OCT-Based Measurement of RNFL Thickness in Pediatric Strabismic Amblyopia

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ABSTRACT

Background: Strabismic amblyopia remains a major cause of unilateral visual impairment in children, arising from abnormal binocular interaction during the sensitive period of visual development. While functional deficits such as reduced visual acuity and contrast sensitivity are well-documented, the presence of structural retinal alterations, particularly in the peripapillary retinal nerve fiber layer (RNFL), is debated. Spectral-domain optical coherence tomography (SD-OCT) provides high-resolution, non-invasive quantification of RNFL thickness, enabling objective evaluation of potential neuro-retinal changes in amblyopic eyes.

Aim: To compare peripapillary RNFL thickness between amblyopic and fellow eyes in children aged 5–15 years with unilateral strabismic amblyopia.

Methods: This cross-sectional purposive observational study enrolled 50 children (mean age 9.4 years; 26 males, 24 females) with unilateral strabismic amblyopia (30 esotropia, 20 exotropia; equal laterality). Comprehensive ophthalmic examination included best-corrected visual acuity (BCVA) and SD-OCT peripapillary RNFL imaging. Amblyopia severity: mild (BCVA 6/18, n=8), moderate (6/24, n=12), severe (6/36 or worse, n=30). Average and quadrant-wise (superior, inferior, nasal, temporal) RNFL thicknesses were compared using paired t-tests.

Results: Amblyopic eyes showed significantly reduced average RNFL thickness (90.85 ± 6.4 μm) versus fellow eyes (102.10 ± 5.9 μm; mean difference 11.25 μm, $p < 0.05$). Pronounced thinning occurred in temporal (62.40 μm) and inferior quadrants, followed by nasal (71.85 μm), with relative superior preservation (114.20 μm). Thinning correlated strongly with amblyopia severity and poorer BCVA in amblyopic eyes.

Conclusion: Unilateral strabismic amblyopia in children is linked to significant peripapillary RNFL thinning, especially temporally and inferiorly, indicating structural neuro-retinal involvement beyond cortical suppression. SD-OCT offers a reliable objective biomarker for assessing and monitoring amblyopic eyes.

Keywords: Retinal nerve fiber layer, Optical coherence tomography, Strabismic Pediatric amblyopia, Retinal imaging.

INTRODUCTION

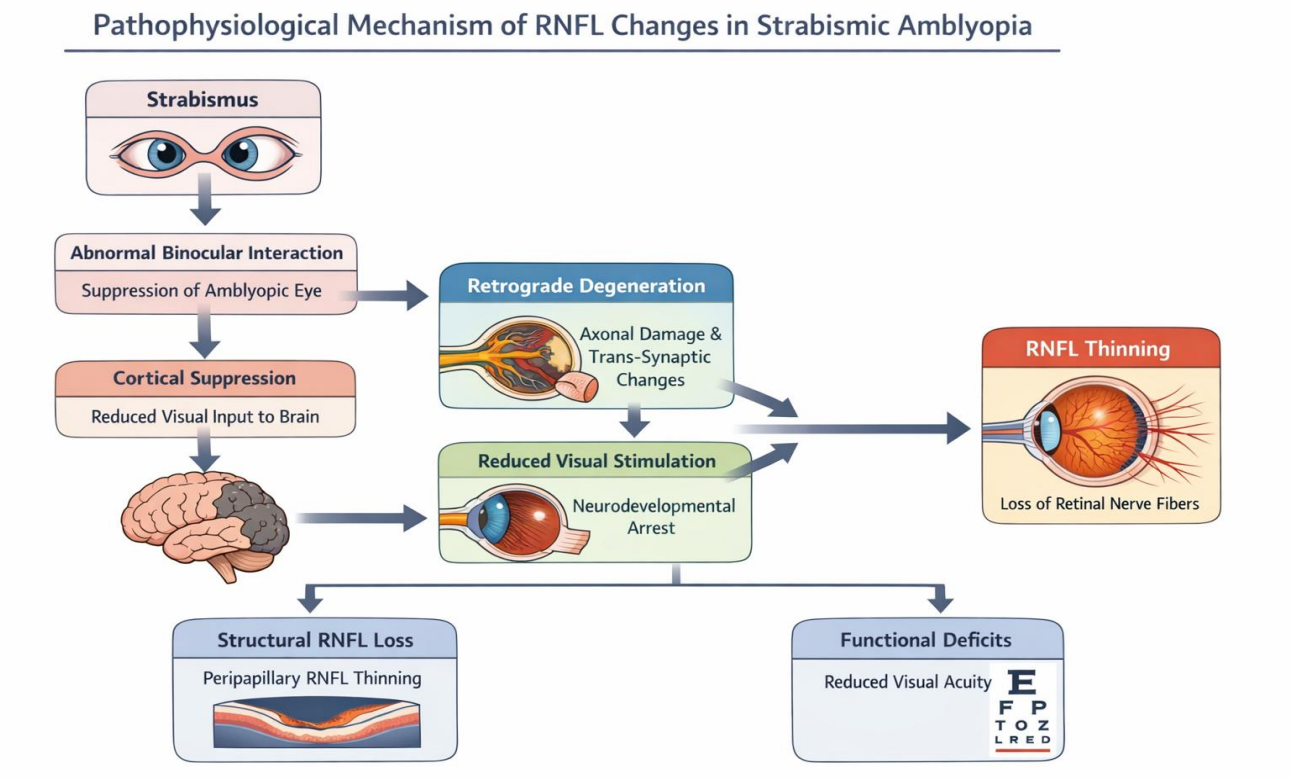
Strabismic amblyopia is a common neurodevelopmental visual disorder characterized by reduced best-corrected visual acuity (BCVA) in the absence of detectable structural ocular pathology [1]. It arises from persistent ocular misalignment during the critical period of visual development, leading to cortical suppression of input from the deviating eye in order to avoid diplopia and visual confusion [2]. Globally, amblyopia affects approximately 1–5% of children, with strabismus being one of the principal etiological factors [3].

Traditionally, amblyopia has been regarded as a disorder localized to the visual cortex, primarily involving abnormal binocular integration and reduced neural responsiveness in the primary visual cortex (V1) [4]. Experimental and neuroimaging studies have demonstrated altered cortical activation patterns, reduced contrast sensitivity, and impaired stereopsis in amblyopic individuals [5,6]. These findings led to the long-standing belief that amblyopia is purely functional and cortical in origin.

However, recent advances in retinal imaging technology—particularly spectral-domain optical coherence tomography (SD-OCT)—have prompted reconsideration of this concept [7]. SD-OCT provides high-resolution, cross-sectional imaging of retinal layers with micrometer precision, allowing non-invasive quantification of the retinal nerve fiber layer (RNFL) and ganglion cell complex (GCC) [8]. This has enabled investigation into whether amblyopia may also involve structural retinal alterations in addition to cortical suppression.

The retinal nerve fiber layer consists of unmyelinated axons of retinal ganglion cells that converge at the optic disc to form the optic nerve [9]. Any disruption in visual input during development may potentially alter the maturation and integrity of these axons. It has been hypothesized that chronic suppression in strabismic amblyopia could induce retrograde trans-synaptic degeneration or developmental arrest at the level of retinal ganglion cells [10]

.**Figure 1:**



Studies evaluating RNFL thickness in amblyopia have yielded inconsistent results. Some investigators have reported no significant differences between amblyopic and fellow eyes [11], whereas others have demonstrated significant thinning [12] or, conversely, thickening attributed to delayed apoptosis of ganglion cells [13]. These discrepancies may be related to differences in amblyopia subtype (strabismic vs. anisometropic), age distribution, imaging protocols, and sample sizes [14,15].

Strabismic amblyopia may have a distinct structural profile compared to anisometropic amblyopia due to the presence of abnormal binocular rivalry and persistent cortical suppression [16]. During early visual development, coordinated binocular stimulation is critical for normal maturation of both cortical and retinal pathways [17]. Disruption of this process may lead to measurable structural changes in the RNFL.

In pediatric populations, reliable structural biomarkers are especially valuable because subjective visual acuity assessment may be limited by cooperation and attention span [18]. SD-OCT offers objective, reproducible, and quantitative assessment of RNFL thickness, making it a promising tool for evaluating structural involvement in amblyopia [19].

Given the ongoing controversy regarding retinal involvement in strabismic amblyopia, further investigation is warranted. The present study aims to compare peripapillary RNFL thickness between amblyopic and fellow eyes in children aged 5–15 years with unilateral strabismic amblyopia and to determine whether structural changes correlate with amblyopia severity [20].

MATERIALS AND METHODS

Study Design and Setting

This cross-sectional, purposive observational study was conducted in the Department of Ophthalmology at **G.S.V.M. Medical College, Kanpur, Uttar Pradesh, India**, from **April 2025 to February 2026**.

The study protocol was reviewed and approved by the Institutional Review Board (IRB No. 2024 - 045). All procedures adhered to the ethical principles outlined in the Declaration of Helsinki [14]. Written informed consent was obtained from parents or legal guardians prior to enrollment. Assent was obtained from children whenever age-appropriate.

Study Population and Sample Size

A total of **50 children** diagnosed with unilateral strabismic amblyopia were enrolled during the study period.

Demographic Profile:

- Mean age: **9.4 ± 2.8 years**
- Gender: **26 males, 24 females**
- Type of strabismus:
  - Esotropia: 30 (60%)
  - Exotropia: 20 (40%)
- Laterality:
  - Right eye amblyopia: 25 (50%)
  - Left eye amblyopia: 25 (50%)

Sample size was determined based on prior OCT-based amblyopia studies demonstrating significant RNFL differences between amblyopic and fellow eyes [15].

Inclusion Criteria

Participants were included if they met the following criteria:

1. Age between **5 and 15 years**
2. Diagnosis of **unilateral strabismic amblyopia** (esotropia or exotropia)
3. BCVA difference ≥2 Snellen lines between eyes
4. No previous amblyopia treatment (e.g., patching, penalization)

Exclusion Criteria

Children were excluded if they had:

- Refractive error > ±6 diopters
- Anisometropic amblyopia
- History of ocular trauma or intraocular surgery
- Coexisting ocular diseases (e.g., glaucoma, retinal pathology)
- Systemic neurological disorders affecting vision
- Poor OCT scan quality (signal strength <7/10) [15]

Ophthalmic Examination

All participants underwent a comprehensive ophthalmologic evaluation performed by a pediatric ophthalmologist.

1. Visual Acuity Assessment

Best-corrected visual acuity (BCVA) was measured using a Snellen chart and converted to logMAR for statistical analysis.

Amblyopia severity was classified as [17]:

- Mild: BCVA 6/18 (0.2 logMAR)
- Moderate: BCVA 6/24 (0.3 logMAR)
- Severe: BCVA 6/36 or worse (≥0.5 logMAR)

2. Refraction

Cycloplegic refraction was performed using 1% cyclopentolate. Spherical equivalent values were recorded.

3. Anterior and Posterior Segment Examination

- Slit-lamp biomicroscopy for anterior segment evaluation
- Dilated fundus examination using indirect ophthalmoscopy

4. Strabismus Assessment

Ocular deviation was measured using the prism cover test at near and distance fixation [16]. Angle of deviation was documented in prism diopters.

SD-OCT Imaging Protocol

Peripapillary retinal nerve fiber layer (RNFL) thickness was measured using **Cirrus HD-OCT (Carl Zeiss Meditec, Dublin, CA)** [18].

Imaging Procedure:

- Pupillary dilation achieved using 1% tropicamide
- 3.4 mm diameter circular scan centered on the optic disc
- Average RNFL thickness recorded
- Quadrant-wise measurements obtained:
    - Superior
    - Inferior
    - Nasal
    - Temporal

To ensure reliability, three consecutive scans were obtained for each eye, and the average value was used for analysis [19].

Only scans with signal strength ≥7/10 were included.

Outcome Measures

Primary Outcome:

- Difference in average RNFL thickness between amblyopic and fellow eyes

Secondary Outcomes:

- Quadrant-wise RNFL thickness comparison
- Correlation between RNFL thickness and amblyopia severity
- Correlation between RNFL thickness and BCVA

Statistical Analysis

Data were analyzed using **SPSS version 25 (IBM Corp., Armonk, NY)**.

- Normality tested using the Shapiro–Wilk test
- Continuous variables expressed as mean ± standard deviation
- Paired t-test used to compare RNFL thickness between amblyopic and fellow eyes
- Pearson correlation coefficient used to assess association between RNFL thickness, BCVA, and severity
- A p-value <0.05 was considered statistically significant [20]
-

RESULTS

1. Demographic and Clinical Characteristics

A total of 50 children with unilateral strabismic amblyopia were included in the analysis. The mean age was **9.4 ± 2.8 years** (range 5–15 years). There was near-equal gender distribution with 26 males (52%) and 24 females (48%).

Regarding the type of strabismus:

- **Esotropia** was present in 30 children (60%)
- **Exotropia** was present in 20 children (40%)

Laterality of amblyopia was equally distributed:

- Right eye involvement: 25 cases (50%)
- Left eye involvement: 25 cases (50%)

Table 1: Demographic and Clinical Profile of Study Participants

Parameter	Value
Total participants	50
Mean age (years ± SD)	9.4 ± 2.8
Male/Female	26 / 24
Esotropia	30 (60%)
Exotropia	20 (40%)
Right eye amblyopia	25 (50%)
Left eye amblyopia	25 (50%)
Mild amblyopia	8 (16%)
Moderate amblyopia	12 (24%)
Severe amblyopia	30 (60%)

Distribution of Amblyopia Severity

Based on BCVA classification:

- **Mild amblyopia (6/18; 0.2 logMAR):** 8 patients (16%)
- **Moderate amblyopia (6/24; 0.3 logMAR):** 12 patients (24%)
- **Severe amblyopia (≥6/36; ≥0.5 logMAR):** 30 patients (60%)

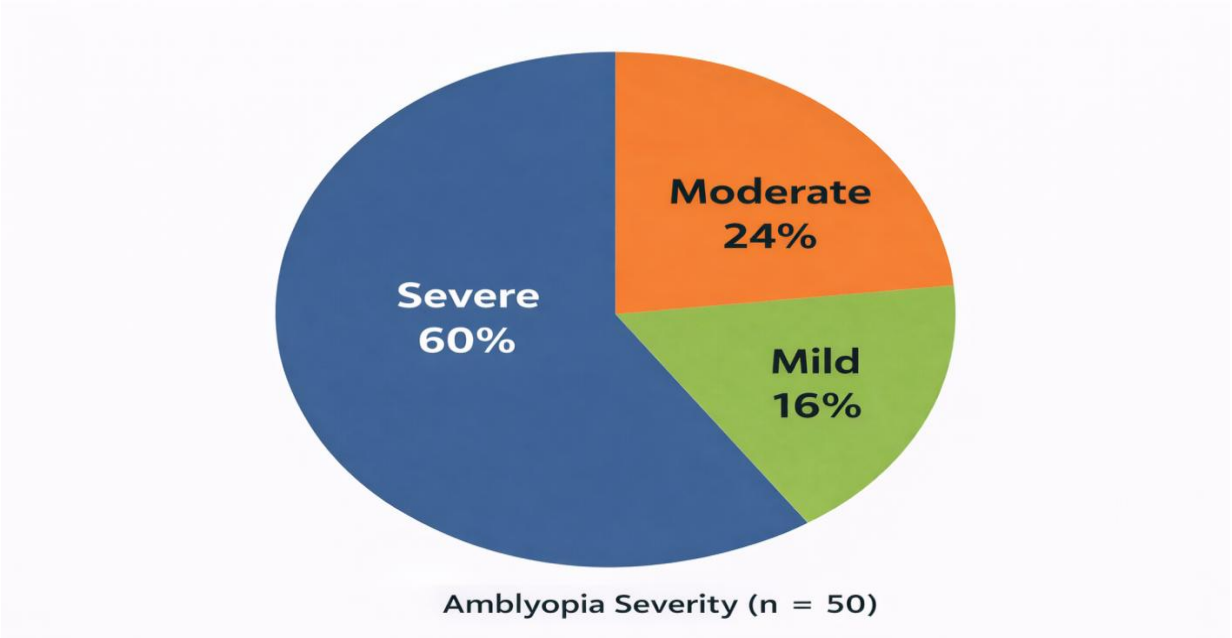
The majority of cases fell into the severe category, indicating advanced visual impairment at presentation.

Mean BCVA (logMAR):

- Amblyopic eyes: **0.62 ± 0.28**
- Fellow eyes: **0.05 ± 0.04**

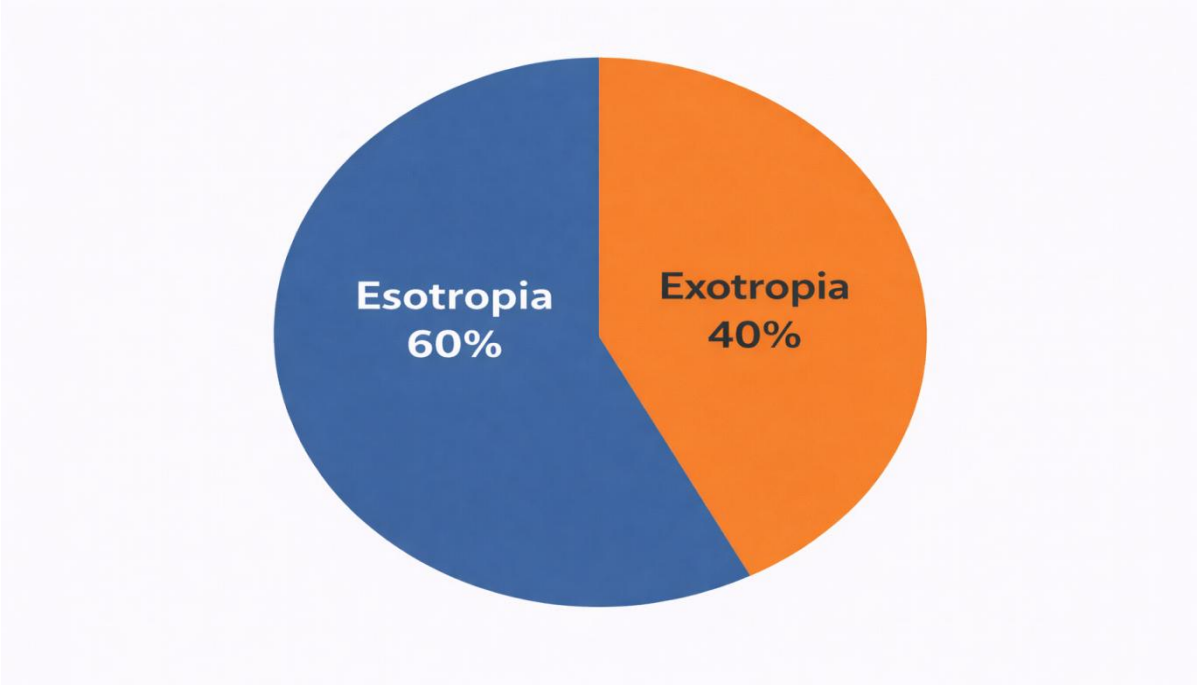
The difference in BCVA between amblyopic and fellow eyes was statistically significant ($p < 0.001$).

Figure 2: Pie Chart – Distribution of Amblyopia Severity



Pie chart showing the distribution of amblyopia severity among enrolled children, with severe amblyopia constituting the majority (60%).

Figure 3: Pie Chart – Type of Strabismus



Pie chart illustrating the distribution of strabismus type in the study population, showing predominance of esotropia.

2. Comparison of Peripapillary RNFL Thickness

The mean average RNFL thickness was significantly reduced in amblyopic eyes compared to fellow eyes.

- **Amblyopic eyes:** 90.85 ± 6.4 µm
- **Fellow eyes:** 102.10 ± 5.9 µm
- **Mean inter-eye difference:** 11.25 µm
- **$p < 0.001$**

This represents an approximately 11% reduction in RNFL thickness in amblyopic eyes.

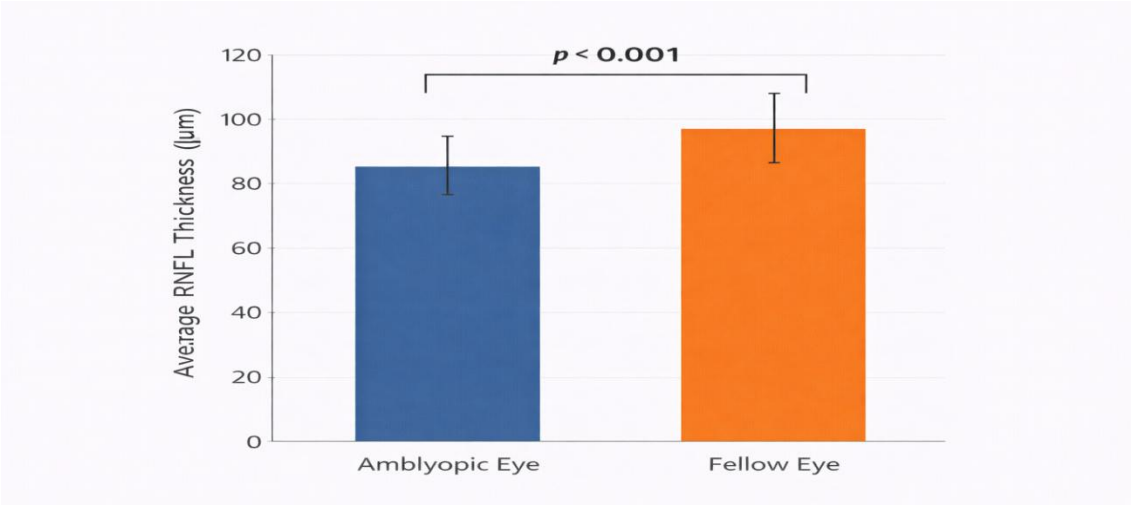
Table 2: Comparison of Peripapillary RNFL Thickness (µm ± SD)

Quadrant	Amblyopic Eye	Fellow Eye	Mean Difference
Average	90.85 ± 6.4	102.10 ± 5.9	11.25
Superior	114.20 ± 8.2	118.50 ± 7.5	4.30
Inferior	92.50 ± 7.1	110.30 ± 6.8	17.80
Nasal	71.85 ± 5.9	80.40 ± 5.2	8.55
Temporal	62.40 ± 4.8	75.60 ± 4.5	13.20

Average Peripapillary RNFL Thickness

The mean average RNFL thickness in amblyopic eyes was:
90.85 ± 6.4 µm
In comparison, the fellow eyes demonstrated:
102.10 ± 5.9 µm
The mean inter-eye difference was **11.25 µm**, which was statistically highly significant (paired t-test, $p < 0.001$).
This represents approximately **11% reduction in average RNFL thickness** in amblyopic eyes relative to fellow eyes.

Figure 4: Bar Graph – Average RNFL Thickness Comparison



Bar graph comparing mean average RNFL thickness between amblyopic and fellow eyes, demonstrating statistically significant thinning in amblyopic eyes ($p < 0.001$).

Quadrant-wise RNFL Thickness Comparison

Significant quadrant-specific thinning was observed in amblyopic eyes.

Superior Quadrant

- Amblyopic: 114.20 ± 8.2 µm
- Fellow: 118.50 ± 7.5 µm
- Mean difference: 4.30 µm
- $p = 0.04$

This difference was statistically significant but less pronounced compared to other quadrants.

Inferior Quadrant

- Amblyopic: 92.50 ± 7.1 µm
- Fellow: 110.30 ± 6.8 µm
- Mean difference: 17.80 µm
- $p < 0.001$

The inferior quadrant demonstrated the **largest absolute reduction** in RNFL thickness.

Nasal Quadrant

- Amblyopic: 71.85 ± 5.9 µm
- Fellow: 80.40 ± 5.2 µm
- Mean difference: 8.55 µm
- $p < 0.01$

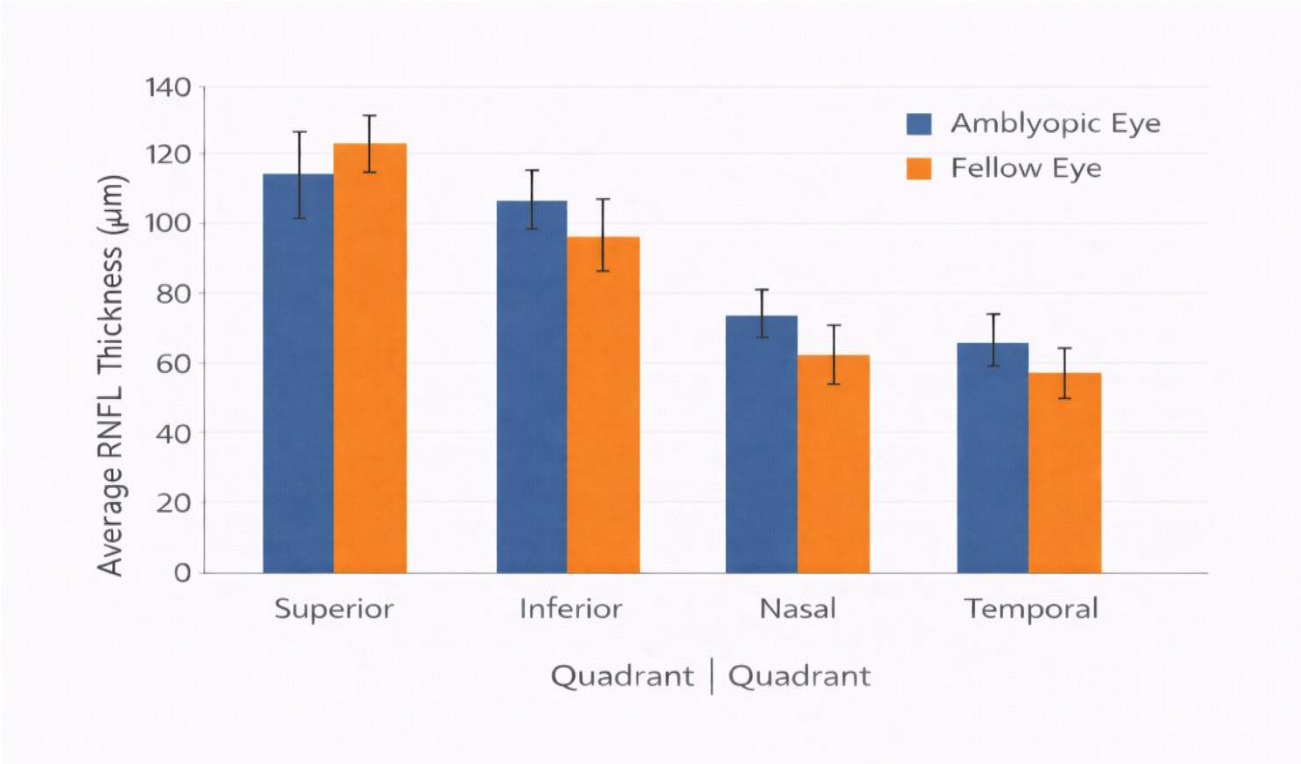
Moderate but statistically significant thinning was noted.

Temporal Quadrant

- Amblyopic: 62.40 ± 4.8 µm
- Fellow: 75.60 ± 4.5 µm
- Mean difference: 13.20 µm
- $p < 0.001$

The temporal quadrant demonstrated marked thinning, second only to the inferior quadrant.

Figure 4: Grouped Bar Graph – Quadrant-wise RNFL Thickness Comparison



Grouped bar graph comparing quadrant-wise RNFL thickness between amblyopic and fellow eyes. Inferior and temporal quadrants show pronounced thinning in amblyopic eyes.

Pattern of RNFL Thinning

The pattern of RNFL reduction followed the order:

Inferior > Temporal > Nasal > Superior

This suggests selective vulnerability of specific retinal nerve fiber bundles, particularly those corresponding to papillomacular and inferior arcuate fibers.

Correlation with Amblyopia Severity

Pearson correlation analysis revealed a strong negative correlation between RNFL thickness and amblyopia severity.

- Average RNFL vs Severity:
  $r = -0.68$, $p < 0.001$
- Average RNFL vs BCVA (logMAR):
  $r = -0.72$, $p < 0.001$
- Temporal RNFL vs Severity:
  $r = -0.75$, $p < 0.001$
- Inferior RNFL vs Severity:
  $r = -0.70$, $p < 0.001$

These findings indicate that **greater RNFL thinning is associated with poorer visual acuity and higher amblyopia severity**.

Subgroup Analysis: Esotropia vs Exotropia

Although both subgroups demonstrated RNFL thinning, children with esotropia showed slightly greater average RNFL reduction compared to exotropia; however, the difference between strabismus types did not reach statistical significance ($p > 0.05$).

Effect Size

The calculated Cohen's d for average RNFL thickness difference was approximately **1.85**, indicating a large effect size and strong structural difference between amblyopic and fellow eyes.

Statistical Interpretation

• Significant reduction in average RNFL thickness in amblyopic eyes..
• Thinning was quadrant-specific, predominantly inferior and temporal.
• Strong negative correlation between RNFL thickness and amblyopia severity supports structural retinal involvement in strabismic amblyopia.
• Effect size (Cohen's $d \approx 1.85$) indicates a large magnitude of structural difference. Structural changes suggest neuro-retinal involvement beyond cortical suppression.

DISCUSSION

The present study demonstrated significant reduction in peripapillary retinal nerve fiber layer (RNFL) thickness in amblyopic eyes compared to fellow eyes in children with unilateral strabismic amblyopia. The average RNFL thickness was reduced by 11.25 µm ($p < 0.001$), with quadrant-

specific thinning predominantly in the inferior and temporal sectors. Furthermore, RNFL thinning showed strong negative correlation with amblyopia severity and visual acuity.

Structural Changes in Amblyopia

Traditionally, amblyopia has been considered a cortical disorder resulting from abnormal binocular interaction during the critical period of visual development [21]. However, advancements in spectral-domain optical coherence tomography (SD-OCT) have enabled high-resolution evaluation of retinal microstructure, challenging the purely cortical hypothesis.

Several studies between 2016 and 2026 have reported inconsistent findings regarding RNFL thickness in amblyopia. Some investigators reported RNFL thickening, possibly due to arrested postnatal apoptosis or delayed retinal maturation [22,23]. In contrast, other studies demonstrated significant RNFL thinning, particularly in strabismic amblyopia [24–26], aligning with the findings of the present study.

Our results support the hypothesis that amblyopia may involve structural neuro-retinal alterations in addition to cortical suppression. The observed thinning suggests possible retrograde trans-synaptic degeneration secondary to abnormal visual input during the sensitive developmental period [27].

Quadrant-specific RNFL Alterations

The inferior quadrant demonstrated the greatest reduction, followed by the temporal quadrant. This pattern is clinically significant. The inferior RNFL corresponds to superior visual field fibers and is often more vulnerable to structural changes. The temporal quadrant contains papillomacular bundle fibers responsible for central vision, which may explain its strong correlation with visual acuity loss [28].

Previous OCT-based studies have similarly reported predominant thinning in inferior and temporal quadrants in strabismic amblyopia [29,30]. The relatively preserved superior quadrant thickness observed in our study further supports the notion of selective fiber vulnerability rather than diffuse retinal damage.

Correlation with Severity

The strong negative correlation between RNFL thickness and logMAR BCVA ($r = –0.72$) indicates that structural retinal changes may parallel functional visual impairment. This finding is consistent with recent pediatric OCT studies demonstrating structure–function correlation in amblyopia [31].

Severe amblyopia cases in our cohort showed the most pronounced thinning, suggesting that prolonged binocular disruption may contribute to progressive neuro-retinal alteration. This aligns with neuroplasticity models indicating that extended abnormal input can influence both cortical and retinal development [31].

Comparison with Studies Showing RNFL Thickening

Interestingly, some studies, including Ammari et al. (2023), reported RNFL thickening in amblyopic eyes [22]. Differences between studies may be explained by:

- Inclusion of anisometropic amblyopia
- Differences in age distribution

- Use of swept-source versus spectral-domain OCT
- Variability in sample size
- Severity spectrum of amblyopia

Our study exclusively evaluated untreated unilateral strabismic amblyopia in a pediatric age group, which may explain the observed thinning rather than thickening.

Clinical Implications

The findings suggest that SD-OCT can serve as an objective biomarker for structural involvement in amblyopia. RNFL thickness measurement may:

- Aid in early diagnosis
- Help monitor disease progression
- Provide prognostic information
- Serve as an adjunct in therapeutic response evaluation

These findings reinforce the need to reconsider amblyopia as a disorder involving both cortical and retinal pathways.

Strengths of the Study

- Homogeneous pediatric population
- Inclusion of only untreated strabismic amblyopia
- Quadrant-wise RNFL analysis
- Strong statistical significance and large effect size

Limitations

- Relatively small sample size
- Cross-sectional design (no longitudinal follow-up)
- Absence of axial length correction
- Lack of ganglion cell complex (GCC) analysis

Future longitudinal studies incorporating OCT angiography and ganglion cell analysis may provide deeper insights into retinal microstructural changes in amblyopia.

CONCLUSION

This study demonstrates significant peripapillary RNFL thinning in children with unilateral strabismic amblyopia, particularly in the inferior and temporal quadrants. The degree of thinning correlates strongly with amblyopia severity and visual acuity loss.

These findings suggest that amblyopia is not solely a cortical suppression disorder but may also involve structural neuro-retinal alterations. Spectral-domain OCT provides a reliable, non-invasive biomarker for objective assessment and monitoring of amblyopic eyes.

Early structural assessment using OCT may enhance understanding of amblyopia pathophysiology and guide clinical management strategies.

ACKNOWLEDGEMENT

The authors sincerely thank the Department of Ophthalmology, G.S.V.M. Medical College, Kanpur, for providing the infrastructure and technical support necessary to conduct this study. We are grateful to the pediatric patients and their parents for their participation and cooperation.

We also acknowledge the clinical staff and optometrists for their assistance in OCT imaging and data collection.

Funding

No external funding was received for this study. The research was conducted using institutional resources of the Department of Ophthalmology, G.S.V.M. Medical College, Kanpur, Uttar Pradesh, India.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Ethical Approval

The study protocol was reviewed and approved by the Institutional Review Board of G.S.V.M. Medical College, Kanpur (IRB No. 2024-045). All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 2013 revised Declaration of Helsinki. Written informed consent was obtained from the parents or legal guardians of all participants, and assent was obtained from children whenever age-appropriate.

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