



**St. Paul's Hospital
Millennium Medical College**

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**DIAGNOSTIC PERFORMANCE OF OPTICAL COHERENCE TOMOGRAPHY
MACULAR GANGLION CELL-INNER PLEXIFORM LAYER & RETINAL NERVE
FIBER LAYER THICKNESS IN GLAUCOMA SUSPECT AND EARLY GLAUCOMA**

**RESEARCH PROPOSAL SUBMITTED TO DEPARTMENT OF OPHTHALMOLOGY IN
PARTIAL FULLFILLMENT FOR SPECIALITY CERTIFICATE IN
OPHTHALMOLOGY**

ADDIS ABABA; ETHIOPIA

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ACRONYMS

AUROC – Area Under Receiver Operating Curve

CDR – cup to disc ratio

GCC – Ganglion Cell Complex

GCIPL – Ganglion Cell Inner Plexiform Layer

HVF – Humphrey Visual Field Analyzer

IOP – Intraocular Pressure

MD- Mean Deviation

OCT – Optical Coherence Tomography

ONH – Optic Nerve Head

POAG – Primary Open Angle Glaucoma

PSD – Pattern Standard Deviation

RNFL – Retinal Nerve Fiber Layer

SPHMMC – Saint Paul's Hospital Millennium Medical Collage

VFT – Visual Field Test

WHO – World Health Organization

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1. ABSTRACT

PURPOSE: To evaluate the glaucoma diagnostic performance of ganglion cell inner plexiform layer (GCIPL) parameters and retinal nerve fiber layer (RNFL) parameters measured with cirrus HD- Optical Coherence Tomography(OCT).

METHOD: A total of 188 eyes were included in this study. 49 eyes of healthy controls, 70 glaucoma suspect eyes and 69 early glaucomatous eyes. Complete ophthalmic examination was done including visual field test with Humphrey field analyzer and optical coherence tomography scanning of GCIPL and RNFL in different quadrants. The area under the receiver operating characteristic curve (AUROC), sensitivity and specificity of each parameter was calculated to provide diagnostic ability between normal controls, glaucoma suspects or early glaucoma.

RESULT: GCIPL and RNFL parameters had strong power in discriminating early glaucoma from healthy controls with all having area under receiver operating curve (AUROC) of above 0.76. Of all the GCIPL and RNFL parameters, the only variable that could discriminate between glaucoma suspect and healthy controls was the combined parameter by OR-logic approach . Of all the parameters, the average and nasal RNFL parameters had the strongest power in discriminating between the two with AUROC of 0.81. All parameters had an overall good diagnostic performance with excellent sensitivity but the specificity was relatively poor. The combined parameter had the best specificity (62.2%) with excellent sensitivity (93.5%).

CONCLUSION: The Or-logic combination of GCIPL and RNFL provides better diagnostic performance than single GCIPL, RNFL or ONH parameter.

DISSEMINATION OF RESULT- The research findings will be submitted to a reputable Journal for publication at the end.

1. INTRODUCTION

2.1 BACKGROUND

Glaucoma represents a group of diseases defined by a characteristic optic neuropathy that is consistent with excavation and undermining of the neural and connective tissue elements of the optic disc and by the eventual development of distinctive patterns of visual dysfunction (1).

Using data collected predominantly in 2002, World health organization (WHO) estimated blindness prevalence for all types of glaucoma was estimated at more than 37 million people, placing glaucoma as the second leading cause of blindness worldwide, following cataract, which previously was estimated to be third cause of blindness. However glaucoma presents an even greater public health challenge than cataract because the blindness it causes is irreversible. More than 82% of all blind people are 50 years and older which shows as the population grows older, the prevalence rises. (2).

Among white aged 40 years and older a prevalence of between 1.1% & 2.1% has been reported based on population based studies performed throughout the world. The prevalence among Black and Latino persons is up to 4 times higher to the prevalence among whites. Black individuals are also at greater risk with increasing age: in persons 46-65 years, the likelihood of blindness from POAG is 15 times higher among blacks than among whites (3).

Glaucomatous damage to the retinal nerve fiber layer can precede future visual field damage by up to 5 yrs. Compared with standard automated perimetry which is a functional and subjective test with greater interest variability, optical coherence tomography provides a highly qualitative, objective and reproducible structural assessment of the optic nerve, retinal nerve fiber layer and macular thickness (4).

2.2 STATEMENT OF THE PROBLEM

Glaucoma preferentially affects the innermost retina layers (nerve fiber layer, ganglion cell and inner plexiform layer) which we call ganglion cell complex. It causes an irreversible gradual loss of ganglion cell axons. Given that approximately 50% of retinal ganglion cells are located within 4.5mm of foveal center and we cannot detect patients at least 40% of retinal ganglion cell loss occur, early detection of macular ganglion cell complex loss could be used for early glaucoma diagnosis or following disease progression.(5) .

The staggering number of patients with glaucoma may be even an underestimate, since visual field loss is required in the definition of glaucoma & many individuals have glaucoma without documented visual field loss. So, early detection is therefore essential for applying pressure-reducing treatment to stop or delay progressive loss of visual function (6).

Diagnostic error accounts more when compared to medication error or failure to monitor patients. Detection of early loss of retinal nerve fiber either clinically or by means of red-free photos is also subjective and may be inconclusive. There is a risk for being inaccurate in clinical detection of suspicious optic nerve head. Looking for proof of disease Progression to confirm glaucoma suspect as having pre perimetric glaucoma is risky (7).

2.3 LITERATURE REVIEW

Macular thickness loss is correlated with estimated retinal ganglion cell count and Humphrey visual field parameters in both glaucomatous and normal eyes. The traditional 24-2 and 30-2 visual field have relatively few spots which represents the macular region so field deficit in this area, if we compare it with peripheral retina, requires a greater number of ganglion cell loss. Small losses of macular thickness are thus not detectable on visual field testing (8).

A study on 76 eyes in patients with glaucoma and normal volunteers with three dimensional (3-D) spectral domain (SD) OCT, 512x128 axial scans acquired in Japanese individuals during the period May2007-August 2007 in Kyoto, Japan found fourteen more defects in retinal nerve fiber layer on OCT than on color fundus photograph (9).

A prospective cross sectional study was done in United States of America at three different states (Florida, Texas and California) to evaluate the diagnostic performance of GCIPL, RNFL and ONH parameters by Cirrus HD-OCT on 50 patients with 49 age matched healthy subjects. They found, among GCIPL parameters, the minimum had the best diagnostic performance (sensitivity of 82.0%, specificity of 87.8%) and inferior quadrant was the best RNFL parameter (sensitivity of 74% & specificity of 95.9%) as was rim area (sensitivity of 68% & specificity of 98%) among ONH parameters. The or-logic combination of minimum GCIPL and average RNFL provided the overall best diagnostic performance (sensitivity of 94% & specificity of 85.7%) as compared with the best RNFL, best ONH, and best and-logic combination (minimum GCIPL and inferior quadrant RNFL; sensitivity of 64% & specificity of 100%) (10).

A study was done in Turkey to determine the discriminating performance of the macular GCIPL parameters and to compare it with RNFL and ONH parameters on 40 healthy eyes, 40 glaucoma suspects and 40 early glaucoma eyes. Cirrus HD-OCT was obtained and diagnostic performances were evaluated by determining the AUROC. All GCIPL parameters discriminated glaucoma suspects from healthy eyes and from early glaucoma patients ($P<0.017$). Also, minimum, inferotemporal and inferonasal GC-IPL parameters discriminated early glaucoma patients from glaucoma suspects, whereas no RNFL or ONH parameter could discriminate between the two. The best parameters to discriminate glaucoma suspects from subjects with healthy eyes were superonasal GCIPL, superior RNFL and average CDR (AUC=0.746, 0.810

and 0.746, respectively). Discriminating performances of all the parameters for early glaucoma and glaucoma suspect comparison were lower with the best parameters being minimum and inferotemporal (AUC=0.669 and 0.662, respectively) (11).

A study done in University of Tulino, Italy on a total study population of 178 eyes in a prospective non randomized observational cohort study between Oct 2009 and Sept 2010 shows papillary and macular parameters were significantly lower in pre perimetric glaucomatous eyes. There was high loss of global and focal loss volume. In terms of diagnosis accuracy, the best GC IPL parameters were GC IPL inferior (0.77, 95% CI 0.70–0.84) and GC IPL OR (0.79, 95% CI 0.71–0.85) (12).

From 137 eyes of 125 patients with glaucoma from ongoing prospective study (the Advanced glaucoma progression study) at University of California Los Angeles in June 2016, found that the highest correlation was observed between inferotemporal GC IPL thickness and corresponding visual field sensitivity in early glaucoma. (13)

Population based study recruited 623 Chinese adults aged 40-80 in August 2012 to determine the distribution, variation and determinants of ganglion cell-inner plexiform layer thickness in non-glaucomatous eyes measured by HD-OCT. The study includes 50.1% male and 49.9% female subjects who had normal visual field with no signs of glaucoma or glaucoma suspect. The GCC thickness was significantly associated with age ($p<0.001$), female sex ($p<0.001$), axial length ($p=0.002$), and mean peri papillary retinal nerve fiber layer thickness ($p<0.001$). The mean overall, superior and inferior GCC thickness were 82.78 ± 7.01 micrometer, 83.30 ± 7.89 micrometer, and 80.16 ± 8.31 micrometer respectively. The study also states that IOP, Central corneal thickness (CCT), disc area, serum glucose level and history of diabetes mellitus had no significant influence on GCC thickness (14).

Lu et al stated that the best stand-alone diagnostic strategy for OCT RNFL data is to classify an eye as glaucomatous if the combination of the overall, inferior quadrant and superior quadrant RNFL thickness average is below normal (15).

Another observational cross sectional study on advanced imaging for glaucoma study between the period 2003 and 2007 included 178 patients which divide them in three groups. 65 normal subjects, 78 patients in perimetric glaucoma group and 52 in preperimetric glaucoma group. FD-

OCT was used to map the macula 7mm by 6mm region and diagnostic power was assessed by area under the receiver operator (AUROC) curve. The FD-OCT GCC average had significantly ($p=0.02$) higher diagnostic power ($AUROC=0.9$) in differentiating between perimetric glaucoma and normal. This study also shows the global loss volume had significantly higher AUROC (0.92) than the overall average ($p=0.01$) when compared to other parameters (16).

A study was done in Nigeria which enrolled 47 patients between January and December 2009 to evaluate GCC in primary open angle glaucoma. It was a comparative analysis on Ganglion Cell Complex parameters in Nigerian Negroes with Glaucoma and Macular disease. All patients underwent GCC analysis with OCT and the mean FLV value was 9.34 ± 7.98 among eyes with glaucoma. Mean GLV value was 18.07 ± 12.86 and the mean average GCC value was 76.55 ± 12.57 . A significant higher percentage of eyes with macular disease had GLV value within normal range when compared with eyes with glaucoma (17).

A retrospective study was done to assess diagnostic consistency and relation between SD-OCT and standard automated perimetry (SAP) in patients with primary open-angle glaucoma on 51 eyes. SD-OCT parameters (RNFL; average, superior, inferior, nasal and temporal], RNFL symmetry, rim area, disc area, average and vertical CDR and cup volume) were compared with SAP parameters and they found 29 eyes (56.9%) had consistent RNFL and VF damage. However, 9 pts (17.6%) showed isolated RNFL damage on SD-OCT and 13 pts (25.5%) had abnormal VF test with normal RNFL. In addition to these parameters, worsening in average, superior, inferior, and temporal RNFL thicknesses and RNFL symmetry was significantly associated with consistent SD-OCT and SAP outcomes (18).

2. JUSTIFICATION OF THE STUDY

Early diagnosis of glaucoma is a challenge because visual field change which is considered as a gold standard for diagnosis do not show changes until significant neuronal loss occur. The problem is confounded by the fact that the patient is also not aware of the problem. Most of our patients wait until they see or feel a difference in their vision which increase the chance that irreversible damage may already be there. As structural damage precede detectable visual field loss, quantitative analysis of optic nerve head and retinal nerve fiber layer along with macular ganglion cells is really important for early assessment. So this study will have great contribution for early detection of the problem and implementing further programs. In addition to this, there is no a single study done in our country regarding this area and we don't know the normal range of OCT parameters specifically to our patients. It will have a role to prevent the detrimental impact of blindness due to glaucoma that place a greater socio-economic burden on the society currently.

3. OBJECTIVES

4.1 General objective

- To assess diagnostic performance of Optical Coherence Tomography Macular Ganglion Cell-Inner Plexiform Layer Thickness measurements in glaucoma

4.2 Specific Objective

- To evaluate the diagnostic value of GCIPL thickness for glaucoma suspects and early glaucoma patients
- To evaluate the diagnostic value of RNFL thickness in glaucoma suspects and early glaucoma patients
- To assess correlation of macular GCIPL & RNFL thickness in healthy subjects, glaucoma suspects & early glaucoma patients

5. METHODS AND MATERIAL

5.1 Study design:

a prospective clinic based comparative study was conducted to assess the macular ganglion cell inner plexiform layer (GCIPL) thickness & retinal nerve fiber layer (RNFL) analysis among healthy subjects, glaucoma suspects and early glaucoma patients.

5.2 Study area and period:

The data was collected from January 2018-may 2019. St Paul's hospital millennium medical college (SPHMMC) was built in 1969 by Emperor Haile Selassie. It has 392 beds, with annual average of 200,000 patients and a catchment population of more than 5 million. There are 1300 clinical and non-clinical staff in over 13 department. The medical school is opened in 2017 with a practical modular and integrated curriculum. The students are selected by written exam and structured interview. The post graduate programs are distinctive in that they are competency based. The school has strong ties to local and international institutions like Addis Ababa University, Jimma University, Tulane University, University of Michigan as well as open University (19).

5.3 Source population:

Patients who present for eye care at SPHMMC

5.4 Study population:

All healthy controls, glaucoma suspects, patients with early glaucoma change and fulfilling the inclusion criteria

5.5 Sample size determination:

Patients per group = $f(\alpha, \beta) \times 2 \times SD^2$

— (20)

$(d)^2$

Where $f(\alpha, \beta) = 7.85$ for 80% (1- β) with 5% or 0.05 significance (α)

SD - Standard Deviation of the outcome of interest

d - The smallest difference in means that it would be clinically meaningful to detect

- SD taken from a similar study..... (21)

-d was taken as 5

The sample size needed for each group were 70

5.6 Sampling Technique

All consecutive patients from the three groups of study population fulfilling the inclusion criteria were included till the sample size is achieved.

5.7 Inclusion and Exclusion criterias

5.71 Inclusion Criteria

- Age ≥ 18 years
- BCVA $\geq 6/18$ with spherical equivalent between -5.00D & +5.00D and a cylinder correction with ± 2.00 D
- Open anterior chamber angle

For healthy subjects

- IOP ≤ 21 mmhg
- A normal Humphrey SITA 24-2 standard reliable visual field test

For glaucoma suspects

- Optic nerve head changes (listed in the scientific definition) or
- RNFL defect with normal Humphrey SITA 24-2 standard reliable Visual field test

For Early glaucoma patients

- Optic nerve head changes (listed in the scientific definition) or
- RNFL defects correlating with Humphrey SITA 24-2 Visual field changes

5.72 Exclusion Criteria (for all groups)

- media opacity such as corneal opacity, cataract which compromises image quality
- retinopathy that could cause VF loss or optic disc abnormalities
- macular pathology such as Age related macular degeneration
- history of chronic ocular or systemic steroid use or cerebro vascular event that could affect the VFT
- High Refractive error (± 5)
- OCT image quality < 6
- any history of intraocular surgery
- angle closure
- Signs suggesting non glaucomatous optic neuropathy such as remaining neuro retinal rim pallor, visual field defect respecting the vertical midline.
- Secondary glaucoma such as Uveitic glaucoma, previous trauma.

5.8 Variables of the study

- Independent Variable -Age

-Sex

-MD

- PSD

- Address

- Dependent Variables

- Average and sectoral GCIPL thickness

- Retinal NFL thickness

5.9Scientific Definition

I. Glaucoma suspects

- patients who have optic nerve or nerve fiber layer defect suggestive of glaucoma like asymmetric cup-disc ratio >0.2 unexplained by the ONH size difference,

enlarged cup-disc ratio >0.6 , notching or narrowing of neuro retinal rim, disc hemorrhage or suspicious alteration in the nerve fiber layer in the absence of an abnormality in visual function determined by perimetry with normal glaucoma hemi field test and normal MD & PSD.

II. Early Primary open angle glaucoma (the above changes plus vertical cup-disc ratio <0.75 clinically

AND – visual field loss with a mean deviation (MD) ≥ -6 db and at least one of the following

- A. On pattern deviation plot, there exists a cluster of 3 or more points in an expected location of the visual field on the Pattern deviation plot depressed below the 5% level, at least 1 of which is depressed below the 1% level on the same side of the horizontal meridian
- B. Pattern standard deviation significant at $p < 0.05$
- C. GHT(glaucoma hemi field test) “outside normal limit”

III. Normal Subjects

- Are those with no history of evidence of intraocular surgery, have no first degree relative with glaucoma, no retinal pathologic feature, VA of 6/18 or better with R/E between $+5.00$ and -5.00 D, IOP of ≤ 21 mmhg, normal appearing ONH without any neuroretinal rim change (peri papillary hemorrhages, notches, localized pallor, or RNFL defect), an open anterior chamber angle; and no history of chronic ocular or systemic corticosteroid use., reliable normal VF test result with normal glaucoma hemi field test and normal MD & PSD.

5.10 Data collecting procedure and instruments

- All patients had undergone a complete ophthalmic examination: past and recent ocular & medical history, VA test with Snellen E chart, non contact tonometer , gonioscopy, slit lamp exam with 90D volk, VFT and OCT
- This was done by ophthalmology resident and ophthalmologist

Visual field examination (Carl Zeiss MeditecInc)

- It was done by one optometrist using Humphrey SITA 24-2 standard Visual field analyzer and was considered reliable when FL<20%, FP<20%, FN<33%. The mean deviation and pattern standard deviation was calculated by the software installed. To minimize learning effect, only the most recent VF test was used in patients having more than one result.

OCT measurements (Carl Zeiss Meditec's proprietary software)

- It was done by an optometrist
 - macular GCIPL and peri papillary RNFL of each participant was taken after pupil dilated with 1% tropicamide.
 - A patient was considered glaucomatous if scans had the average or at least one sector measurement flagged at 1% to 5% or less than 1%.
- GCIPL scan– Cirrus HD-OCT was used to map the macula over a 7mm×6 mm region by using macular cube 512x128 scan mode
- Average, minimum, superior, inferior, superonasal, superotemporal, inferonasal and inferotemporal GCIPL thickness was taken into account.
- RNFL - The peri papillary RNFL thickness was measured in the optic disc cube 200x200 scan mode, which scan a 6x6mm square centered on the ONH. Average RNFL thickness and each quadrant thickness (Superior, Inferior, Nasal, and Temporal) was measured.
- For all parameters, the instrument provides classification in a color - coded manner. All comparison was adjusted with age.
- 🚦 If value falls in green color, probability value is within 5%-95% and classified as “within normal limit”
 - 🚦 If value falls in yellow color, probability value is < 5% but > 1% and classified “borderline”
 - 🚦 If value falls in red color, probability value is <1% and classified “outside normal limit”

5.11 Data quality control

All data relevant to the study was collected using pre-prepared and printed data collection forms. The data was collected by optometrists and ophthalmology residents, then it was checked for completeness and correctness. Identified issues were cleared. Then glaucoma specialist has checked on the filled data sheet for completeness.

5.12 Data processing and analysis

Statistical analyses were performed with SPSS version 23.0. Proportions, summary statistics like mean and standard deviation was calculated for the variables. The Or-logic approach requires at least one of the parameters (sectors in GCIPL or RNFL) in consideration to be abnormal to be considered abnormal and suggestive of glaucoma. Main outcome measures, sensitivities and specificities were compared with the chi-square test. Comparison of variables between groups was determined using the independent sample t test. Receiver operating characteristic (ROC) curve displayed the relationship between sensitivity and 1-specificity for the result of a given diagnostic test. Area under the receiver operating characteristic curve (AUROC) was used to assess the discriminating ability between the groups. An AUROC of 1.0 represents perfect discrimination, whereas an AUROC of 0.5 represents chance discrimination. P value of less than 0.05 was used to indicate a statistically significant difference.

5.13 Ethical consideration

Before conducting the study, ethical clearance was obtained from the Ethical Review committee of SPHMMC. Informed consent of each participant was taken after explaining the purpose of the study. Patients' personally identifying information was not extracted to ensure confidentiality of patient information. The right of the respondents not to participate in the study was respected.

6. RESULT

A total of 188 eyes were involved in this study including early glaucoma 36.7%, Glaucoma Suspect 37.2%, and Healthy control 26.1%. The mean age of participants for early glaucoma patients was 58 years (SD±10), for glaucoma suspects 49 years (SD±12) and for healthy controls 44 years (SD±12). Male proportion was 52.1% & female 47.9%. Hypertension was present among 22.3% & diabetes among 18.1%. Family history of glaucoma was present in only 6 pts (3.2%), and no participant was taking steroid. (Table 1)

TABLE 1. Demographic characteristics of participants

parameters	Early Glaucoma	Glaucoma Suspect	Healthy Control
Participant no.	69(36.7%)	70(37.2%)	49(26.1%)
Age (mean±SD)	58±10	49±12	44±12
Sex (Male/Female)	44/25	31/39	23/26
IOP(mmHg)	20.6±5.6	19.1±5.3	16±3.9
MD(dB)	-5.9±3.5	-3.8±1.7	-3.4±1.8
PSD(dB)	3.2±2.5	2.1±0.9	1.8±0.7
VCDR	0.61±0.1	0.64±7.8	0.59±0.1
Rim area	1.21±0.2	1.21±0.2	1.29±0.2

On comparison of GCIPL parameters among glaucoma suspects and healthy controls, all GCIPL quadrant thicknesses were significantly different except superior and temporal quadrant. In contrast to the observed large differences in GCIPL parameters among early glaucoma and controls, the differences were less marked among glaucoma suspects and controls. All RNFL parameters were different significantly in these groups. Compared to GCIPL parameters, the

observed differences were larger in RNFL parameters (22.3) among suspects and controls. Of the RNFL parameters, the largest difference was observed on the inferior quadrant.

TABLE 2 Mean $\pm$ SD of GCIPL and RNFL parameters

PARAMETER	MEAN $\pm$ SD		
	EG	HC	GS
GCIPL Average	74.2 $\pm$ 12.4	83.4 $\pm$ 5.7	79.8 $\pm$ 10.9
GCIPL Minimum	67.2 $\pm$ 16.2	79.9 $\pm$ 6.6	74.9 $\pm$ 14.5
GCIPL Superior	74.9 $\pm$ 14.7	84 $\pm$ 6.3	80.9 $\pm$ 11.1
GCIPL Inferior	72.7 $\pm$ 13.7	81.7 $\pm$ 6.7	77.5 $\pm$ 11.5
GCIPL Superonasal	76.5 $\pm$ 14.4	85.2 $\pm$ 6.8	81.5 $\pm$ 11.2
GCIPL Inferonasal	74.7 $\pm$ 13.5	84.1 $\pm$ 7.0	80.3 $\pm$ 12
GCIPL Superotemporal	73.6 $\pm$ 13.2	82.8 $\pm$ 5.3	79.5 $\pm$ 10.8
GCIPL Inferotemporal	73.2 $\pm$ 12.9	82.5 $\pm$ 7.1	79.1 $\pm$ 12.1
RNFL Average	84.0 $\pm$ 7.35	97.8 $\pm$ 9.3	92.3 $\pm$ 9.9
RNFL Superior	101.8 $\pm$ 8.41	121.6 $\pm$ 15.5	114.9 $\pm$ 17.2
RNFL Inferior	105.7 $\pm$ 536	128.0 $\pm$ 16.7	118.3 $\pm$ 16.8
RNFL Nasal	70.1 $\pm$ 8.84	78.2 $\pm$ 12.0	73.8 $\pm$ 10.5
RNFL temporal	57.8 $\pm$ 5.51	63.3 $\pm$ 8.3	61.3 $\pm$ 8.8

TABLE 3 The association of mean difference of GCIPL and RNFL parameters

PARAMETER	MEAN DIFFERENCE			
	EG Vs HC	P1	GS Vs HC	P2
GCIPL Average	-9.2	.000	-3.6	.036
GCIPL Minimum	-12.7	.000	-5.07	.024
GCIPL Superior	-9.1	.000	-3.1	.086
GCIPL Inferior	-9.0	.000	-4.2	.024
GCIPL Superonasal	-8.7	.000	-3.7	.043
GCIPL Inferonasal	-9.2	.000	-3.8	.047
GCIPL Superotemporal	-9.2	.000	-3.2	.054
GCIPL Inferotemporal	-9.2	.000	-3.4	.079
RNFL Average	-13.7	.000	-5.5	.003
RNFL Superior	-19.7	.000	-6.7	.033
RNFL Inferior	-22.3	.000	-9.7	.002
RNFL Nasal	-8.0	.004	-4.4	.036
RNFL temporal	-5.5	.005	-2.0	.212

The overall diagnostic accuracy of combined and individual GCIPL and RNFL parameters were tested by receiver operating curve (ROC). For detection of early glaucoma from healthy controls, the combined or logic and all of the GCIPL parameters had good diagnostic performance with all having area under receiver operating curve (AUROC) above 0.7. Among the RNFL parameters, superior and temporal thickness had poor performance (0.682, 0.647 respectively) while, the average, inferior and nasal thickness had good diagnostic performance (0.647, 0.813, 0.726 respectively). Overall, RNFL inferior and nasal thickness were the most accurate parameters in detecting early glaucoma from healthy control with AUROC of 0.81. The combined GCIPL and RNFL parameters and the GCIPL parameters individually had lower

performance compared to the RNFL average and nasal thickness, but overall accuracy is still good with AUROC of 0.77 and 0.76 each.

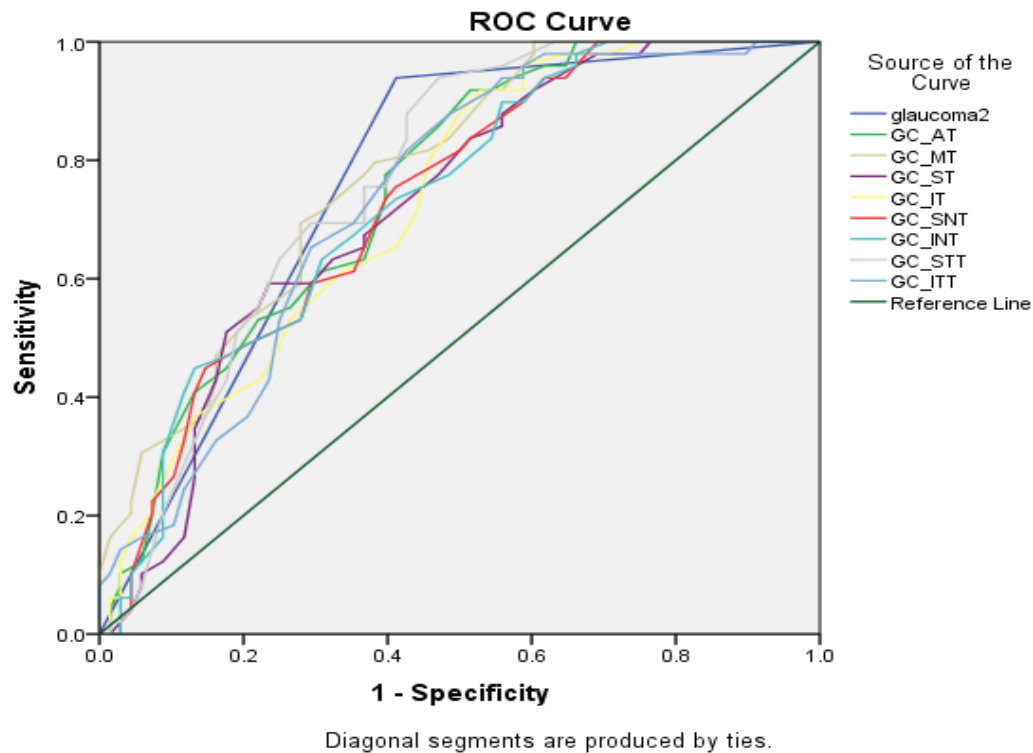


FIGURE 1. ROC curve of GCIPL showing healthy versus glaucoma suspect & early glaucoma

All GCIPL and RNFL parameters had poor performance in detecting glaucoma suspects from healthy control except the combination of both parameters by or logic (sensitivity 88.5%, specificity 49.5%). The AUC for this parameter was 0.63, which had relatively better, but still weak discriminating power. TABLE 3 shows AUROC, sensitivity and specificity of the GCIPL and RNFL parameters in diagnosis of early glaucoma and glaucoma suspects.

There was statistically significant difference b/n sex only in the superior quadrant from GCIPL parameter ($p=0.02$) and inferior quadrant from RNFL parameter ($p=0.027$).

TABLE 4. AUROC, Sensitivity and Specificity of the GCIPL parameters in diagnosis of Early glaucoma and Glaucoma suspect

	EG Vs HC				GS VS HC			
	AUROC	P	Sensitivity	Specificity	AUROC	P	Sensitivity	Specificity
Or - logic	0.764	0.000	93.2%	62.2%	0.634	0.013	88.5%	49.5%
GCIPL Average	0.749	0.000	100.0%	51.6%	0.594	0.080	100.0%	45.0%
GCIPL Min	0.774	0.000	87.5%	52.3%	0.591	0.093	80.0%	45.5%
GCIPL Sup	0.722	0.000	100.0%	50.5%	0.580	0.140	100.0%	43.8%
GCIPL Inferior	0.727	0.000	95.2%	49.5%	0.599	0.067	90.0%	44.0%
GCIPL Superonasal	0.729	0.000	100.0%	50.5%	0.579	0.141	100.0%	45.0%
GCIPL Inferonasal	0.732	0.000	100.0%	50.0%	0.573	0.175	100.0%	43.0%
GCIPL Superotemporal	0.764	0.000	100.0%	52.1%	0.596	0.076	100.0%	43.8%
GCIPL Inferotemporal	0.735	0.000	95.7%	51.1%	0.564	0.238	90.9%	44.4%

TABLE 5. AUROC, sensitivity, and specificity of the RNFL parameters in diagnosis of EG and GS

EG VS HC					GS Vs HC			
	AUROC	P	Sensitiv ity	Specificity	AUROC	P	Sensitiv ity	Specificity
RNFL	0.819	0.006	95.8%	51.6%	0.684	0.111	85.7%	42.9%
Average								
RNFL	0.682	0.115	100.0%	50.0%	0.504	0.972	100.0%	44.1%
Superior								
RNFL	0.813	0.007	100.0%	50.0%	0.715	0.062	100.0%	42.2%
Inferior								
RNFL	0.726	0.050	100.0%	43.0%	0.684	0.111	100.0%	41.5%
Nasal								
RNFL	0.647	0.202	90.9%	44.9%	0.517	0.880	83.3%	42.5%
Temporal								

7. DISCUSSION

Glaucoma management involves visual field testing and morphologic assessment of ONH and the RNFL. It is known that structural damage precedes detectable visual field loss measured with the standard automatic perimetry. The quantitative morphometric analysis of ONH and RNFL contribute to a more reliable, reproducible, and early assessment in glaucoma (8).

Ishikawa et al developed a macular segmentation algorithm to measure sub layer thickness for glaucoma diagnosis and they showed that macular inner retinal complex (ganglion cell layer, inner plexiform layer, inner nuclear layer) was thinner in eyes with perimetric glaucoma (9).

Leung et al used the Stratus TD-OCT to evaluate macular nerve fiber layer thinning in glaucoma. They reported a reduction in macular nerve fiber layer thickness in glaucomatous eyes compared with normal eyes (22). Different previous studies have been done to determine which OCT analysis protocol yields the best discrimination performance in detecting glaucoma. GCIPL and RNFL measurements by using OCT has been suggested as one means of early diagnosing glaucoma patients. In our study we assessed diagnostic performances of GCIPL, and RNFL parameters individually or in combination among cases of early glaucoma, glaucoma suspects, and healthy controls. The mean thickness of all the parameters was significantly lower in early glaucoma and glaucoma suspects compared to healthy controls. The differences were large in early glaucoma, while it is moderate in suspects compared to healthy controls.

The overall accuracy of each parameter were determined by using the AUROC. The AUROC assesses the overall accuracy of a diagnostic test by plotting the rate of true positive against that of false positive rate. An ideal test will have the AUROC of 1, which means 100% sensitive and specific. Practically, this is rare and there will always be a tradeoff between sensitivity and specificity. But, a test to be considered diagnostically useful, it is expected to have AUROC above 0.7 and its accuracy increase as the value approaches 1. While a test with AUROC of 0.5 and lower is considered to be not better than a chance and has limited diagnostic utility (23).

In our study, specificity for most of the parameters was in the range of 40% to 60%, and the maximum specificity was 62.2%. While the parameters had excellent sensitivity with most having sensitivity of 90%-100%, and no parameter had sensitivity of below 70%. One study

shows Or- logic combination of infero temporal sector alone with inferior quadrant RNFL resulted in significant increase of sensitivity while maintaining good specificity. In contrast to our study, the or- logic combination of minimum GCIPL and rim area resulted in a diagnostic performance equivalent to that of the or-logic combination of minimum GCIPL and average RNFL thickness (10). Another study done in Italy showed high specificity for all parameters but sensitivity was poor for eyes with preperimetric glaucoma. In this study the RNFL parameters with the widest AUC were RNFL inferior (0.76, 95% CI 0.66–0.82) and RNFL OR (0.79, 95% CI 0.72–0.86); the best GCIPL parameters in terms of diagnostic accuracy were GCIPL inferior (0.77, 95% CI 0.70–0.84) and GCIPL OR (0.79, 95% CI 0.71–0.85) (12).

In our study for glaucoma suspect, sensitivity ranges 80%-100% and specificity ranges 43%-49.5% for GCIPL and for RNFL sensitivity ranges from 83.3%-100% and specificity from 41.5%-44.1%. For early glaucoma sensitivity ranges 87.5%-100% and specificity ranges 49.5%-62.2% for GCIPL and for RNFL sensitivity ranges from 90.9%-100% and specificity from 43.0%-51.6% . In a study done at multi centers (Florida, Texas and California) for single GCIPL parameters, the minimum was the best discriminant with 82% sensitivity and 87.8% specificity. The other GCIPL parameters had low sensitivities ranging between 24% and 60%. Among single RNFL parameters, the inferior quadrant outperformed the other parameters with sensitivity and specificity values of 74% and 95.9% respectively, followed by average RNFL (64% and 95.9% respectively). All other RNFL parameters had a sensitivity range of 10% to 48% and specificity range of 87.8% to 100%. Combining any abnormal GCIPL or RNFL parameters had sensitivity of 96% and specificities of 63.6%. Combining the best performing GCIPL or RNFL had the best performance with sensitivity of 94% & specificity of 85.7%. The sensitivity and specificity of combining any abnormal GCIPL with any abnormal RNFL parameter in this study was 96% and 63.6% which was very close to our finding. The other similarity between this study and our is, the fact that the average and inferior RNFL are good parameters. On the other hand, a sharp contrast between this study and our was observed in sensitivity which was low as 10%, and for most of the parameters specificity was very good as high as 95%. (10).

In another study from Turkey, all the GCIPL parameters could discriminate early glaucoma from healthy controls. All the GCIPL parameters had the AUROC of 0.68-0.76. This is very

similar to the findings in our study (AUROC 0.647-0.819). Of the RNFL parameters, except temporal quadrant, all other could discriminate early glaucoma from healthy controls. The highest of them was RNFL superior quadrant with AUROC of 0.81. In this similar study, all of the GCIPL parameters except the superotemporal quadrant could discriminate glaucoma suspects from health controls, while none of the RNFL parameters could discriminate the two. In contrast, except the combined parameter, none of the GCIPL or RNFL parameters could discriminate glaucoma suspects from healthy controls in our study ($p>0.05$). Although this study mentioned that most of GCIPL parameters could discriminate b/n the two groups, the AUROC was still weak with all in the range of 0.61-0.67.

These findings suggest the diagnostic performance of GCIPL and RNFL parameters are relatively weak in discriminating glaucoma suspects from health controls. This is possibly due to the blurred grey zone for normal and abnormal in the two conditions (11).

8. CONCLUSION AND RECOMMENDATION

GCIPL and RNFL parameters can be useful to make early diagnosis of glaucoma. Results from different studies show inconsistent results on sensitivity and specificity of the individual and combined parameters. This inconsistency is due to difference in study design, the study participants and differences in measurement of the parameters. To conclude, high sensitivity means if we found a result “within normal limit” we can be sure that there is no structural damage and low specificity means if we found “outside normal limit” result we can not correlate it with structural damage. The value of cutoff point to define an abnormality should be adjusted to improve the specificity while maintaining an acceptable level of sensitivity is the recommendation from our study.

9. LIMITATIONS OF THE STUDY

Limitations include lack of bioengineer (familiar with this new machine) to do maintenance was the main problem faced in conducting this research and this affects the sample size of the normal controls to be fewer in our study.

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11. ANNEXES

11.1 INFORMMATION SHEET AND INFORMED CONSENT

Title of the research project - Diagnostic performance of optical coherence tomography macular ganglion cell-inner plexiform layer and retinal nerve fiber layer thickness measurement in glaucoma suspect and early glaucoma.

Name of principal investigator – Dr Addishiwot Abera

Name of organization and sponsor – St Paul Hospital Millennium Medical College

Benefits, Risks and/of Discomfort - By participating in this research project you may waste a maximum of 30 minutes. However, your participation is definitely important to decrease the burden of glaucoma by early detection. There is no risk in participating in this research project. You will be benefited to get free examination.

Incentives/Payments for Participating - You will not be provided incentives or payment to take part in this research.

Confidentiality - your name will not be written and the information collected from you will be kept confidential and stored in a file, by assigning a code number to it.

Right to Refusal or Withdraw - You have the full right to refuse from participating in this research.

Person to contact - This research project has been reviewed and approved by the ethical committee of SPHMMC. If you have any question you can contact the investigator at any time (Dr Addishiwot Abera 0929280052)

Consent form for interview and eye examination

You are participating in a study to assess diagnostic performance of OCT macular ganglion cell-inner plexiform layer and retinal nerve fiber layer thickness measurement in glaucoma. Glaucoma is a condition that causes damage to your optic nerve, which transmits image to your brain, and gets worse over time. Without treatment it can cause total permanent blindness within a few years. Since glaucoma have no early symptom or pain, it can go unnoticed until late in the disease. Detecting glaucoma early is one reason

you should have a complete eye exam. Visual field test is a subjective measure of the area of vision or how wide you can see. Complementary to this, optical coherence tomography which is non invasive and more accurate imaging technique tells information about optic nerve tissue loss and macular ganglion cell loss, which is affected early. So it is more useful in glaucoma suspects and early glaucoma. The result of your eye examinations will be collected for research and program planning purposes by SPHMMC, Department of Ophthalmology.

With your permission, we would like to:

1. Conduct interview
2. Conduct eye examination
3. Collect demographic and related information of VFT and OCT result.

You don't have to agree to do these things if you don't want to. All information that we collect will be confidential and no identifiable information will be released.

I acknowledge that I have understood this consent and the reasons for the study have been explained to me. I give my consent to participate in the study.

Name ----- sign and date -----

Researcher/witness ----- sign and date -----

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11.3 DATA COLLECTION FORMAT

SUBJECT CODE: _____

Part 1 -CLINICAL HISTORY

1. Age in Years-----

2. Sex ☐ Male ☐ Female

3. Address -----

4. Family history of glaucoma

☐yes ☐ No

5.If your answer is yes for question no 5, mention your relationship-----

6.Steroid use for more than 3 weeks / history of systemic chloroquine

☐yes ☐ No

7.Systemic illness - Diabetes mellitus: Yes ☐ No ☐

- Hypertension Yes ☐ No ☐

Part 2-OCULAR EXAMINATION OD/OS

1. BCVA (by snellen E chart)----- (R/E if any-----)

2. IOP-----mmhg

3. Visible anterior chamber angle structure-----

4. Optic nerve head

4.1 VCDR -----

4.2 Glaucomatous changes (tick all that apply)

- asymmetric cup-disc ratio >0.2 unexplained by the ONH size difference
- enlarged cup-disc ratio 0.5
- notching or narrowing of neuro retinal rim

- disc hemorrhage
- suspicious alteration in the nerve fiber layer

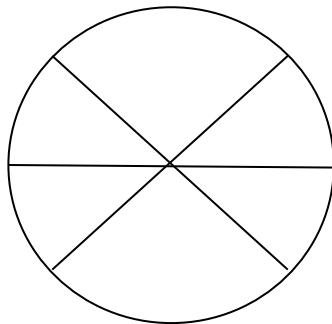
Part 3 –Visual Field Test & Optical Coherence Tomography

1. VFT (please attach the print out)

- Mean Deviation -----
- Pattern Standard Deviation -----
- GHT -----

2. OCT

- **Macula cube 512x128** scan mode, 7mm * 6mm
- GCIPL (in μm) - Average ----- (Green/Yellow/Red)
 - Minimum ----- (Green/Yellow/Red)
 - Nasal - Superonasal ----- (Green/Yellow/Red)
 - Inferonasal----- (Green/Yellow/Red)
 - Temporal -superotemporal--- (Green/ Yellow/Red)
 - Inferotemporal----- (Green/Yellow/Red)
 - Superior thickness ----- (Green/Yellow/Red)
 - Inferior thickness----- (Green/Yellow/Red)



- Optic disc cube 200x200 scan mode

-6mm*6mm square centered on the optic nerve head

RNFL (in μm)	- Average -----	(Green/Yellow/Red)
	-Superior -----	(Green/Yellow/Red)
	-Inferior -----	(Green/Yellow/Red)
	-Nasal -----	(Green/Yellow/Red)
	-Temporal -----	(Green/Yellow/Red)

