 0.77) between early glaucoma and normal eyes. In another study by Medeiros and colleagues,[40] it was concluded that the best pRNFL thickness parameter (inferior quadrant, AROC: 0.91) had a significantly larger AROC than the best macular thickness parameter (inferior outer macular thickness, AROC: 0.81). Leung and colleagues[41] showed no significant difference in AROC's for the detection of glaucoma or glaucoma suspects when the macular nerve fiber layer and total macular thickness were compared. However pRNFL thickness had better discriminative performance than total macular thickness and macular nerve fiber layer thickness.[15] In contrast, our study showed a comparable performance of best retinal and macular parameters for the discrimination of NTG and POAG, and further established that inferior outer macular thickness and total volume are the best macular parameters for the evaluation of glaucomatous damage.The different results found in this study may be attributable to the use of different devices, study designs, stage of glaucoma patients as well as to the fact that none of the above mentioned studies looked at NTG to establish the findings.

In conclusion, this study establishes that macular parameters offer comparable performance to pRNFL parameters for the discrimination of NTG and POAG from healthy population, serving further to add to the paucity of data on the same. Further, it also identifies the best structural parameters that could aid in the early distinction of NTG and POAG. This could provide a new model to the management of NTG, outside the current practice of basing the management as a subset of POAG in light of the variance in clinical features of these entities . Results from our study may be somewhat limited in their generalizability as all the patients included in our study were recruited from hospital based glaucoma

practice and may not represent the RNFL and macular characteristics of the population as a whole. Large population based studies with a much larger sample size would aid in our understanding of the strength of this relationship in various cohorts and as a whole. This study would further serve as a basis for the much needed longitudinal studies dealing with the evaluation of average RNFL thickness, total macular volume and inferior outer macular thickness that need to be conducted to determine if a model could be established to further understand the structure and function relationship in glaucoma.

Table 1. Baseline characteristics of the study groups.

	Normal (n = 30)	NTG (n = 30)	POAG (n = 30)	*P*
Age (yrs)	47.00 ± 8.16	50.97 ± 10.03	52.00 ± 9.58	0.10*
Sex (M/F)	0.88:1	0.67:1	0.88:1	0.84¶
BCVA (logMAR)	0.07 ± 0.10	0.05 ± 0.10	0.09 ± 0.12	0.33*
Refractive error (D)	0.26 ± 0.53	0.16 ± 0.73	0.33 ± 0.56	0.16*
MD (dB)	0.38± 0.82	3.10 ± 0.87	5.88 ± 4.07	<0.001‡
LV (dB)	2.68 ± 0.90	8.49 ± 3.59	23.4 ± 16.2	<0.001‡

*One way ANOVA analysis
¶ Chi-square test
‡ One way ANOVA analysis using Games-Howell adjustment for pairwise comparisons
GS indicates glaucoma suspects; NTG, normal tension glaucoma; POAG, primary open angle glaucoma; BCVA, best corrected visual acuity; MD, mean deviation; LV, loss variance

Table 2. Average and quadrantic RNFL thickness in different study groups

Quadrant	Normal (n=30)	NTG (n=30)	POAG (n=30)	P^1	P^2	P^3
*Superior	139.0 (133.0-144.8)	106.3 (101.0-111.7)	81.33 (73.02-89.65)	<.001	<.001	<.001
ǂNasal	82.97 (78.97-86.96)	64.97 (59.16-70.78)	53.17 (46.48-59.85)	<.001	<.001	<.001
*Inferior	141.9 (137.5-146.2)	117.3 (111.0-123.7)	73.73 (64.42-83.05)	<.001	<.001	<.05
ǂTemporal	74.23 (70.44-78.02)	58.67 (55.47-61.86)	48.93 (45.48-52.39)	<.001	<.001	<.001
*Average	109.8 (106.7-112.9)	85.43 (81.78-89.09)	64.30 (58.90-69.70)	<.001	<.001	<.01

Values are expressed in micrometers as Mean (95%CI of the mean).
NTG, normal tension glaucoma; POAG, primary open angle glaucoma; RNFL, retinal nerve fiber layer
* One way ANOVA analysis using Games-Howell adjustment for pairwise comparisons (equal variances not assumed).
ǂ One way ANOVA analysis using Tukey HSD adjustment for pairwise comparisons (equal variances assumed).
P^1, Normal vs POAG; P^2, Normal vs NTG; P^3, POAG vs NTG

Table 3. Mean Macular thickness parameters in all study groups.

Analysis of variance using Tukey HSD adjustment for multiple comparisons.
P^1, p value of normal-POAG comparison group; P^2, p value of normal-NTG comparison group; P^3, p value of NTG-POAG comparison group

	Groups								
	Normal		NTG		POAG				
	Mean	SD	Mean	SD	Mean	SD	P^1	P^2	P^3
Center	228.8	16.98	212.1	11.81	210.6	13.39	<.001	<.001	.975
Nasal outer	319.1	12.37	295.3	11.49	275.9	13.92	<.001	<.001	<.001
Superior outer	300.2	11.30	280.0	12.22	266.1	14.90	<.001	<.001	<.001
Temporal outer	280.8	9.54	268.7	10.68	252.7	11.72	<.001	<.001	<.001
Inferior outer	286.4	10.22	270.2	9.86	247.1	12.90	<.001	<.001	<.001
Nasal inner	334.8	12.18	322.3	16.55	301.7	18.53	<.001	<.05	<.001
Superior inner	334.1	15.23	320.6	11.00	297.5	20.52	<.001	<.01	<.001
Temporal inner	319.0	14.27	307.9	10.74	283.9	13.10	<.001	<.01	<.001
Inferior inner	329.7	18.76	316.8	12.18	287.2	16.29	<.001	<.01	<.001
1 mm thickness	254.8	16.89	247.3	15.21	243.4	20.92	.06	.346	.820

Table 4. Mean macular volume parameters in all study groups

	Groups								
	Normal		NTG		POAG				
	Mean	SD	Mean	SD	Mean	SD	P^1	P^2	P^3
1 mm volume	.200	.014	.195	.013	.191	.016	.074	.495	.730
Inferior inner	.518	.031	.497	.019	.440	.060	<.001	.138	<.001
Temporal inner	.502	.024	.484	.018	.446	.020	<.001	<.05	<.001
Superior inner	.524	.024	.505	.018	.472	.028	<.001	<.05	<.001
Nasal inner	.525	.020	.508	.022	.475	.030	<.001	<.05	<.001
Inferior outer	1.518	.053	1.432	.051	1.310	.069	<.001	<.001	<.001
Temporal outer	1.489	.050	1.426	.057	1.339	.062	<.001	<.001	<.001
Superior outer	1.591	.060	1.478	.070	1.409	.078	<.001	<.001	<.001
Nasal outer	1.690	.067	1.561	.060	1.464	.074	<.001	<.001	<.001
Total volume	8.572	.233	8.103	.275	7.566	.283	<.001	<.001	<.001

Analysis of variance using Tukey HSD adjustment for multiple comparisons.
P^1, p value for normal-POAG comparison group; P^2, p value for normal-NTG comparison group; P^3, p value for NTG-POAG comparison group

Table 5. AROC and specificity at >90% sensitivity for RNFL parameters for comparing normal and POAG; normal and NTG; NTG and POAG

RNFL parameters	Comparison groups								
	Normal - POAG			Normal – NTG			NTG-POAG		
	AROC	P^1	Sn/Sp	AROC	P^2	Sn/Sp	AROC	P^3	Sn/Sp
Average	1.000	<.001	100/100	0.979	<.001	93/91	0.900	<.001	60/90
Superior	0.989	<.001	100/91	0.940	<.001	72/91	0.811	<.001	40/90
Inferior	0.999	<.001	100/91	0.876	<.001	65/93	0.917	<.001	63/90
Nasal	0.918	<.001	73/91	0.831	<.001	67/91	0.688	<.01	20/90
Temporal	0.987	<.001	95/91	0.876	<.001	58/91	0.761	<.001	47/90

P^1, p value for normal-POAG comparison group; P^2, p value for normal-NTG comparison group; P^3, p value for NTG-POAG comparison group

Table 6. AROC and specificity at >90% sensitivity for macular thickness parameters for comparing normal and POAG; normal and NTG; NTG and POAG

Macular thickness parameters	Comparison groups								
	Normal - POAG			Normal - NTG			NTG-POAG		
	AROC	P^1	Sn/Sp	AROC	P^2	Sn/Sp	AROC	P^3	Sn/Sp
Center thickness	0.788	<.001	15/92	0.788	<.001	13/91	0.512	.878	17/90
1 mm thickness	0.652	<.05	32/91	0.650	.05	22/91	0.563	.420	7/90
SIMT	0.922	<.001	77/91	0.804	<.001	18/91	0.844	<.001	20/90
IIMT	0.937	<.001	83/91	0.779	<.001	9/91	0.916	<.001	40/90
NIMT	0.929	<.001	81/91	0.752	<.001	27/91	0.819	<.001	40/90
TIMT	0.959	<.001	80/91	0.757	<.001	5/92	0.913	<.001	37/90
SOMT	0.972	<.001	80/91	0.798	<.001	37/92	0.752	<.001	33/90
IOMT	0.987	<.001	92/92	0.862	<.001	47/91	0.910	<.001	53/90
NOMT	0.929	<.001	79/91	0.918	<.001	23/91	0841	<.001	40/90
TOMT	0.924	<.001	81/91	0.800	<.001	32/91	0.844	<.001	47/90

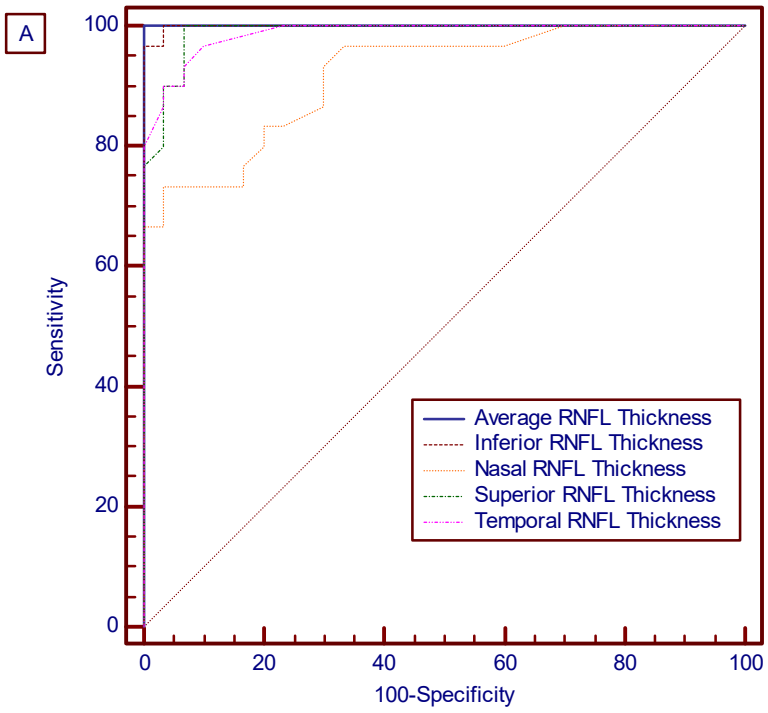
SIMT, superior inner macular thickness; IIMT, inferior inner macular thickness; NIMT, nasal inner macular thickness; TIMT, temporal inner macular thickness; SOMT, superior outer macular thickness; IOMT, inferior outer macular thickness; NOMT, nasal outer macular thickness; TOMT, temporal outer macular thickness.

P^1, p value for normal-POAG comparison group; P^2, p value for normal-NTG comparison group; P^3, p value for NTG-POAG comparison group

Table 7. AROC and specificity at >90% sensitivity for macular volume parameters for comparing normal and POAG; normal and NTG; NTG and POAG

Macular volume parameters	Comparison groups								
	Normal - POAG			Normal - NTG			NTG-POAG		
	AROC	P^3	Sn/Sp	AROC	P^2	Sn/Sp	AROC	P^3	Sn/Sp
1 mm volume	0.654	<.05	31/91	0.621	.087	15/91	0.571	.341	8/90
SIMV	0.920	<.001	73/91	0.791	<.001	18/91	0.833	<.001	31/90
IIMV	0.935	<.001	83/91	0.772	<.001	10/91	0.921	<.001	48/90
NIMV	0.913	<.001	75/91	0.736	<.001	33/93	0.816	<.001	37/90
TIMV	0.914	<.001	80/91	0.754	<.001	4/91	0.917	<.001	49/90
SOMV	0.903	<.001	81/92	0.800	<.001	32/91	0.732	<.001	32/90
IOMV	0.987	<.001	93/91	0.867	<.001	47/91	0.908	<.001	53/90
NOMV	0.914	<.001	81/92	0.819	<.001	39/91	0.832	<.001	41/90
TOMV	0.921	<.001	80/91	0.796	<.001	50/91	0.839	<.001	50/90
Total Volume	0.997	<.001	97/91	0.898	<.001	67/91	0.922	<.001	77/90

SIMV, superior inner macular volume; IIMV, inferior inner macular volume; NIMV, nasal inner macular volume; TIMT, temporal inner macular volume; SOMT, superior outer macular volume; IOMV, inferior outer macular volume; NOMT, nasal outer macular volume; TOMT, temporal outer macular volume.
P^1, p value for normal-POAG comparison group; P^2, p value for normal-NTG comparison group; P^3, p value for NTG-POAG comparison group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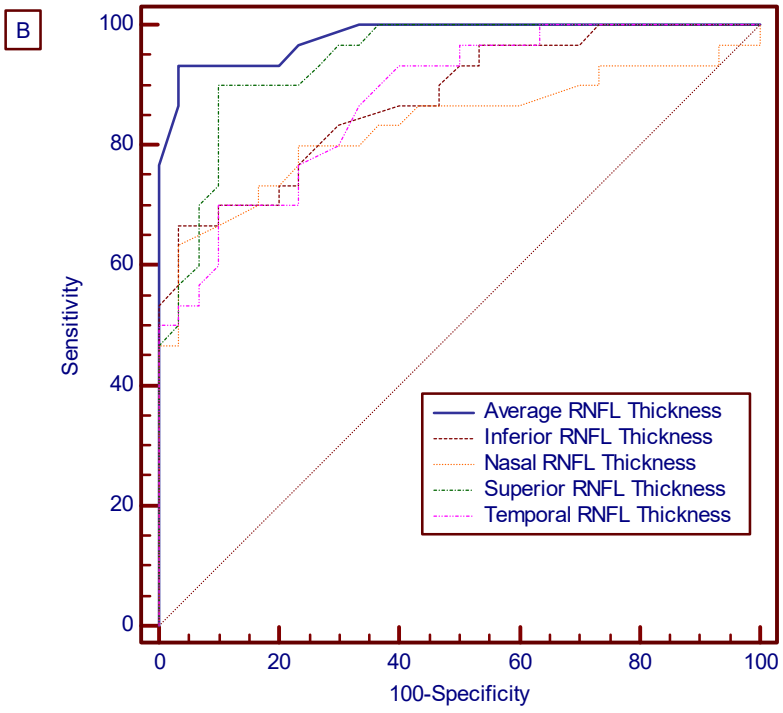
Nasal RNFL Thickness
Superior RNFL Thickness
Temporal RNFL Thickness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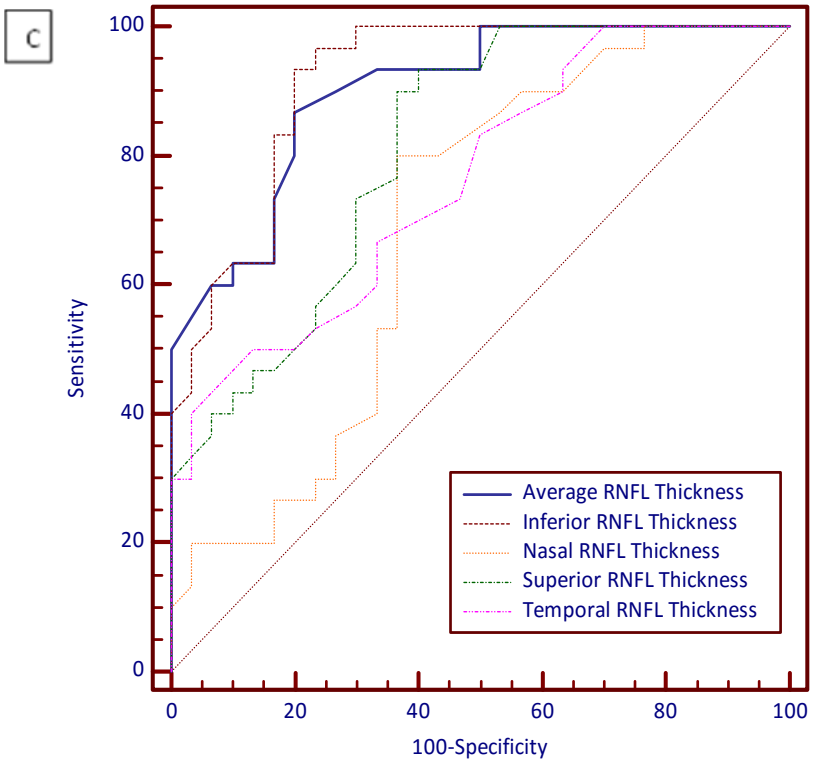
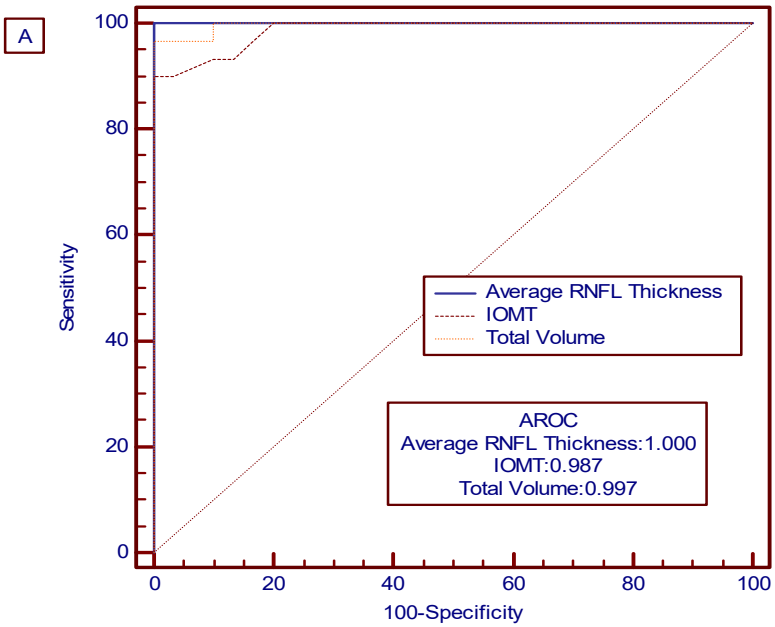
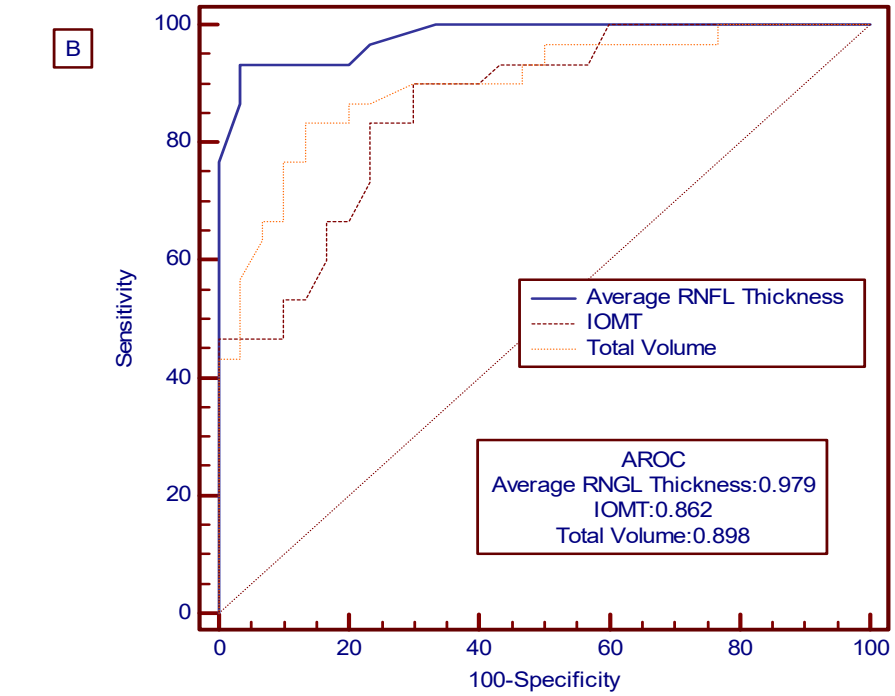


Figure 1. ROC curves for Average RNFL thickness for A) Normal-POAG; B) Normal-NTG; C) NTG-POAG





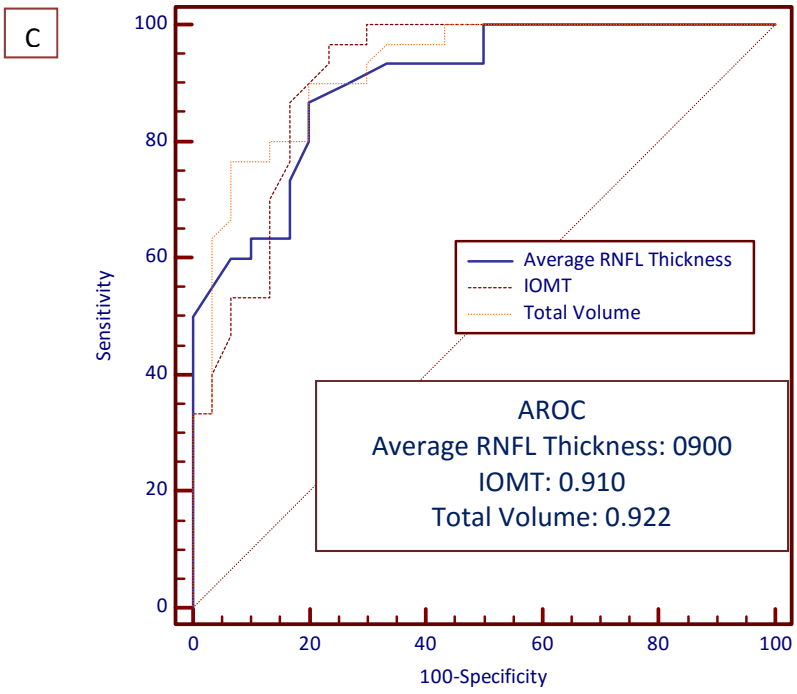


Figure 2. AROC's for average RNFL thickness, inferior outer macular thickness (IOMT) and total volume between A) Normal – POAG; B) Normal – NTG; C) NTG-POAG

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