Comparison of Macular and RNFL Thickness in Anisometropic Amblyopia as Compared to Normal Fellow Eyes

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Abstract

Purpose: To compare the macular and peripapillary RNFL thicknesses of the amblyopic and normal eye in patients with anisometropic amblyopia. Methods: This is a hospital based cross-sectional study for which we studied 36 amblyopic subjects aged between 5 to 16 years in which the amblyopic eye was taken as the case and the unaffected eye was taken as control eye. The inclusion criteria included children having unilateral strabismic or anisometropic amblyopia who underwent macular and RNFL thickness assessment by SD-OCT. Results: Central Macular thickness as well as the thickness of the other macular segments was significantly greater in the affected eye (p<0.001) as compared to the normal eye. The average RNFL thickness was also found to be increased in the affected eye but the difference was not statistically significant (p=0.106), however it was statistically significant in quadrantic analysis (p<0.05). The Central Macular thickness showed a negative correlation with the BCVA (r=-0.252), although it was not statistically significant (p=0.139). Conclusion: A significant increase was found in the macular as well as RNFL thickness in the amblyopic eyes as compared to the normal fellow eye which support the hypothesis of decreased apoptosis of the ganglion cells postnatally.

Keywords: RNFL thickness, amblyopia, macular thickness, anisometropia, nerve fibre layer.

INTRODUCTION

Amblyopia is the most frequent cause of unilateral poor visual acuity (VA) in children, with an incidence of 0.20% to 6.2% in preschool and school-age children1. Amblyopia involves loss of Snellen and grating acuity, loss of contrast sensitivity and creation of distortions in the shape of a stimulus2. Amblyopia is thought to occur during the period of neuronal development of the retina and the cerebral cortex. Therefore, it frequently arises during the first 2-3 years of childhood. However, it may also occur in children up to 8-9 years and persists life-long.

Optical coherence tomography (OCT) is a non-invasive, noncontact technique that visualizes the retinal structure in vivo newer spectral domain machines may approach 3 micron resolution3, and can measure the thickness of both peripapillary retinal nerve fiber layer (RNFL) and macula retinal layer which can be used as a helpful tool in assessing retinal changes in amblyopia.

During fetal development, there is a rapid decline in cell density in the retinal ganglion cell layer toward
and had minimal segmentation errors. We excluded scans of signal strength <7 and those with centration errors. The proprietary Cirrus segmentation algorithm was used to produce retinal thickness maps, which were then averaged over nine retinal subfields within a 6-mm diameter circle centered at the true foveal location, as defined by the Age-Related Eye Disease Study. Macular thickness measurements were obtained in macular cube 512 x128 combination scan mode. The 6 x 6 mm circle corresponds to the Early Treatment Diabetic Retinopathy Study subfield, which is segmented by a concentric circle into central, inner, and outer circles (1, 3, and 6 mm). The inner and outer circles were both subdivided into four quadrants (superior, inferior, nasal, and temporal) to give nine areas in total. The thickness of each area was measured. The optic disk cube 200 x 200 scan mode was used to image the optic disk and RNFL over a 6 x 6 mm optic nerve head using 200 x 200 axial measurements.

The RNFL thickness of the four quadrants (superior, inferior, nasal, and temporal) was then measured. Data was analyzed using IBM-SPSS Version 21.0 software. Data has been represented as numbers and percentages and mean±SD. Paired ‘t’-test was used for comparison. A ‘p’ value less than 0.05 indicates a significant difference.

**OBSERVATION AND RESULTS**

This is a hospital based cross-sectional study carried out at a tertiary centre of north India for which 36 subjects with unilateral amblyopia were enrolled.

A total of 36 patients (18 males and 18 females) aged between 5 to 16 years (mean age 11.58±4.96 years) were enrolled in the study. In 69.4% cases right eye was affected as opposed to only 30.6% subjects in whom left eye was affected. Mean best corrected visual acuity of affected eye was 1.21±0.32 LogMAR as compared to 0.56±0.21 LogMAR for the unaff ected eye (Table 1).

Total mean of total RNFL thickness of affected side (106.81) was greater than that of unaff ected side (98.58) but difference was not found to be significant (p=0.106). An increase in the RNFL thickness was observed in the affected eye for superior (136.19), temporal (61.97) and inferior (100.89) quadrants as compared to the unaff ected side, where the thickness of the respective quadrants were found to be 96.31, 85.03 and 85.03 respectively but the difference was statistically significant for superior and inferior quadrants.
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In the correlative study of BCVA (LogMAR) with macular thickness, the Central Macular thickness showed a negative correlation with the BCVA (r=-0.252), although it was not statistically significant (p=0.139). There was a positive correlation in inner superior (r=0.066; p=0.701), inner temporal (r=0.708; p=0.000), inner inferior (r=0.145; p=0.400), outer superior (r=0.159; p=0.353) and outer inferior (r=0.099; p=0.566) quadrants, while inner nasal (r=-0.029; p=0.869), outer temporal (r=-0.173; p=0.313) and outer nasal (r=-0.156; p=0.364) quadrant showed negative correlation. The statistical significance was reached in the inner temporal quadrant (p=0.000), while the correlation with the rest of the quadrants was statistically insignificant (Table 5).

**DISCUSSION**

In the present hospital based observational study conducted at a tertiary care hospital of north India, we have attempted to study the effect of amblyopia on macular and RNFL thickness in subjects aged between 5 to 16 years.

We studied 36 patients with unilateral amblyopia with equal number of males and females (18 males and 18 females) which is in contrast with other studies which had greater number of male subjects as compared to the female subjects. More number of subjects had right side affected as compared to the left eye [25 (69.4%) and 11 (30.6%) respectively]. No other study, to the best of our knowledge, has reported such comparison. Although we could explain this discrepancy but it may be the result of embryonal developmental behaviour.

In comparing the RNFL thickness between the amblyopic and unaffected eye, we found significant difference in the values of both. The RNFL thickness was greater in the amblyopic eye as compared to the unaffected side (68.00; p=0.001) (Table 2).

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| SN | Characteristic | Statistic |
|----|---------------|-----------|
| 1. | Mean Age±SD (Range) in years | 11.58±4.96 (8-15) |
| 2. | Sex | Male (50.0%) Female (50.0%) |
| 3. | Affected eye | Left (30.6%) Right (69.4%) |
| 4. | Mean±BCVA (LogMAR) | Affected side 1.21±0.32 Unaffected side 0.56±0.21 |

Table 2: Comparison of RNFL Thickness (μm) between affected and unaffected eye

| SN | Segment | Affected side (n=36) | Unaffected side (n=36) | Significance of difference (Paired t-test) |
|----|---------|---------------------|-----------------------|---------------------------------------|
|    |         | Mean | SD   | Mean | SD   | 't' | 'p' |
| 1. | Total peripapillary RNFL thickness | 106.81 | 16.88 | 98.58 | 18.30 | 1.662 | 0.106 |
| 2. | Superior | 136.19 | 12.86 | 96.31 | 15.81 | 9.489 | <0.001 |
| 3. | Temporal | 61.97 | 7.45 | 58.58 | 7.52 | 2.207 | 0.034 |
| 4. | Inferior | 100.89 | 8.81 | 85.03 | 5.96 | 8.434 | <0.001 |
| 5. | Nasal | 54.22 | 16.84 | 68.00 | 14.39 | -3.589 | 0.001 |

(p<0.001). In nasal segment average RNFL of affected side was significantly lower (54.22) as compared to that of unaffected side (68.00; p=0.001) (Table 2).

Macular thickness also showed significant differences between affected and unaffected side. The thickness of central subfield area (267.89), inner superior (341.58), inner inferior (343.31), inner nasal (340.75), outer superior (316.94), outer temporal (292.25), outer inferior (292.36) and outer nasal (316.97) segment of the affected eye were higher than those of the unaffected eye (241.86, 333.81, 319.03, 327.97, 321.94, 307.19, 285.42, 287.81 in the respective segments) which was statistically significant (p<0.001) in all but inner superior segment (p=0.035) (Table 3).

In the study, comparison of BCVA, with the Macular and peripapillary RNFL thickness between normal and abnormal eye in children with unilateral amblyopia was done. It was observed that while assessing the relationship between the RNFL thickness and BCVA, there was a positive correlation in the average (r=0.262, P=0.123), superior (r=0.192, p=0.262), inferior (r=0.169, p=0.325) and nasal quadrants (r=0.118, p=0.492) except temporal, which showed a negative correlation (r=-0.065, p=0.707) although it was not statistically significant. Since the worse vision is indicated by a higher LogMAR value, hence, the Pearson correlation came out to be positive when compared with the increased RNFL thickness (Table 4).
in central macular thickness between eyes with moderate to severe amblyopia and the external controls (P = 0.037). In a similar comparison drawn by Hyuhn et al.10 amblyopic eyes had slightly greater foveal minimum thickness than the normal fellow eye (by 5.0 μm; 95% confidence interval 0.1–9.9). Amblyopic eyes were found to have a slightly thicker central macula (1 mm diameter region) in both comparisons, although these differences were not statistically significant. He also compared the RNFL thickness among the two, but did not find any significant difference between amblyopic and normal fellow eyes or normal eyes of non-amblyopic children. Chatterjee et al.11 studied the difference in the macular thickness of the anisometropic and strabismic amblyopic eyes in comparison to the normal fellow eye and found that the macular thickness of the amblyopic eye was greater than that in the normal eye ([247.8947 +/- 34.3926 in amblyopic eye versus 222.2386 +/- 31.1919 in normal eye, p = 0.001]) but failed to reach a statistical significance in the mean macular thickness (in microns) 233.75 +/- 31.5224 in strabismic amblyopic eye versus 223.00 +/- 31.4266 in normal eye a (p = 0.4118).

Table 3. Comparison of macular Thickness (μm) between affected and unaffected eye

| Segment          | Affected side (n=36) | Unaffected side (n=36) | Significance of difference (Paired ‘t’-test) |
|------------------|----------------------|------------------------|-------------------------------------------|
|                  | Mean | SD   | Mean | SD   | ‘t’ | ‘p’   |
| CST*             | 267.89 | 33.56 | 241.86 | 28.57 | 7.448 | <0.001 |
| Average Cube     | 297.56 | 12.44 | 278.92 | 13.10 | 8.347 | <0.001 |
| Volume Cube      | 9.77 | 0.21 | 9.80 | 0.24 | -0.952 | 0.348 |
| Inner superior   | 341.58 | 21.12 | 333.81 | 10.86 | 2.187 | 0.035 |
| Inner temporal   | 328.08 | 5.27 | 319.03 | 7.55 | 7.035 | <0.001 |
| Inner inferior   | 343.31 | 6.11 | 327.97 | 10.77 | 7.644 | <0.001 |
| Inner nasal      | 340.75 | 11.07 | 321.94 | 9.72 | 7.158 | <0.001 |
| Outer superior   | 316.94 | 9.91 | 307.19 | 8.18 | 4.828 | <0.001 |
| Outer temporal   | 292.25 | 6.80 | 285.42 | 5.70 | 5.751 | <0.001 |
| Outer inferior   | 292.36 | 4.45 | 287.81 | 4.19 | 5.886 | <0.001 |
| Outer nasal      | 316.97 | 3.31 | 310.81 | 3.76 | 7.098 | <0.001 |

*ST (central subfield thickness)

Table 4: Relationship between RNFL thickness the amblyopic eye with the BCVA

| Average | RNFL QUADRANT |
|---------|---------------|
|         | Superior | Temporal | Inferior | Nasal |
| Pearson correlation (r) | 0.262 | 0.192 | -0.065 | 0.169 | 0.118 |
| Significance (p) | 0.123 | 0.262 | 0.707 | 0.325 | 0.492 |
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Wang et al.12 studied 14 subjects with hyperopic anisometropic amblyopia and reported no significant differences in peripapillary retinal nerve fiber layer, central macular thickness, and macular volume between amblyopic eyes and fellow eyes of the participants. Similarly, Dickmann et al.13 reported no significant difference between retinal nerve fiber layer thickness, macular thickness, and foveal volume in amblyopic versus fellow eyes in patients with unilateral amblyopia. However, Pang et al.14 reported that amblyopic children with unilateral high myopia tend to have a thicker fovea and thinner inner and outer macular ring in the amblyopic eye as compared to their normal fellow eye.

We also found an inverse correlation between the RNFL and Macular thickness and Best corrected visual acuity in the amblyopic eye. There was a positive correlation between the BCVA (LogMar) and RNFL thickness, average as well as in all the quadrants except in the temporal quadrant. Similar positive correlation was found between the BCVA (LogMAR) and the macular thickness, except in the inner nasal, outer temporal and outer nasal quadrants. The higher the LogMAR value worse is the visual acuity, hence there was a positive correlation with the RNFL and macular thickness. To the best of our knowledge, no other study has derived the correlation between the RNFL and Macular thickness with the visual acuity.

Yen et al.7 hypothesised that amblyopes have thicker retina and that the amblyopic process have effects at multiple levels in the visual pathway. Experiments have proved that retinal ganglion cells suffer changes with light deprivation from birth. These changes may include some or all of these: cell loss or apoptosis,15 mean nucleolar volume diminution in ganglion cell layers, and inner plexiform layer thinning in rats and cats. Changes also include reduction in optic nerve head area in mice. Arden15 showed that pattern ERG was reduced in various types of amblyopia in human. The amblyopic process may have an effect on various levels of the visual pathway.

Histopathologic changes in the lateral geniculate nucleus and visual cortex have been reported. Histologic study of the lateral geniculate nucleus of monkeys with strabismic, anisometropic, and visual deprivation amblyopia reveals marked shrinkage of cells that receive input from the amblyopic eye.5,8,9 There are similar findings in the lateral geniculate nucleus in human anisometropic amblyopia and strabismic amblyopia11. But we also think that at least in humans amblyopia may be associated with changes that affect retinal function at the level of production of Pattern ERG deficit, which is assumed to be preganglionic, however other studies 16 have refuted this claim, especially those in which fixation and focus were pre adjusted.

CONCLUSION

In our study we found a significant increase in the macular as well as RNFL thickness in the amblyopic eyes as compared to the normal fellow eye which support the hypothesis of decreased apoptosis of the ganglion cells postnatally. This baseline evaluation can be used to monitor the occlusion therapy thereafter and hence, changes in the macular and RNFL thickness can be evaluated after a successful occlusion therapy.

Compliance with ethics requirements: The authors declare no conflict of interest regarding this article. The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study.

| Table 5: Relationship between macular thickness in the amblyopic eye with the BCVA |
|---------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                                 | CST            | AVG Cube       | Volume Cube   | Inner Superior | Inner Temporal | Inner Nasal    | Outer Superior | Outer Temporal | Outer Nasal    |
| Pearson correlation (r)         | -0.252         | 0.319          | 0.130         | 0.066          | 0.708          | 0.145          | -0.029         | 0.159          | -0.173         |
| Significance (p)                | 0.139          | 0.058          | 0.448         | 0.701          | 0.000          | 0.400          | 0.869          | 0.353          | 0.313          |
|                                 |                |                |              |               |               |               |               |               |               |
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