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*by* Habibah Setyawati Muhiddin

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# Ganglion Cell Complex thickness as an early predictor of microstructural changes in varying degrees of myopia in comparison with Retinal Nerve Fiber Layer (RNFL) thickness



Andi Pratiwi,\* Andi Muhammad Ichsan,\* Habibah Setyawati Muhiddin

## 15 ABSTRACT

**18** **pose :** To compare the Ganglion Cell Complex (GCC) thickness with Retinal Nerve Fiber Layer (RNFL) thickness in correlation with visual field (VF) in varying degrees of myopia.

**Design:** a Cross-Sectional study.

**Participants:** Ninety-six eyes of 49 patients with myopia.

**Methods:** Patients with myopia examined with streak retinoscope to obtained the best corrected visual acuity based on spherical equivalent. Patients then divided into low (50 eyes=50.1%), moderate (31 eyes=32.3%), and high (15=15.6%) myopia. Axial length measured with A-Scan ultrasound divided into short (4=4.2%), moderate (74=77.1%), and long (18=18.7%). SD-OCT was used to measured GCC thickness in 9 areas including fovea (R1), parafovea (R2-R5), and perifovea (R6-R9) according to early treatment diabetic retinopathy study (ETDRS), and RNFL thickness in five areas (G, TS, TI, NS, NI). VF analysis with Humphrey field analyzer with SITA 24-2 pattern.

**Main outcome measures:** GCC thickness has higher correlation with degree of myopia particularly in perifovea area and means deviation than RNFL thickness.

**Results:** GCC thickness decreased significantly along with the elevation of myopia degree in almost entire area except R1 and R5 . Parafovea (R2-R4) and perifovea (R6-R9) were significantly correlated with  $p=0.001, 0.005, 0.006$  and  $p=0.000, 0.000, 0.000, 0.003$  respectively. In contrast, RNFL thickness obtained lower correlation with myopia degree whereas the only significant result was found in Nasal area ( $p=0.045$ ). VF found to be strongly correlated with the degree of myopia in which  $p$ -value for Mean Deviation = 0.000.

**Conclusions:** GCC thickness may compare the predictive value of RNFL in predicting early retinal microstructural changes even in low degree myopia.

**Financial Disclosure:** Author has no financial disclosure.

**Keywords:** Retina, Myopia, Ganglion Cell

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Department of Ophthalmology,  
Faculty of Medicine, Hasanuddin  
University

## INTRODUCTION

**3** Myopia is the refractive anomaly of the eye in which the conjugate focus of the retina is at some finite point in front of the eye when the eye is not accommodating. It can also be described as the refractive condition in which parallel light rays from an object at optical infinity are focused by the eye in front of the retina, with accommodation relaxed. Myopia is derived from the term “muopia” which, in Greek, means to close the eyes. It manifests itself as blurred distance vision, hence, the popular term “near sightedness.”<sup>1</sup>

With very large increases in refractive diopter (D), the retina can degenerate and become thinner, and the retinal microstructures change, especially at the posterior pole. Myopia is a risk factor for a number of retinal pathologies such as retinal detachment, macular holes, choroidal neovascularization, and retinoschisis. Thus, complications related to myopia are one of the leading causes of blindness. As a result, early detection of the changes

**1** in intraretinal structures is of utmost importance for the myopic eye. Understanding the abnormal patterns of retinal structures will help in assessing early stage, myopia- related complications.<sup>2</sup>

Several studies have used OCT to investigate relationships between variations in macular thickness and myopia. The thicknesses of the retinal nerve fiber layer (NFL), ganglion cell layer (GCL), and inner plexiform layer (IPL) were reported by some authors to be thinner in myopic eyes compared to normal eyes. Furthermore, the thickness of the outer retinal layers, including the retinal pigment epithelium (RPE) and the photoreceptor layer vary according to the axial length (AL).<sup>3</sup>

Additionally, it is important to determine if there is any correlation between the thickness of specific retinal layers and the AL and/or the refractive error in myopic patients and to determine if such correlations are predictive of developing myopia-related complications.<sup>2</sup>

\*Correspondence to: Andi Pratiwi and Andi Muhammad Ichsan have an contribution to this research, Department of Ophthalmology, Faculty of Medicine, Hasanuddin University  
am\_ichsan@yahoo.com

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## RESEARCH DESIGN AND METHOD

The present study was an analytic observational study with cross-sectional design conducted in Hasanuddin University Hospital, Makassar, Indonesia. Period of study: April-June 2016

### Inclusion Criteria

1. Age 20-50-year-old
2. Patients with refractive error  $\geq 0.50$  D (SE)
3. Intra Ocular Pressure  $\leq 21$ mmHg
4. Patient with normal optic disc without glaucomatous changes.
5. Agreed to be part of the study and signed the inform consent application.

### Exclusion Criteria

1. The macular diseases such as age related macular degeneration, macular edema, epiretinal membrane.
2. History of diabetes or neurological diseases such as stroke, intracranial tumor, multiple sclerosis, intracranial infection.
3. History of ocular trauma
4. Corneal disease such as keratopathy.
5. History of intraocular surgery.
6. Uncooperative patient.

### Data collection

All patients who met the inclusion criteria will be informed about the study procedure and if they were agreed then signed the consent application. 49 patients (96 eyes) were included in our study. The enrolled subjects were divided into the following 3 subgroups according to their spherical equivalents (SEs): low myopia ( $< -3.00$  D,  $n = 47$ ), moderate myopia (from  $-3.00$  D to  $-6.00$ D,  $n = 34$ ), and high myopia ( $> -6.00$  D,  $n = 15$ ) and their AL : Short ( $< 22.5$  mm,  $n = 4$ ), Moderate (22.51–25.5 mm,  $n = 74$ ), Long ( $> 25.51$  mm,  $n = 18$ ).

Each subject underwent a complete ocular examination, which included measurements of best-corrected visual acuity with Snellen chart and streak retinoscope (Welch-Allyn WA-11710 3.5V), afferent pupillary reflex, anterior segment examination (Topcon SL-D7 Digital Ready Slit Lamp), intraocular pressure (IOP) by Goldmann applanation tonometry, funduscopy with 90D lens (Volk Optical, Mentor, Ohio, USA), AL measurement using an ultrasound biometer (Tomey, 25 rasonic A/B Scanner and Biometer, UD-6000), macular ganglion complex thickness and peripapillary retinal nerve fibre layer thickness using Spectral Domain Optical Coherence Tomography (Spectralis HRA+OCT, Heidelberg Engineering,

Germany), and visual field testing with Humphrey Field Analyzer SITA 24-2 (Humphrey Field Analyzer II; Carl Zeiss Meditec, Dublin, California, USA)

### SD-OCT Imaging

Imaging was performed using Heidelberg SD-OCT (Heidelberg Engineering, Germany)

The Ganglion Cell Complex thickness was measured with Heidelberg Spectral Domain OCT according to Early Treatment Diabetic Retinopathy Study (ETDRS) grid (6x6 mm) to obtain the values for each sector. The grid was divided into nine independent sectors; the inner and outer rings, with semidiameters of 1500  $\mu$ m and 3000  $\mu$ m, respectively, were segmented into four quadrants (superior, inferior, nasal, and temporal). The central sector was defined as being within 1000  $\mu$ m of the center of the fovea.<sup>3</sup>

Segmentation of GCC thickness were divided into; R1: Sentral, R2: inner superior, R3: Inner nasal, R4: Inner inferior, R5: Inner temporal, R6: Outer superior, R7: Outer nasal, R8: Outer inferior, R9: Outer temporal.<sup>3</sup>

SD-OCT RNFL thickness measurements were performed using circular scans (software version 5.1.2; Heidelberg engineering) along with a peripapillary circle of 360 degrees. Peripapillary nerve fiber layer thickness segmentation divided into; G: Cup, TS: Temporal Superior, NS: Nasal Superior, N: Nasal NI: Nasal Inferior, TI: Temporal Inferior, T: Temporal.

### Visual Field Examination

Standardized visual field testing was performed with static automated perimetry (Swedish Interactive Threshold Algorithm standard 24-2, Humphrey Field Analyzer II; Carl Zeiss Meditec, Dublin, California, USA). A visual field was defined as reliable when fixation losses were less than 20%, and false-positive and false-negative rates were less than 33%. A glaucomatous visual field defect was defined as the presence of three or more significant ( $p < 0.05$ ) non-edge contiguous points with at least one at the  $p < 0.01$  level on the same side of the horizontal meridian in the pattern deviation plot, and classified as 'outside normal limits' on the Glaucoma Hemifield Test, confirmed on two consecutive visual field examinations.<sup>4</sup>

### Statistical Analyses

Statistical analyses were done by calculation of range, mean, median, standard deviation, percentage, one-way ANOVA, Pearson correlation test, and p-value.

### Statistical Software

RNFL thickness of all sectors, GCC thickness in 9 regions, axial length and visual field testing were examined with Statistical Package for Social Science (SPSS statistics, ver.22; IBM Corporation, New York, NY), and Microsoft word and excel have been used to generate graphs, and tables.

### RESULT

Ninety-six eyes from 49 patients are included in our study. There are 32 men and 64 women. The patients are divided into 3 groups according to their SE and AL.

Table 1 consists of comparison for mean GCC (R1-R9) and RNFL thickness according to myopia degree and AL. The table shows a significant negative correlation ( $p < 0.05$ ) in which GCC thickness in low, moderate, and high myopia markedly different in parafovea (paracentral) and perifovea (peripheral) except in R1 ( $p = 0.315$ ) and R5 ( $p = 0.451$ ). In line with table 1, figure 1 reveal R6, R7, and R8 turns out to be the most significant area ( $p = 0.000$ ). In contrast, AL express insignificant

correlation with GCC thickness in most areas except in R1 ( $p = 0.043$ ).

Comparison of peripapillary RNFL based on myopia degree and axial length is also shown in table 1. One way ANOVA comparative test indicate an insignificant correlation between RNFL with myopia degree and axial length. This table points that the thickest area of low, moderate, and high myopia are TS, TI, and TI, respectively. This result signifies that the temporal area is not interfered by myopia because there are no thickness changes regardless of the myopia degree. Similarly, AL showed the insignificant correlation among all degrees in all areas. Nevertheless, peripapillary RNFL indicates that there is a tendency to thinned in accordance with myopia degree elevation although the nasal area is the only area which shows a significant correlation (Figure 2).

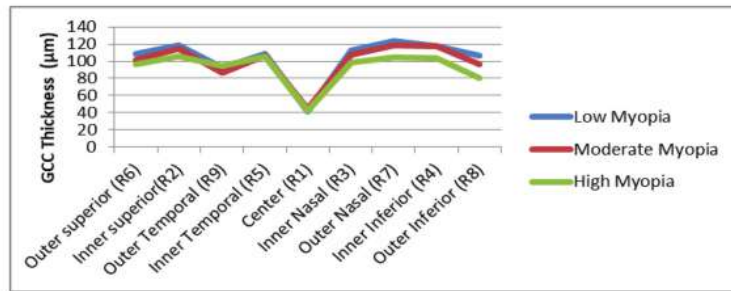
Comparison of visual field test result based on myopia degree and AL are shown in table 2. Based on myopia degree, visual field test which is represented with Mean Deviation (MD) and Pattern Standard Deviation (PSD) revealed a significant result  $p = 0.000$  and  $0.029$  respectively. Mean MD

**Table 1** Comparison for mean GCC (R1-R9) and RNFL thickness according to myopia degree and axial length

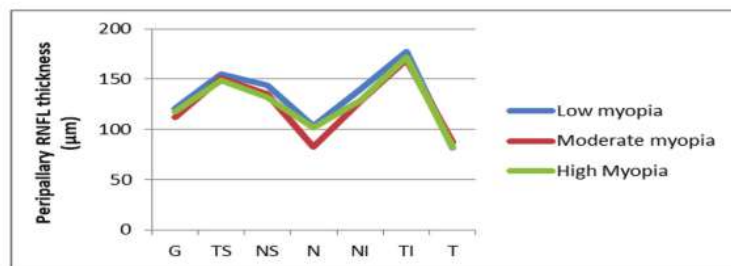
GCC and RNFL Thickness	Myopia Degree				Axial Length			
	Low	Moderate	High	p	Short	Moderate	Long	p
R1	41.234	44.0588	42.8	0.315	44	41.3649	42.4792	<b>0.043</b>
R2	118.1064	114.9706	105.4	<b>0.001</b>	116	114.8649	115.3889	0.973
R3	112.3191	107.6471	98.8	<b>0.005</b>	113.5	108.7703	106.5556	0.667
R4	118	117.3824	103.4667	<b>0.006</b>	115.5	114.8108	118.3889	0.707
R5	108.1702	106.1471	105.2	0.451	105	106.9595	107.5556	0.883
R6	108.5532	101.9412	96.4667	<b>0.000</b>	105.75	104.8514	101.8333	0.574
R7	124.0638	118.3529	104.1333	<b>0.000</b>	118.5	119.5	116.6667	0.816
R8	106.1702	96.8235	80.2	<b>0.000</b>	103.75	100.6757	90	0.063
R9	92.5532	86.3235	94.3333	<b>0.003</b>	89.5	91.3784	87.7778	0.347
G	120.766	112.1765	117.8	0.338	117.75	117.0676	117.9444	0.991
TS	154.9787	151.5	148.8	0.710	156	153.8514	147.6667	0.674
NS	143.6596	135.4412	131.9333	0.504	135.25	140.527	133.1111	0.768
N	102.9362	82.7353	101.5333	<b>0.045</b>	83.25	94.5811	102.3333	0.593
NI	139.6596	128.2353	128.6667	0.374	130.5	132.9324	138.6111	0.849
TI	177.4255	169.4412	171.6667	0.402	160	174.7162	172.5556	0.559
T	82.1489	87.1176	82.8667	0.403	86.5	83.2162	86.7778	0.691

**Table 2** Comparison of visual field test result based on myopia degree and axial length

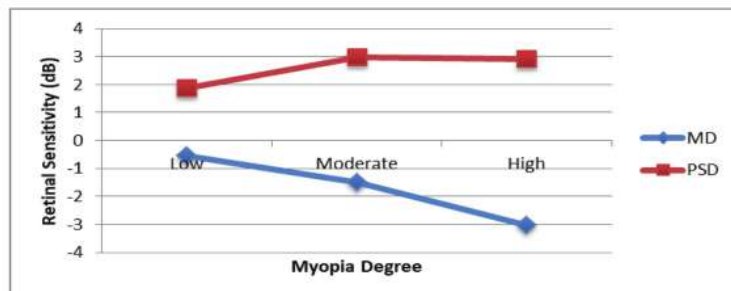
Visual Field	Myopia Degree				Axial length			
	Low	Moderate	High	p	Short	Moderate	Long	p
MD	-0.5621	-1.5026	-3.026	<b>0.000</b>	0.1300	-1.2286	-1.806	0.385
PSD	1.8709	2.9918	2.9007	<b>0.029</b>	1.322	2.5828	2.0411	0.186



**Figure 1** Correlation between retinal GCC thickness with myopia degree according to ETDRS area



**Figure 2** Correlation between peripapillary RNFL thickness with myopia degree



**Figure 3** Correlation between MD and PSD with myopia degree

in low, moderate, and high myopia are -0.5621, -1.5026, and -3.026, respectively. Mean PSD are 1.8709, 2.9918, and 2.9007 in low, moderate, and high myopia, respectively. However, based on AL, there is no significant correlation with MD ( $p=0.385$ ) and PSD ( $p=0.186$ ). Correlation between myopia degree and visual field showed a negative correlation in which the higher the myopia degree, the lower the MD and PSD value. (Figure 3)

## DISCUSSION

Correlation between retinal GCC thickness based on myopia degree showed a significant result between both variables. Almost all macula areas, except R1 and R5, experience a marked decreased in thickness along with the increase of myopia degree.

This result is in line with some other studies related to retinal GCC, where they stated in their research that foveal thickness (R1) was not decreased in myopia, nonetheless, parafovea (R2-R5) and perifovea (R6-R9), were positively correlated with myopia degree, the further away from the fovea, the thinner the macula as myopia progress.<sup>2,5,6</sup>

Pearson correlation test for GCC thickness with AL indicate a significant result in some areas that is R1 ( $p=0.017$ ), R6 ( $p=0.001$ ), R7 ( $p=0.023$ ), R8 ( $p=0.000$ ). This result denotes that there is a negative correlation between those two variables, although not evenly in all areas. However, one-way ANOVA test revealed almost no significant correlation between GCC thickness with AL except R1 (Fovea,  $p=0.043$ ). This matter represents that fovea is the area most affected by the increase of axial length.

As already known, R1 is a central area that only consists of outer retinal layer segments and has no inner layers. Previous studies using OCT to figure out the relationship between macular thickness variability with myopia. Some of those reported that nerve fiber layer, ganglion cell layer, and inner plexiform layer are thinned due to myopia in comparison with normal people, whilst axial length has more influence to outer retinal layers including photoreceptor and retinal pigment epithelium (RPE).<sup>2</sup> A study by Lam et al. found that macular thinning only occur in the outer ring (3-6mm). The reason is peripheral retina seem to be less resistant to stretching due to lack of large vessels. But different mechanism ruled in the posterior pole whereas perifovea area which is closer to the vascular arcade thinned faster than parafovea which lies farther away.<sup>9</sup> Other mechanism stated by Zhao et al., that foveal area has the highest RPE permeability so that the thickness will not decrease dramatically.<sup>5</sup> Numerous studies also state different result with this study. One thing that can lead to this is most of those studies using total retinal thickness as the parameter instead of specifically put GCC as the particular layer to be measured.

Correlation between peripapillary RNFL and myopia degree showed that temporal superior (TS) and temporal inferior (TI) is the thickest among all, whilst temporal (T) is the thinnest. The same result published in 2010 by Kim et al. In their study they found that peripapillary RNFL decreased significantly but not evenly in all areas, with temporal superior, and temporal inferior became the thickest area. They stated that these results raise the possibility that peripapillary RNFL redistributed along with the axial stretching in myopia. When the axial length is stretched, the retina will be dragged to the temporal horizon. In the process, the RNFL will be

<sup>5</sup> compressed against the bundles originating from the opposite hemisphere at the horizontal raphe. This would result in thickening of the RNFL in the temporal quadrant. In contrast, the nasal area would become thinner as it is stretched.<sup>6</sup>

After using ANOVA test, according <sup>1</sup> the degree of myopia only nasal area that shows a significant difference between low degree myopia, moderate degree myopia, and high degree myopia ( $p = 0.045$ ), meanwhile according to the axial length of eyeballs, there is no significance in all the area. The relationship between peripapillary retinal nerve thickness with the degree of myopia can be seen that temporal superior (TS) area and temporal inferior (TI) area is the thickest layer, and temporal (T) area become the thinnest layer.

Structure-function relationship between myopia degree and axial length with <sup>13</sup> visual field revealed a significant correlation for Mean Deviation (MD), and Pattern Standard Deviation (PSD) whereas MD value decreased ( $p = 0.000$ , one-way ANOVA test) and PSD increased ( $p = 0.029$ , one-way ANOVA test) as the myopia degree elevated. These result denoted that the decreased of retinal sensitivity (MD and PSD value) is quite specific to be used as a parameter of microstructural changes in the retina.

Statistical analyses for visual field and axial length relationship in this study found to be insignificant. However, it is clearly showed that MD value decreased and PSD increased along with axial length. These results indicate decreased retinal sensitivity that interfered by axial elongation. <sup>12</sup> Varies explanation related to decreased of visual field sensitivity in myopia had been submitted. Retinal stretching due to axial elongation will broaden the interphotoreceptor distance, thus, triggered distortion of the photoreceptor, and lead to decreased of retinal sensitivity.<sup>10</sup>

## CONCLUSION

<sup>16</sup> In summary, we have demonstrated that there is a significant correlation between myopia degree with macular GCC thickness in almost all areas based on ETDRS criteria, except R1 and R5. In contrast, peripapillary retinal nerve fiber layer showed results in which the only <sup>20</sup> significant correlation was found in the nasal area. There was no significant correlation between axial length with GCC and peripapillary RNFL. Nevertheless, MD and PSD markedly different in low, moderate, and high myopia. These results indicate that GCC thickness may be used as a predictive value to compare the RNFL in order to predict microstructural changes even in low myopia. and PSD markedly different in low, moderate, and high myopia. These results indicate that

GCC thickness may be used as a predictive value to compare the RNFL in order to predict microstructural changes even in low myopia.

A limitation of our study is the relatively small numbers in each group of myopia and axial length. In addition, we use SE to define myopia criteria, so the astigmatism part could have introduced potential selection bias. Other than that, AL measurements using contact instead of optical biometer could possibly bias the AL results.

## AUTHOR CONTRIBUTIONS

Andi Pratiwi and Andi Muhammad Ichsan have an equal contribution to this research. Habibah Setyawati Muhiddin as the first co-author contributed in sampling and designing the research.

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