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Can retinal nerve fiber layer thickness be an indicator of amblyopia – Opening new horizons

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ABSTRACT

Aims: To study the RNFL thickness in amblyopia at Institute of Ophthalmology, JNMCH, AMU, Aligarh.**Design of Study:** Prospective cross-sectional study.**Material and Methods:** This study was conducted on 30 amblyopic children of age ranging between 6-16 years at Institute of Ophthalmology, Jawaharlal Nehru Medical College and Hospital, AMU, Aligarh after taking institutional ethical clearance and informed consent from parents of the patients. A brief clinical history was taken and detailed eye examination was done for visual acuity. Evaluation and quantification of strabismus was done. SD- OCT was performed after dilatation of pupil with 1% cyclomid eye drop on all enrolled children for evaluation of RNFL thickness and GCC thickness. RNFL thickness and GCC thickness of amblyopic eyes were compared with the fellow eyes.**Statistical Analysis:** Data entry and analysis was done using paired t-test and p- values <0.05 was considered statistically significant. JASP application version 0.16.4.0 was used for statistical analysis of data and results.**Results:** Out of 30 children, 23 were anisometric amblyopic and 7 were mixed amblyopic. Average RNFL thickness was more in amblyopic eye as compared to fellow eye. This difference in RNFL thickness between the eyes remained statistically significant in anisometric amblyopic eyes ($p < 0.001$) but not in mixed amblyopic eyes ($p = 0.50$). No significant difference was found on comparing GCC thickness between the eyes in both the groups (anisometric amblyopic and mixed amblyopic) ($p = 0.88, 0.30$).**Conclusion:** Amblyopic eyes have more RNFL thickness than fellow eyes. Therefore, we conclude that amblyopia may involve retinal structure, also exploding the old age myth that only cortical changes are responsible.This is an Open Access (OA) journal, and articles are distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License](https://creativecommons.org/licenses/by-nc-sa/4.0/), which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.For reprints contact: reprint@ipinnovative.com

1. Introduction

The term “amblyopia” refers to impaired vision in one or both the eyes due to unusual visual encounter during cortical plasticity and visual system development. Clinically it is considered disparity between the eyes of at least 2 or more lines on visual acuity chart.¹ With a worldwide prevalence of approximately 1-5%, amblyopia is considered the most frequent cause of unilaterally impaired visual

acuity in children.²⁻⁵ Causes includes strabismus usually esotropia during infancy or early childhood, anisometropia, combination of both strabismus and anisometropia and visual deprivation. It has a psychosocial impact on individuals and has a significant economic burden.⁶⁻⁸ It may result in a irreversible impairment of vision if it is left untreated or because of delayed treatment. Early diagnosis and treatment play a vital role in preventing blindness and visual impairment due to amblyopia.

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Amblyopia develops during critical period, estimated to be 7-8 years, when the connection between retina and the cerebral cortex is growing and maturing.^{6,9,10} After this critical period of neuroplasticity, the decrease in visual acuity is irreversible as demonstrated by Holmes et al.¹¹ Following Hubel and Wiesel's pioneering work in the understanding of the pathophysiology of amblyopia, studies have demonstrated distinct alterations in the visual cortex and lateral geniculate body areas in amblyopic eyes. It is hypothesized that apoptosis is further inhibited in amblyopic eyes, so retinal nerve fiber layer thickness is likely to be higher.^{12–15}

With the advancement of new modality in imaging, Optical Coherence Tomography (OCT) provides a new horizon in understanding the etiopathology of amblyopia and highlights the morphological and structural changes in the retina of amblyopic eye. It is a high-resolution, cross-sectional tomographic imaging technique of retina and optic nerve head and it is non-invasive too.

2. Materials and Methods

It was a prospective cross-sectional study carried out on 30 children having unilateral amblyopia at Institute of Ophthalmology, Jawaharlal Nehru Medical College and Hospital, A.M.U, Aligarh from December 2020 to September 2022 after taking clearance from Institutional Ethical Committee which was according to the Declaration of Helsinki. Informed consent was taken from parents of patients. SD-OCT was performed on all 30 recruited children to measure RNFL thickness of both the eyes and amblyopic eye RNFL thickness compared with the fellow eye.

2.1. Inclusion criteria

All cooperative children aged 6-16 years visiting ophthalmology OPD, with parents consenting and fulfilling the following criteria: Unilateral strabismic amblyopia or mixed amblyopia, anisometropic amblyopia

2.2. Exclusion criteria

Parent not consenting, Children less than 6 and more than 16 years Patients with history of glaucoma and cataract, Patients with neurological disease Patients with no media clarity (corneal opacity, vitreous haemorrhage) & all uncooperative patients A brief clinical history was taken including, detailed history of onset of poor vision, history of wearing glasses and its compliance, history of strabismus, nystagmus, previous intraocular surgery, family history of strabismus. General and systemic examination were done to rule out any associated illnesses. Detailed eye examination was done including visual acuity with logMAR chart, best-corrected spectacle visual acuity was recorded and the appropriate correction was prescribed

to the patients, cycloplegic refraction was done with age appropriate cycloplegics, evaluation of strabismus and its quantification was done. Slit lamp examination and fundus examination were done to rule out any anterior segment and posterior segment pathology. SD-OCT (Cirrus HD-OCT, Carl Zeiss Meditec Inc, Dublin CA) was done on all patients, and retinal nerve fiber layer thickness and ganglion cell complex thickness were recorded for both the eyes. All OCT measurements were taken by the same investigator after dilatation of pupil to at least 5 mm. Multiple scans were taken and scans were accepted only if they were free from artifacts and had signal strengths of ≥ 6 . For analysis of RNFL thickness, each eye was scanned with the optic disc cube 200×200 scan and average RNFL thickness around a circle of 3.4 mm diameter. For ganglion cell analysis, data from the same scans were processed and evaluated. JASP application version 0.16.4.0 was used for statistical analysis. Comparison of RNFL thickness and GCC thickness was made by applying paired t-test. The categorical data was expressed in percentage, whereas continuous data was expressed in mean and standard deviation, and p-values <0.05 was considered statistically significant.

3. Results

This study included 30 children of the mean age 13.36 (2.81) years, ranged between 6-16 years. Out of these, 12 (40%) were females and 18 (60%) were males.

The mean (SD) BCVA in logMAR of 30 children in amblyopic eyes was 0.58 (0.21), and in fellow eyes was 0.09 (0.11), while median in amblyopic eyes was 0.55, and in normal fellow eyes was 0.00.

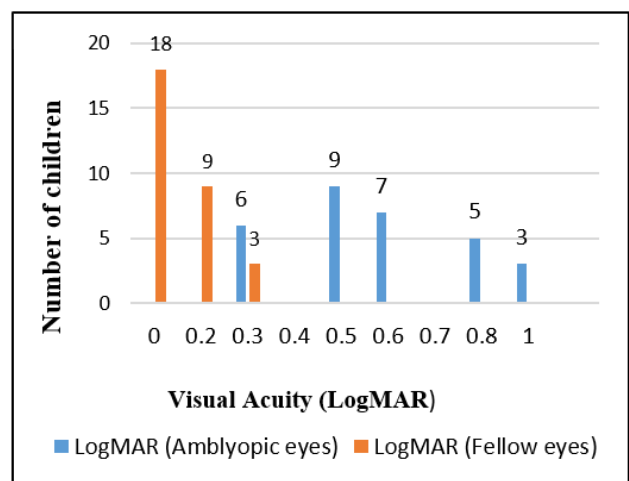


Figure 1: Distribution plot for BCVA in LogMAR for amblyopic eyes and fellow eyes

Out of 30 children, 23 (76.7%) of them were noted as anisometropic amblyopia while 7 (23.3%) of them were of mixed amblyopia. Among anisometropic amblyopia,

Table 1: Statistical analysis of comparison of BCVA (logMAR) between amblyopic eyes and fellow eyes

Variables	Mean Difference	Standard Error Difference	95% CI of the Difference		p- value
			Lower	Upper	
BCVA [logMAR] (amblyopic eyes- fellow eyes)	0.49	0.04	0.41	0.57	<0.001

6 were myopes and 17 were hypermetropes. The mean BCVA in logMAR in their amblyopic eyes was 0.57 ± 0.18 (median 0.60) and in their fellow eyes was 0.08 ± 0.11 (median 0.00). The mean spherical equivalents in myopes was 5.70 ± 1.70 and in hypermetropes was 2.97 ± 1.95 in their amblyopic eyes while 4.41 ± 2.36 and 1.23 ± 1.57 in their fellow eyes. Among mixed amblyopia 4 were noted as esotropia and 3 were noted as exotropia. The mean BCVA in logMAR in their amblyopic eyes was 0.60 ± 0.29 (median 0.50) and in their fellow eyes was 0.13 ± 0.12 (median 0.20). The mean spherical equivalents of myopic correction was 4.41 ± 3.62 and of hypermetropic correction was 3.25 ± 1.89 in amblyopic eyes while 1.75 ± 2.41 and 1.87 ± 1.93 in their fellow eyes respectively.

3.1. Retinal nerve fiber layer thickness assessment by using SD-OCT

Mean of average RNFL thickness in amblyopic eyes of 30 children was 95.00 ± 13.13 and in their fellow eyes was 88.03 ± 12.63 .

Table 2 shows that the mean of average RNFL thickness in amblyopic eyes was more as compared to fellow eyes. This difference was statistically significant ($p < 0.00$).

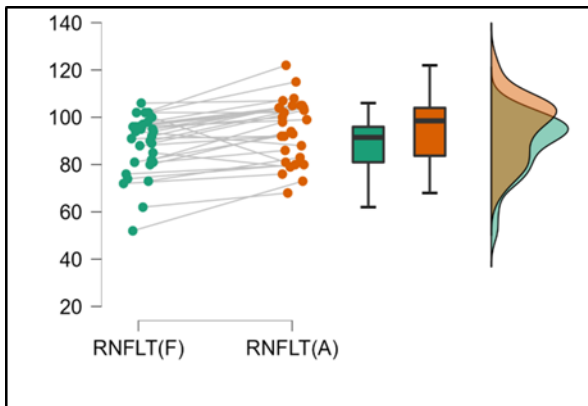


Figure 2: Comparison of average RNFL thickness for amblyopic eyes and fellow eyes

Raincloud plot shows mean of average RNFL thickness in amblyopic eyes was more as compared to normal fellow eyes.

3.2. Analysis of RNFL thickness in anisometric amblyopic children

Mean \pm SD of average RNFL thickness in amblyopic eyes was 96.78 ± 12.58 and in normal fellow eyes was 88.56 ± 12.12 .

Table 3 shows that the mean of average RNFL thickness in amblyopic eyes was more as compared to normal fellow eyes in anisometric amblyopic eyes ($p < 0.001$).

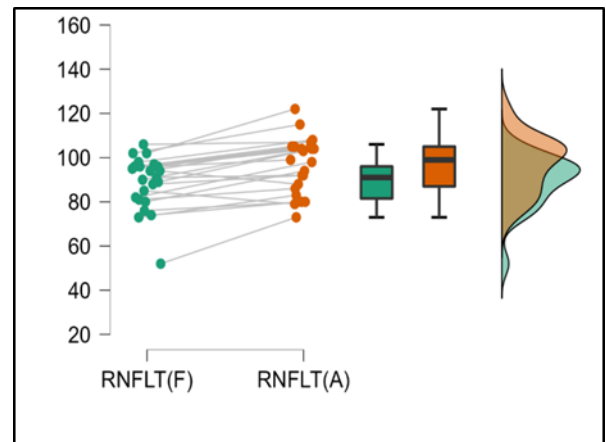


Figure 3: Comparison of average RNFL thickness for amblyopic eyes and fellow eyes in anisometric amblyopic group

Raincloud plot shows mean of average RNFL thickness in amblyopic eyes was more as compared to normal fellow eyes in anisometric amblyopic group.

3.3. Analysis of RNFL thickness in mixed amblyopic children

Mean \pm SD of average RNFL thickness in amblyopic eyes was 89.14 ± 14.17 and in normal fellow eyes was 86.28 ± 15.08 .

Table 4 shows that the mean of average RNFL thickness in amblyopic eyes was slightly more as compared to fellow eyes. This difference was statistically insignificant ($p < 0.50$).

Raincloud plot shows mean of average RNFL thickness in amblyopic eyes was more as compared to normal fellow eyes.

Table 2: Statistical analysis of comparison of average RNFL thickness in amblyopic eyes and fellow eyes

Variables	Mean Difference	Standard error Difference	t	95% CI for Mean Difference Lower Upper	p-value
Average RNFL Thickness (Amblyopic eyes-fellow eyes) (μm)	6.96	1.48	4.70	3.93 9.99	<0.001

Table 3: Comparison of mean RNFL thickness between amblyopic eyes and fellow eyes in anisometropic amblyopic group

Variables	Mean Difference	Standard error Difference	t	95% CI for Mean Difference Lower Upper	p-value
Average RNFL thickness (Amblyopic eyes-fellow eyes) (μm)	8.21	1.46	5.60	5.17 11.25	<0.001

Table 4: Comparison of mean RNFL thickness between amblyopic eyes and fellow eyes in mixed amblyopic group

Variable	Mean Difference	Standard error Difference	t	95% CI for Mean Difference Lower Upper	p-value
Average RNFL thickness (Amblyopic eyes fellow eyes)	2.85	3.99	0.71	-6.90 12.62	0.50

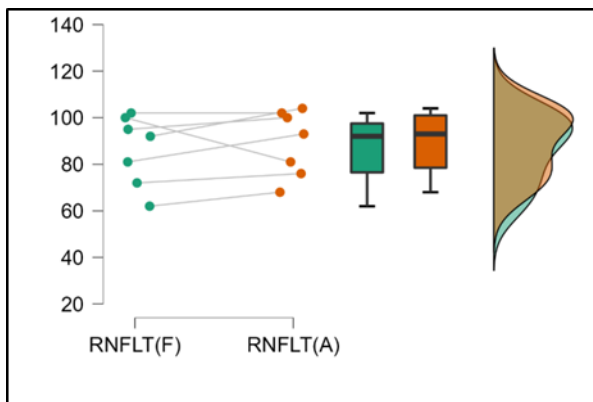


Figure 4: Comparison of average RNFL thickness for amblyopic eyes and fellow eyes in mixed amblyopic group

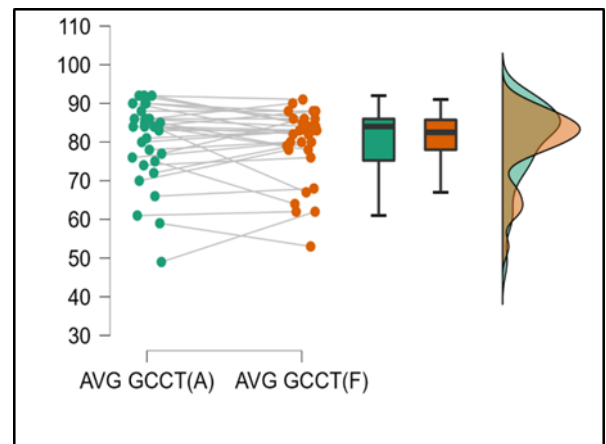


Figure 5: Comparison of average GCC thickness for amblyopic eyes and fellow eyes

3.4. Ganglion cell complex thickness assessment by using SD-OCT

Mean \pm SD of average GCC thickness in amblyopic eyes of 30 children was 79.96 ± 10.57 and in their fellow eyes was 79.30 ± 9.43 . Mean difference was 0.66 ± 1.17 . This difference was statistically insignificant ($p=0.57$).

Raincloud plot shows that the mean of average GCC thickness in amblyopic eyes was slightly more as compared to fellow eyes.

Table 5 shows that difference in GCC thickness between amblyopic eyes and fellow eyes among both the group is insignificant ($p=0.88$, $p=0.30$).

4. Discussion

Amblyopia is not just a disease to be treated, or not just a topic to be studied. It is a wide variety concept that is debatable and that needs to be explored. Earlier amblyopia was thought to be the condition that results from retinal abnormality but current research suggests that it is at the level of higher function of brain including lateral geniculate body and cerebral cortex that is responsible. Several studies and researches demonstrated that retinal ganglion cells can undergo changes with light deprivation from birth. It is yet to be determined whether there are any significant changes at the level of retina including retinal nerve fiber

Table 5: Analysis of GCC thickness in anisometropic amblyopic group and mixed amblyopic group

Average GCC thickness (Amblyopic eyes- fellow eyes)	Mean Difference	Standard error Difference	t	p-value
Anisometropic amblyopic group	0.17	1.20	-0.14	0.88
Mixed amblyopic group	3.42	3.04	1.12	0.30

layer and ganglion cell complex. In the present study, we included 30 children having anisometropic amblyopia and mixed amblyopia. In this study, mean of average RNFL thickness in amblyopic eyes of 30 children was 95.00 ± 13.13 and in their fellow eyes was 88.03 ± 12.63 . It was observed that, amblyopic eyes had thicker average RNFL thickness than the fellow eyes and difference in RNFL thickness between amblyopic eyes and fellow eyes was significant ($p < 0.001$). Our result was consistent with the study of Yen et al,¹⁶ Yoon et al,¹⁷ who reported thicker RNFL thickness in amblyopic eyes as compared to fellow eyes. Different results were observed by Kee et al,¹⁸ Repka et al¹⁹ and Ferat et al.²⁰ Further on comparing RNFL thickness in both the groups (anisometropic amblyopia and mixed amblyopia) we found that in anisometropic amblyopia, there was a significant difference in average RNFL thickness between amblyopic eye and their fellow eyes ($p < 0.001$). This significant difference was not noticed in mixed amblyopic group ($p = 0.50$). Yen et al¹⁶ also reported a significant difference in RNFL thickness between amblyopic eyes and normal fellow eyes in anisometropic amblyopia. They came to the conclusion that mechanism of ganglion cell apoptosis during development might be reduced in amblyopia producing a thicker RNFL. A variety of outcomes have been reported by prior studies on peripapillary retinal nerve fiber layer thickness, central macular thickness, foveal thickness and perifoveal area assessment. Al- Haddad et al,²¹ Alotaibi et al,²² Dickmann et al,²³ Andalib et al,²⁴ Jingjing et al²⁵ reported a wide variety of results on central macular thickness, foveal thickness and peripapillary retinal nerve fiber layer thickness. Contrary to previous research, Yalcin and Balci²⁶ reported significantly thicker mean foveal thickness but similar RNFL thickness in anisometropic amblyopes. In present study, mean of average GCC thickness in amblyopic eyes of 30 children was 79.96 ± 10.57 and in fellow eyes was 79.30 ± 9.43 . This difference in GCC thickness between the eyes was insignificant ($p < 0.57$). Also no significant difference in average GCC thickness in both the groups (anisometropic amblyopia and mixed amblyopia) was found ($p = 0.88, 0.30$). Our result was in agreement with the study of Firat et al,²⁰ Araki et al.²⁷ Firat et al²⁰ published their work on assessment of RNFL thickness, GCC thickness in unilateral amblyopic patient using SD-OCT. They reported no significant difference in GCC thickness among the eyes. In our study, we found a significant difference in RNFL thickness between amblyopic eyes and fellow eyes. Following previous study and researches, it is hypothesized

that apoptosis is further inhibited in amblyopic eyes. On comparing anisometropic amblyopia and mixed amblyopia with their fellow eyes, we found a significant difference in RNFL thickness in anisometropic amblyopia not in mixed amblyopia. In accordance to this Yen et al¹⁶ indicated that various process involved in the development of type of amblyopia is the reason being. Anisometropic amblyopia does not influence the vernier acuity while in strabismic amblyopia vernier acuity is reduced. Furthermore crowding effect is seen in strabismic amblyopia for vernier acuity. This might be the reason for thicker RNFL thickness in anisometropic amblyopia. Also we studied GCC thickness simultaneously to see its role in the pathogenesis of amblyopia. We have found no significant difference in GCC thickness in amblyopic children between amblyopic eyes and fellow eyes in both the group (anisometropic amblyopia and mixed amblyopia). In the current work, we used SD-OCT to evaluate the ganglion cell complex (GCC), and this OCT device provides data on GCC thickness but not on GCC count. Therefore, in near future when new features and advancements in OCT machines will arrive, and we actually start counting number of photoreceptors or ganglion cells, the role of adaptive optics may become more clear in early diagnosis and management of amblyopia.

5. Limitation of the study

1. Sample size of the study was small.
2. We used SD-OCT in our study, and the SD-OCT provide normative data for adults but not for children.

6. Novelty of the Study

1. Role of adaptive optics may throw more light when we actually start counting number of photoreceptors or ganglion cells.
2. It may be of useful tool for diagnosis and assessing prognosis of amblyopia on the basis of thickness of retinal nerve fiber layer.
3. Very few studies have been undertaken in India to analyze the retinal nerve fiber layer changes in amblyopia.
4. No studies precisely recognized the ganglion cell complex thickness and its correlation with different types or grades of amblyopia.
5. This study may be a pioneer work for understanding the basic pathophysiology of amblyopia and its remedial steps.

7. Future Research

1. In future this study may provide diagnostic and prognostic value for amblyopia. A proposed nomogram based on OCT may be made that objectively classify amblyopia into mild, moderate, and severe on the basis of retinal nerve fiber layer thickness.
2. It may herald future research in early diagnosis and management of amblyopia.

8. Conclusion

1. On comparing average RNFL thickness between amblyopic eyes and fellow eyes, amblyopic eyes was found to have thicker average RNFL thickness than the fellow eyes. This difference was statistically significant ($p < 0.001$).
2. Among anisometric amblyopic group, average RNFL thickness was found thicker in amblyopic eyes. This difference was statistically significant ($p < 0.001$).
3. Among mixed amblyopic group, no statistically significant difference in average RNFL thickness between amblyopic eyes and fellow eyes was noticed ($p = 0.50$).
4. On comparing average GCC thickness between amblyopic eyes and fellow eyes there was no significant difference noticed ($p < 0.57$).
5. On comparing average GCC thickness in anisometric and mixed amblyopic group between amblyopic eyes and fellow eyes no significant difference in average GCC was found ($p = 0.88, 0.30$). The present study therefore establishes that amblyopia may involve retinal structure.

9. Source of Funding

There are no financial support.

10. Conflict of Interest

None.

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