DOI: 10.7860/JCDR/2023/65264.18785

Correlation Between Axial Length and Peripapillary Retinal Nerve Fiber Layer Thickness Determined by Spectral Domain Optical Coherence Tomography: A Cross-sectional Study

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ABSTRACT

Introduction: Peripapillary retinal nerve fiber layer (pRNFL) thickness is an important indicator for the diagnosis and monitoring of glaucoma. Optical Coherence Tomography (OCT) allows for accurate assessment of pRNFL thickness, but previous studies have shown that axial length can affect pRNFL thickness. Hence, this study aimed to confirm this hypothesis.

Aim: To determine the correlation between axial length and pRNFL thickness in healthy adults.

Materials and Methods: This was a cross-sectional study conducted on 200 eyes of healthy adults aged 18-30 years. All subjects underwent a complete ophthalmic evaluation. Average pRNFL thickness and quadrant pRNFL thickness were recorded using Topcon Spectral Domain OCT (SD-OCT) in all subjects. Axial length measurements were performed using optical biometry with the Topcon IOL Master, and subjects were divided into three groups according to axial length: Group 1 (<23.5 mm), Group 2 (23.5-25.5 mm), and Group 3 (>25.5 mm). pRNFL thickness values were subjected to Littmann's correction for ocular magnification. Data was analysed using a one-way ANOVA test, and the correlation between pRNFL thickness and axial length, before and after correction for ocular magnification, was determined using the Pearson correlation coefficient.

Results: There was a significant negative correlation between uncorrected pRNFL thickness and axial length in the average pRNFL ($r=-0.05$, $p<0.001$), superior quadrant ($r=-0.26$, $p<0.001$), nasal quadrant ($r=-0.44$, $p<0.001$), and inferior quadrant ($r=-0.48$, $p<0.001$). Uncorrected temporal quadrant pRNFL thickness showed a positive correlation with axial length ($r=0.17$, $p=0.015$). After applying Littmann's formula, the negative correlation between uncorrected pRNFL thickness and axial length disappeared in the average, superior quadrant, and inferior quadrant.

Conclusion: A negative correlation was established between pRNFL thickness and axial length, but this correlation disappeared after applying correction for ocular magnification. Thus, to avoid misdiagnosis of glaucoma in individuals with varying axial lengths, the authors recommend using correction methods for the effects of ocular magnification induced by axial length when considering pRNFL thickness values obtained from OCT.

Keywords: Glaucoma, Ocular magnification, Ophthalmic evaluation

INTRODUCTION

Glaucoma is one of the leading causes of irreversible blindness worldwide. It leads to progressive damage to retinal ganglion cells, resulting in changes in the optic disc structure and thinning of the Retinal Nerve Fiber Layer (RNFL) [1,2]. Numerous studies have demonstrated that loss of peripapillary RNFL occurs before the development of Optic Nerve Head (ONH) and Visual Field (VF) abnormalities [3,4]. Imaging devices such as Optical Coherence Tomography (OCT) enable quantitative and objective assessment of peripapillary RNFL thickness, aiding in the early diagnosis of glaucoma [5]. RNFL thickness is also influenced by age, gender, race, and ethnicity [6,7].

Several studies have investigated the relationship between RNFL thickness, axial length, and refractive error in both adults and children [8-10]. While some studies have found a negative correlation between axial length and peripapillary RNFL thickness, recent research has reported no effect of axial length variation on peripapillary RNFL thickness after correcting for the magnification effect induced by axial length variation [11-14]. However, there are discrepancies in the findings of these studies and in the methods used to correct for ocular magnification [6,13,15].

Considering the diverse results from previous studies [13,16,17], the present study aims to examine the correlation between peripapillary RNFL thickness and axial length in normal healthy subjects, taking into account various variables. Littmann's formula is utilised in this study to correct for the effect of ocular magnification induced by axial length variation [18-20]. The objective is to establish a definitive correlation by addressing the limitations and inconsistencies observed in previous research.

MATERIALS AND METHODS

This cross-sectional study was conducted at the Department of Ophthalmology, Bharati Hospital and Research Centre, Pune, Maharashtra, India, from August 2020 to October 2022. Written informed consent was obtained, and the procedures followed were in accordance with the Declaration of Helsinki and the standards of the Ethical Committee of Bharati Vidyapeeth Deemed to be University (BVDUMC/IEC/37).

Inclusion criteria: The study included healthy participants aged between 18 and 30 years.

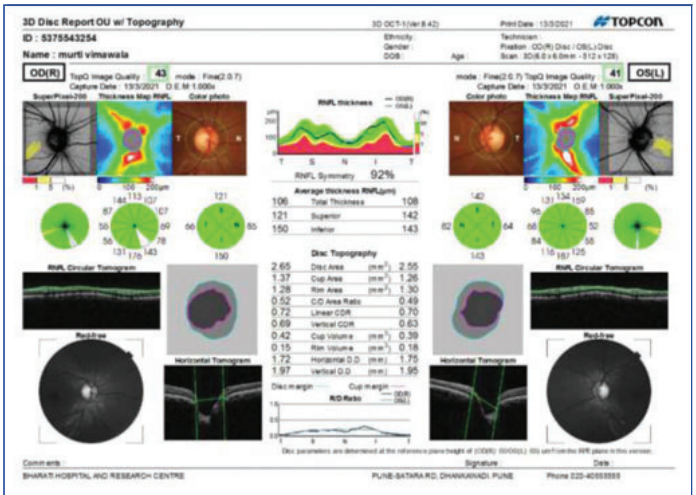
Exclusion criteria: Those participants with astigmatism greater than 1.5 D, visual acuity less than 20/20, Intraocular Pressure (IOP) lower than 21 mm Hg, a history of ocular trauma, prior intraocular/refractive surgery, retinal lasers, glaucoma or ocular hypertension, anterior or posterior segment pathology, neurological disorders, uncooperative individuals, and cases with poor OCT image quality were excluded.

Sample size: Sample size estimation was performed based on the correlation coefficient (r) between axial length and pRNFL thickness, which was found to be 0.201 in a previous study by Kausar A et al., [13]. With a power of 80% and a confidence interval of 95%, a minimum sample size of 192 was calculated [13]. Hence, a sample size of 200 eyes was chosen for this study.

Procedure

The study included 200 eyes from 104 healthy adults aged 18-30 years. Each eye was considered as a separate subject. Out of the 208 eyes initially considered, four eyes had undergone refractive surgery, and four eyes had undergone retinal laser treatment, so they were excluded. All subjects underwent a comprehensive ophthalmic evaluation, including visual acuity testing, refraction, slit lamp examination, fundus examination, and intraocular pressure evaluation using Goldmann applanation tonometry.

Average pRNFL thickness, as well as superior, inferior, nasal, and temporal quadrant pRNFL thickness, were recorded using spectral domain OCT (Topcon 3D OCT-1 Maestro) imaging in all participants [Table/Fig-1]. Uncorrected pRNFL thickness values were then subjected to Littmann's correction to account for the magnification induced by different axial lengths, resulting in corrected pRNFL thickness values.



[Table/Fig-1]: Sample of OCT report obtained using Spectral Domain-Optical Coherence Tomography (OCT) (Topcon 3D OCT-1 Maestro).

Axial length measurements were performed using the Topcon IOL Master (Topcon, Tokyo, Japan). Participants were divided into three subgroups based on axial length as follows:

Group 1: <23.5 mm

Group 2: 23.5 to 25.5 mm

Group 3: >25.5 mm [16].

Correction for ocular magnification was done using Littmann's formula:

$$t = p \times q \times s$$

where t is the actual fundus dimension, p is the magnification factor of the imaging system (3.394 for Topcon 3D OCT-1 Maestro), q is the magnification factor for the individual eye, and s is the value obtained from the imaging device. The value of q is calculated as 0.01306×(axial length-1.82) [20].

STATISTICAL ANALYSIS

The data analysis was performed using IBM SPSS Statistics for Windows, Version 25.0 (Armonk, NY: IBM Corp). Descriptive statistics were used to present the results for continuous variables, while frequency and percentages were used for categorical variables. One-way analysis of variance (ANOVA) was used to compare the variables among the three groups. The Karl Pearson correlation coefficient was employed to determine the correlations between axial length and peripapillary RNFL thickness. A significance level of 5% was used throughout the analysis. A p-value less than 0.05 was considered statistically significant.

RESULTS

In this study, the mean age of the participants was 24.28±2.37 years. Among the participants, 50 were males (48.07%) and 54 were females (51.93%). The mean axial length was 24.4±1.5 mm. The majority of eyes belonged to Group-2 of axial length, with a mean length of 24.5±0.6 mm [Table/Fig-2].

Axial length groups	Number of eyes (Total N=200)	Mean±SD (mm)	Minimum (mm)	Maximum (mm)
Group-1 (<23.5 mm)	56	22.5±0.8	19.8	23.49
Group-2 (23.5-25.5 mm)	86	24.5±0.6	23.54	25.49
Group-3 (>25.5 mm)	58	26±0.5	25.51	27.4

[Table/Fig-2]: Axial length measurement in groups; Total participants N=104.

The average uncorrected pRNFL thickness was 103.73±9.29 μm. On inter group comparison of mean values of pRNFL parameters, the mean average pRNFL thickness and thickness values in three quadrants were significantly different among the three groups, except for the Temporal quadrant (p=0.052) [Table/Fig-3].

Parameter	Group-1 (μm) n=56	Group-2 (μm) n=86	Group-3 (μm)n=58	p-value (ANOVA)
Average PRNFL	109.7±11.2	102.9±7	99.2±7.2	**<0.001**
Superior quadrant PRNFL	135.8±18.5	131.2±11.6	126.8±10	**0.002**
Inferior quadrant PRNFL	140.9±15.7	130.5±12.7	125.7±13.4	**<0.001**
Nasal quadrant PRNFL	86.2±14.5	77.7±14.2	71.3±19.3	**<0.001**
Temporal quadrant PRNFL	72.5±10.6	72.1±12.9	77.1±14	**0.052**

[Table/Fig-3]: Mean values of pRNFL parameters of groups (mean±SD) before applying Littmann's formula.

After correction for the effect of ocular magnification by applying Littmann's formula, the inter group comparison revealed that the mean average pRNFL thickness and thickness values in two quadrants, Superior and Temporal, were significantly different among the three groups (p<0.001) whereas, in the Inferior and Nasal quadrants, the difference was not statistically significant (p>0.05) [Table/Fig-4].

Parameter	Group-1 (μm) n=56	Group-2 (μm) n=86	Group-3 (μm) n=58	p-value (ANOVA)
Average PRNFL	100.7±10.1	103.3±6.8	106.4±7	**<0.001**
Superior quadrant PRNFL	124.8±17.6	131.8±11.6	136±10.4	**<0.001**
Inferior quadrant PRNFL	129.3±13.9	131±12.1	134.7±13.4	0.077
Nasal quadrant PRNFL	79±12.7	77.9±13.9	76.3±20.1	0.659
Temporal quadrant PRNFL	66.6±10.3	72.5±13.3	82.7±15.6	**<0.001**

[Table/Fig-4]: Mean values of pRNFL parameters of groups (mean±SD) after applying Littmann's formula.

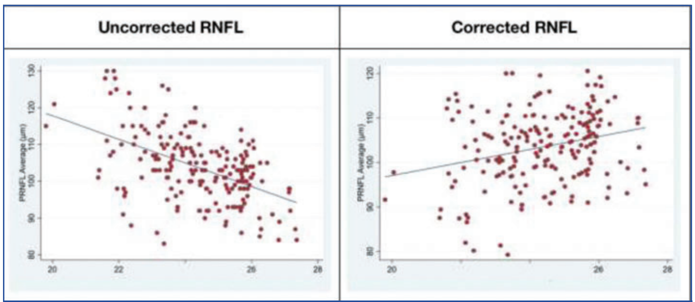
Analysing the correlation between pRNFL thickness and axial length using Pearson's correlation coefficient revealed a statistically significant negative correlation in uncorrected pRNFL thickness (r=-0.05, p<0.001) and axial length in the average as well as in all quadrants [Table/Fig-5].

pRNFL Thickness	Uncorrected		Corrected	
	r	p-value	r	p-value
PRNFL average	-0.50	**<0.001**	0.26	**<0.001**
PRNFL superior quadrant	-0.26	**<0.001**	0.35	**<0.001**
PRNFL nasal quadrant	-0.44	**<0.001**	-0.15	**0.037**
PRNFL inferior quadrant	-0.48	**<0.001**	0.12	0.103
PRNFL temporal quadrant	0.17	**0.015**	0.48	**<0.001**

[Table/Fig-5]: Correlation between axial length and pRNFL thickness (n=200) before and after correction for ocular magnification.

However, analysing the correlation between corrected pRNFL thickness and axial length using Pearson's correlation coefficient revealed a positive correlation that was statistically significant in the average (r=0.26, p<0.001), superior (r=0.35, p<0.001), and temporal (r=0.48, p<0.001) quadrant values. The correlation was statistically insignificant in the inferior quadrant value (r=0.12, p=0.103). There remained a statistically significant negative correlation between corrected pRNFL thickness and axial length in the nasal quadrant [Table/Fig-5].

Scatter plots showing the correlation between average pRNFL thickness and axial length before and after correction for the effect of ocular magnification are shown in [Table/Fig-6].



[Table/Fig-6]: Scatter Plots of correlation between average pRNFL thickness and axial length.

DISCUSSION

The aim of this study was to investigate the correlation between axial length and pRNFL thickness in healthy adults. The average axial length was 24.39±1.46 mm, ranging from 19.8 mm to 27.6 mm, which is consistent with findings from other studies conducted in Indian populations [21-23]. The largest number of subjects belonged to the axial length subgroup of 23.5 to 25.5 mm (n=86), which can be attributed to the random selection of subjects.

The average uncorrected pRNFL thickness in the present study was 103.73±9.29 µm, and the quadrant-wise uncorrected pRNFL thickness followed the ISNT (inferior-superior-nasal-temporal) rule, with the inferior quadrant having the highest thickness, followed by the superior, nasal, and temporal quadrants [24]. Similar studies conducted on healthy Indian populations by Ramakrishnan R et al., and Dhami A et al., reported mean pRNFL thicknesses of 105±38.79 µm and ranging from 104.9±59.89 µm to 108.58±8.55 µm, respectively [11,25]. These findings are comparable to the present study.

The pRNFL thickness is measured by the OCT machine at a fixed angular distance (approximately 12°) centered on the optic disc. The location of the measurement circle on the peripapillary retina is influenced by the ocular optical system's magnification. A longer eye will result in a larger measurement circle diameter, which will shift the RNFL measurement away from the center of the optic disc. Conversely, for smaller eyes, the opposite effect occurs [26,27]. Therefore, higher axial length can lead to falsely low pRNFL thickness measurements, while lower axial length can result in falsely high pRNFL thickness measurements.

To correct for this error caused by ocular magnification resulting from variations in axial length, Littmann's formula was applied to all pRNFL thickness values. Littmann's formula has traditionally been used to correct ocular magnification in OCT measurements [18]. This formula can be used to calculate both the actual scan radius and the RNFL thickness measurement, as used in the present study [19].

In the present study, a significant negative correlation was found between uncorrected average pRNFL thickness and axial length, as well as for all quadrant-wise pRNFL thickness except the temporal quadrant. Similar results of a negative correlation between axial length and pRNFL thickness have been reported in studies conducted by Nagai-Kusuhara AN et al., (r=-0.20, p=0.011) and by Chen CY et al., who also reported that global RNFL thickness decreases by 3.086 µm for each additional millimeter of axial length (β=-3.086; p<0.001) [6,28].

According to Dhami A et al., who also grouped the samples based on axial length, RNFL thinning increased with increasing axial length from 22.50 mm to >25.51 mm in all quadrants [11]. Porwal S et al., also found a negative correlation in all quadrants, except for the temporal quadrant (p=0.75) [15]. In this study, a positive correlation between axial length and uncorrected pRNFL thickness was observed in the temporal quadrant (r=0.17, p=0.015). It has been found that the Ganglion Cell Complex (GCC) thickness value in OCT is least affected by the ocular magnification error induced by axial length [29]. Since the temporal pRNFL is closer to the macula, its value may be less affected by the magnification error induced by axial length. This could explain the absence of a negative correlation between uncorrected temporal quadrant pRNFL thickness and axial length [30,31]. It is important to note that all the aforementioned studies did not apply correction for the ocular magnification error induced by axial length.

In this study, after applying Littmann's formula for the correction of ocular magnification, the previously observed negative correlation between uncorrected pRNFL thickness and axial length disappeared in the average, superior quadrant, and inferior quadrant. However, a statistically significant negative correlation between corrected pRNFL thickness and axial length persisted in the nasal quadrant. This can be attributed to the fact that the nasal side of the disc is maximally distant from the macula, where the readings of OCT are least affected by ocular magnification induced by axial length [30,31].

In a study conducted on 120 children in Turkey using RTVue SD-OCT, it was found that a negative correlation existed between axial length and pRNFL (r=0.818, p<0.001), which was eliminated after applying Littmann's formula (p>0.05) [9]. In another study conducted in Pakistan on 93 adult subjects using Topcon SD 1-Maestro OCT, similar results were found. RNFL thickness was negatively associated with axial length in the average as well as all four quadrants. However, after applying Littmann's formula for ocular magnification, the negative correlation was eliminated (all p>0.05) [13].

A major study conducted by Savini G et al., in Italy included 45 eyes from individuals aged 25 to 55 years. The eyes were divided into three categories: short (<22.5 mm), medium (22.51 to 25.5 mm), and long (>25.51 mm) axial lengths. They found a statistically significant negative correlation (r=-0.69, p<0.001) between axial length and RNFL thickness, which disappeared after applying Littmann's formula [16].

Another study conducted in Turkey with 154 subjects using Stratus OCT also found similar results. They observed a negative correlation between RNFL thickness and axial length in myopic individuals (r=-0.763, p<0.001) and hypermetropic individuals (r=-0.266, p<0.05). However, after applying Littmann's formula, the correlation disappeared [17].

A comparative assessment of similar studies is presented in [Table/Fig-7] [6,9,11,13,15-17,28].

No.	Author's name and year	Place of study	No. of eyes	Objective	Conclusion
1.	Dhami A et al., 2012 [11]	Dehradun, India	298 eyes	To correlate RNFL thickness and axial length in normal individuals with Fourier domain OCT.	RNFL thickness has an inverse correlation with axial length.
2.	Nagai-Kusuhara AN et al., 2007 [6]	Kobe, Japan	162 eyes	To investigate the influence of age, disc size and axial length on RNFL thickness measurements of HRT and OCT.	Axial length influenced RNFL thickness and Disc area measured by OCT.

3.	Chen CY et al., 2012-2014 [28]	Puzih, Taiwan	143 eyes	To interpret how thickness of pRNFL changes with increasing age , axial length or anterior chamber depth as measured by SD-OCT in normal elderly population in Taiwan.	Inverse correlation between RNFL thickness and axial length.
4.	Porwal S et al., 2013-2015 [15]	Tertiary care hospital, India	100 eyes	To evaluate RNFL thickness by OCT and correlate it with axial length and refractive error in myopes.	Significant decrease in RNFL thickness with an increase in the grade of myopia and axial length.
5.	Aykut V et al., 2013 [9]	Turkey	120 eyes of children	To evaluate influence of axial length on pRNFL thickness in Myopic, Hyperopic and emmetropic children by RTVue OCT.	Axial length influences pRNFL thickness. This appears to be due to ocular magnification effects associated with axial length and can be corrected for with the application of Littmann's Formula.
6.	Kausar A et al., 2016 [13]	Islamabad, Pakistan	93 eyes	To evaluate the effect of refractive error and axial length on pRNFL thickness in myopic, hypermetropic and emmetropic eyes by Topcon SD-OCT.	RNFL thickness measurements were found to vary with refractive status and axial length of the eye. Ocular magnification significantly affected RNFL thickness and should be considered in diagnosing glaucoma.
7.	Savini G et al., Oct 2009-June 2010 [16]	Bologna, Italy	45 eyes	To evaluate influence of axial length on measurement of RNFL thickness and Optic Nerve Head (ONH) parameters in healthy subjects.	Axial length influences the measurements of both RNFL thickness and Optic Nerve Head (ONH) parameters. Caution is recommended when comparing measured values of myopic and hyperopic eyes with normative database of the instrument.
8.	Öner V et al., 2011 [17]	Turkey	154 eyes	To investigate pRNFL thickness of myopic and hyperopic eyes in comparison with emmetropic control eyes and to evaluate the correlation between pRNFL thickness with axial length and spherical equivalent.	pRNFL thickness profile differed with refractive status and axial length of the eye. Ocular magnification effect should be taken into account.
9.	Present study, August 2020-October 2022	Pune, India	200 eyes	To determine the correlation between axial length and pRNFL thickness in healthy adults.	A negative correlation was established between pRNFL thickness and axial length which disappeared after applying correction for ocular magnification. The authors recommend using correction methods for effect of ocular magnification induced by axial length before considering the pRNFL thickness values of OCT to avoid misdiagnosis of Glaucoma in varying axial lengths.

[Table/Fig-7]: Review of similar studies [6,9,11,13,15-17,28].

Limitation(s)

The subjects were not evenly distributed among the three axial length subgroups due to the random selection criteria used in this study.

CONCLUSION(S)

A negative correlation was established between average pRNFL thickness and axial length. However, after applying correction for ocular magnification induced by axial length to the OCT pRNFL thickness measurements, there was no longer a negative correlation between average pRNFL thickness and axial length. The authors recommend the use of correction methods to account for the effect of ocular magnification induced by axial length before considering peripapillary retinal nerve fiber layer thickness values from OCT, in order to avoid misdiagnosis of glaucoma in individuals with varying axial lengths.

Further studies with larger sample sizes and greater variability in axial lengths are needed to improve our understanding of the correlation between pRNFL thickness and axial length. Additionally, it would be beneficial for newer OCT machines to have built-in software for correction of the effect of ocular magnification.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: May 09, 2023
- Manual Googling: Aug 16, 2023
- iThenticate Software: Oct 05, 2023 (17%)

ETYMOLOGY: Author Origin

EMENDATIONS: 6

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **May 06, 2023**
Date of Peer Review: **Aug 08, 2023**
Date of Acceptance: **Oct 07, 2023**
Date of Publishing: **Dec 01, 2023**