·Clinical Research·

# Evaluation of peripapillary retinal nerve fiber layer, macula and ganglion cell thickness in amblyopia using spectral optical coherence tomography

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## Abstract

• **AIM: To investigate peripapillary retinal nerve fiber layer (RNFL), macula and ganglion cell layer thicknesses (GCC) in amblyopic eyes with spectral domain optical coherence tomography (SD–OCT).**

• **METHODS: Thirty six patients with a history of unilateral amblyopia and thirty two children who had emmetropia without amblyopia were included in this study. In this institutional study, 36 eyes of 36 patients with amblyopia  (AE), 36 fellow eyes without amblyopia (FE), and 32 eyes of 32 normal subjects  (NE) were included. RNFL, GCC and macular thickness measurements were performed with RS –3000 OCT Retina Scan (Nidek Inc CA. USA).**

• **RESULTS: The mean global thicknesses of the RNFL were 113.22±21.47, 111.57±18.25, 109.96±11.31μm in the AE, FE, and NE, respectively. There was no statistically significant difference for mean global RNFL thickness among the eyes  ($P$=0.13). The mean thicknesses of the macula were 258.25±18.31, 258.75±19.54, 248.62±10.57μm in the AE, FE and NE, respectively. There was no statistically significant difference for thickness of macula among the eyes ($P$=0.06). The GCC was investigated into two parts: superior and inferior. The mean thicknesses of superior GCC were 102.57 ±13.32, 103.32 ±10.64, 100.52± 5.88μm in the AE, FE, and NE, respectively. The mean thicknesses of inferior GCC were 103.82 ±12.60, 107.82± 12.33, 105.86±10.79μm in the AE, FE and NE, respectively. There was no statistically significant difference for thickness of superior and inferior GCC between the eyes ($P$=0.63,  $P$=0.46).**

• **CONCLUSION: The macular thicknesses of AE and FE were greater than the NE, although it was not statistically significant. Amblyopia does not seem to have a profound effect on the RNFL, macula and GCC.**

• **KEYWORDS:** amblyopia; retinal nerve fiber layer; macula; ganglion cell complex

## INTRODUCTION

Amblyopia is a disorder where visual acuity does not develop properly in one or both eyes during childhood. The condition is seen in about 2% -5% of the general population [1]. Amblyopia is attributed to either abnormal binocular interactions or visual deprivation. Common causes of the disease include strabismus, anisometropia, and ptosis [2]. 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 [3]. The structures involved in amblyopia are under investigation. Some studies have suggested that the site responsible for the visual deficit may be located in the lateral geniculate nucleus (LGN) [4]. Dysfunction and atrophy of the LGN have been demonstrated in patients with amblyopia [5-7].

The retina is another region in amblyopia that is under investigation. The retinal changes in the amblyopic eyes have not been enlightened yet. It has been suggested that abnormalities in the retinal ganglion cells may be attributable to the effect of amblyopia on the process of postnatal reduction of ganglion cells [8]. Red-free ophthalmoscopy, scanning laser polarimetry (SLP), and optical coherence tomography (OCT) can evaluate retinal nerve fiber layer thickness (RNFL). Spectral domain OCT (SD-OCT) has increasingly been used in ophthalmology. Contradictory findings have been reported for the involvement of the retina in amblyopia. OCT analysis of the RNFL and macular thickness has shown that amblyopic eyes may present as normal [9, 10], and abnormal [11] thickness. The RS-3000 OCT Retina Scan is a high-speed SD-OCT/confocal ophthalmoscope system. However, as yet, the RS-3000 has not been used to compare the RNFL, macula, and ganglion cell layer thickness in amblyopia. In sensory retina, the ganglion cell complex (GCC) consists of three layers: a nerve fiber layer, a ganglion cell layer, and an inner plexiform layer. In amblyopic eyes GCC is the region that has been under investigation for retinal changes. Therefore, we used SD-OCT to investigate
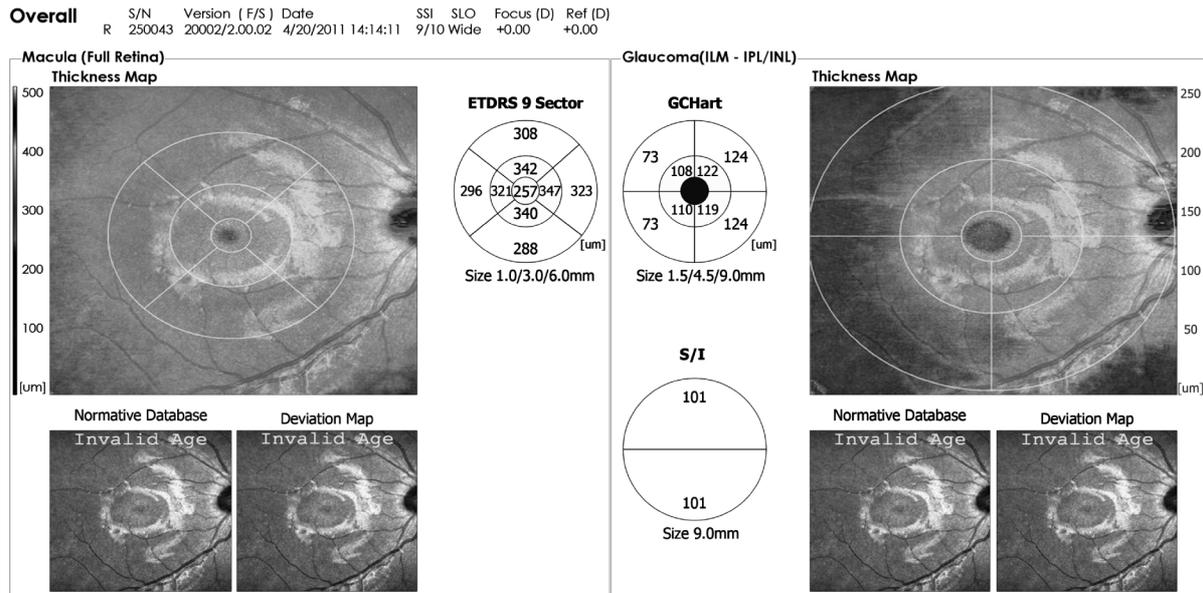
**Figure 1 Spectral-domain optical coherence tomography showing the macula thickness map (on the left) and GCC thickness map (on the right). Near the maps analysis charts were seen. There is no normodative database of the children so the analysis charts are in black and white colour. Scanning laser ophthalmoscopy images of the retina were below the maps.**

whether there was a difference in the RNFL, macular and the GCC thickness of between amblyopic and normal individuals.

**SUBJECTS AND METHODS**

**Subjects** This was a prospective observational cross-sectional study, performed from December 2010 to May 2011. The study was approved by the institutional medical ethics committee. Written informed consent was obtained from each individual or from the individual's parents. The study group comprised 36 patients with a history of unilateral amblyopia and 32 children who had emmetropia without amblyopia. Unilateral amblyopic patients with a corrected visual acuity difference of ≥2 lines between the eyes were consecutively recruited. The causes of amblyopia were strabismus or anisometropia. Healthy subjects were recruited from hospital staff children. Only one eye of each normal subject was evaluated. If both eyes have the inclusion criteria right eye was evaluated. Visual acuities were converted to the logMAR scale. The eyes of the participants were divided into three groups: 36 eyes of 36 patients with amblyopia (AE), 36 fellow eyes of the same patients without amblyopia (FE), and 32 eyes of 32 normal subjects (NE). Furthermore, the eyes with amblyopia were divided into two subgroups according to the cause of amblyopia: patients with strabismus (SA) and patients with anisometropia (AA). All of the subjects underwent a complete eye examination. Patients with any systemic or ocular disease, such as nystagmus or glaucoma were excluded from this study. In addition, to reduce the effect of refractive error eyes, those with a refractive spherical equivalent > ±5D or high astigmatism >3D were excluded.

**Methods**

**RS-3000 OCT Retina Scan measurements** The thickness of the RNFL, macula, and GCC was measured with the RS-3000 OCT Retina Scan (Nidek Inc., CA, USA), which is a high-speed SD-OCT/confocal ophthalmoscope system. Real-time, high-contrast, and wide-view (40°×30°) confocal scanning laser ophthalmoscope (SLO) imaging ensures the accuracy of OCT scanning of the pathological target. It provides 53 000 A-scans/sec and a 4µm OCT axial resolution, showing the discrete retinal layers. Mapping a wide area (9mm×9mm) enables the GCC status to be observed, even in peripheral regions. The OCT scanning position is precisely matched with the SLO fundus image (Figure 1).

The macula map x-y, disc map x-y scanning protocols were performed for all subjects in this study. Superior, and inferior hemiretinal GCC, macular thickness and global, inferior, superior, nasal, temporal RNFL, values were included for the analysis. All of the SD-OCT measurements were obtained by the same clinician (PF). Submitted scans were assessed for signal strength index, image centration and color cross section. Signal strength index greater than 50 were included. Scans that were decentered or had poor color cross-sections were excluded.

**Statistical Analysis** The Shapiro-Wilk test was used to examine the distribution of the numerical data. Normally distributed data of the groups were compared by one-way ANOVA and a post hoc procedure. The Chi-square test was used to compare categorical data.

**RESULTS**

The mean age of the patients with amblyopia was 12.6±5.4 years (range 5 to 23 years), and the mean age in the control group was 11.4 ±5.4 years (range 4 to 24 years). No significant difference between the mean age of the groups was observed ($P$= 0.63). The mean BCVA in logMAR of the AE was 0.45±0.32 (range 1.3 to 0.1), the mean BCVA in logMAR of the FE and NE were 0.00±0.00 (range 0 to 0) and

Table 1  Baseline characteristics and GCC and macular thicknesses of the eyes $\bar{x} \pm s$

|  | AE (n=36) | FE (n=36) | NE (n=32) | P |
|---|---|---|---|---|
| Age (a) | 12.67±5.4 | 12.67±5.4 | 11.41±5.4 | 0.636 |
| Gender (male/female) | 13/15 | 13/15 | 11/13 | [1]0.613 |
| Refractive error, (D) | 1.02±2.08 | 1.00±1.40 | 0.07±0.24 | 0.127 |
| BCVA (logMAR) | 0.45±0.32 | 0.00±0.0 | 0.00±0.0 | <0.0001 |
| RNFL (μm) |  |  |  |  |
| Global | 113.22±21.47 | 111.57±18.25 | 109.96±11.31 | 0.131 |
| Inferior | 131.71±22.55 | 137.0±17.50 | 144.44±15.58 | 0.400 |
| Superior | 154.42±32.37 | 145.14±23.44 | 142.66±7.28 | 0.570 |
| Nasal | 95.71±17.43 | 92.57±20.80 | 81.22±11.89 | 0.206 |
| Temporal | 73.42±13.55 | 71.57±11.29 | 71.55±10.50 | 0.940 |
| GCC (μm) |  |  |  |  |
| Superior | 102.57±13.32 | 103.32±10.64 | 100.52±5.88 | 0.635 |
| Inferior | 103.82±12.60 | 107.82±12.33 | 105.86±10.79 | 0.464 |
| Macula (μm) | 258.25±18.31 | 258.75±19.54 | 248.62±10.57 | 0.062 |

GCC: ganglion cell complex, AE: amblyopic eye, FE: fellow eye, NE: normal eye, D: diopters, BCVA: best corrected visual acuity, μm: micrometer, P: one-way ANOVA,[1]: Chi-Square test.

Table 2  RNFL, GCC and macular thickness of eyes with strabismus and anisometropia $\bar{x} \pm s$

|  | SA (n=17) | FE (n=17) | P | AA (n=19) | FE (n=19) | P | P* |
|---|---|---|---|---|---|---|---|
| RNFL (μm) |  |  |  |  |  |  |  |
| Global | 114.10±21.76 | 112.23±17.21 | 0.766 | 113.52±21.21 | 111.26±18.36 | 0.861 | 0.956 |
| Inferior | 133.43±20.51 | 138.52±20.76 | 0.456 | 130.17±23.35 | 136.89±19.82 | 0.342 | 0.745 |
| Superior | 152.68±33.09 | 146.17±25.64 | 0.567 | 155.86±31.27 | 145.36±27.25 | 0.365 | 0.732 |
| Nasal | 95.43±17.86 | 92.77±18.23 | 0.679 | 95.77±17.26 | 93. 59±20.17 | 0.702 | 1.00 |
| Temporal | 74.86±15.61 | 72.62±13.21 | 0.731 | 72.28±12.96 | 71.52±12.38 | 0.812 | 0.863 |
| GCC (μm) |  |  |  |  |  |  |  |
| Superior | 104.33±11.06 | 104.41±10.68 | 1.00 | 101.25±15.01 | 102.82±10.33 | 0.982 | 0.732 |
| Inferior | 106.33±10.89 | 109.63±11.22 | 0.652 | 101.93±13.79 | 105.93±10.76 | 0.657 | 0.598 |
| Macula (μm) | 258.75±20.81 | 257.72±18.92 | 0.899 | 257.87±16.91 | 258.64±19.23 | 0.987 | 0.988 |

GCC: ganglion cell complex, SA: amblyopic eye with strabismus, AA: amblyopic eye with anisometropia, FE: fellow eye; μm: micrometer, P: paired t test, P*: comparison of SA and AA.

0.00 ±0.00 (range 0 to 0), respectively. The cause of amblyopia was identified as strabismus in 17 cases and anisometropia in 19 cases. Table 1 shows a summary of the results of the SD-OCT analysis. There was no statistically significant difference in the thickness of the RNFL among the groups. (P=0.13) (Table 1). There was no statistically significant difference in the thickness of the macula among the groups (P=0.06) (Table 1). There was no statistically significant difference in the thickness of the superior and inferior ganglion cell layer among the groups (P=0.63, P=0.46) (Table 1). The mean thicknesses of the RNFL, and GCC in SA and AA groups were shown in Table 2. There was no statistically significant difference in these parameters between the SA and the AA groups(Table 2).

**DISCUSSION**
Amblyopia had been thought to be a disease associated with an abnormality of the retina [12]. However, amblyopia-induced cerebral changes were later shown to mainly occur in the visual cortex and the lateral geniculate body [13]. In an experimental study Von Noorden and collegeus have suggested that the mechanism responsible for amblyopia may be inadequate visual stimulation of the fovea during early childhood, abnormal binocular interaction or incompatibility in the visual information received by the two eyes, or a mixture of these problems [14].

In many studies retinal changes were investigated using imaging devices. Several OCT studies have evaluated the RNFL thickness in amblyopia. To date, OCT studies of RNFL thickness in amblyopia have yielded different findings. Repka et al [10] measured the thickness of the peripapillary RNFL in amblyopic and fellow eyes with OCT. They found no meaningful difference in the RNFL thickness. Altintas et al [15] reported that the RNFL thickness was 2.5μm thicker in amblyopic eyes but that this difference was not statistically significant. Kee et al [9] reported that there was no statistically significant difference in the RNFL thickness between amblyopic and normal fellow eyes in 26 children with amblyopia. In contrast, using OCT 2000, Yen et al [11] found

that the global RNFL was 7.7μm thicker in 38 eyes of patients with unilateral amblyopia compared with fellow eyes. In this study we compared the global and four quadrants' RNFL thickness of AE, FE, and NE groups with SD-OCT and we found no statistically significant difference among the groups. Our results are in agreement with those reported by Repka et al[10], Altintas et al[15], and Kee et al[9] but are different from the results reported by Yen et al[11]. Although there was no statistically significant difference in the thickness of the RNFL among the groups in our study, the sequences of RNFL thickness among the groups were different. In healthy eyes it has been accepted as that the superior and inferior quadrants are the thicker than the temporal and nasal quadrants [16, 17]. In our study, the sequence of RNFL thickness in amblyopic patients (both the amblyopic and fellow eye) was; superior, inferior, nasal, and temporal quadrants. However, the sequences of RNFL thicknesses in normal subjects were as follows: inferior, superior, nasal, and temporal quadrants. There are many studies regarding the different thickness variation of the quadrants [18, 19]. The results of our study also showed that RNFL thickness sequence may vary among the amblyopic and normal subjects.

Macular nerve fiber layer is another parameter that has been investigated by several studies. Kee et al [9] reported that there were no statistically significant differences in the fovea thickness between normal and amblyopic children. They also found that the thickness of the RNFL of the amblyopic eyes was thicker than that of the normal fellow eyes, although this is not statistically significant. Yoon et al [20] used OCT to measure the macular and peripapillary RNFL in patients with anisometropic amblyopia. They reported that the RNFL in patients with amblyopia was significantly thicker but that there was no significant difference in macular thickness. They concluded that the amblyopic process may involve the peripapillary RNFL, but not the macula [20]. In many animal studies retinal ganglion cell changes was investigated [21-24]. Retinal ganglion cells found to be normal in most of these studies [22-24]. We measured the macular, superior, and inferior GCC thickness in amblyopic, fellow, and control eyes by SD-OCT. There was no statistically significant difference among the amblyopic, fellow, and control eyes in the thickness of these three parameters in agreement with these animal studies. Huynh et al [25] using TD-OCT demonstrated slightly thicker central macula in amblyopic eyes, although the difference was not statistically significant. Similarly, we found an approximately 10μm thicker macular thickness in AE and FE than NE which was not statistically significant. A possible explanation for this difference can be the slow-down of the normal postnatal reduction of ganglion cells [9]. In a pilot study, Park et al [26] measured the thickness of each retinal layer of the amblyopic and fellow eye with SD-OCT. They found that there was a significant thinning in the ganglion cell layer plus the inner plexiform layer in amblyopic eyes. In this study researchers measured the ganglion cell layer plus the inner plexiform layer manually

using the SD-OCT calipers. Also the structures that were measured in our study and the study of Park et al [26] are not the same ones. Using automatic measurements with RS-3000 SD-OCT we measured the GCC that is composed of nerve fiber layer, ganglion cell layer and inner plexiform layer. The difference between the results of the study by Park et al[26] and our study may be explained by the study design like the structures that were measured and how they were measured. Another factor that affects the OCT measurements may be the age of the patients. Park et al[26] included the patients who were between 4 years and 20 years with a mean age of 9.0± 4.03 years. The patients included in our study were between 5 years and 23 years with a mean age of 12.67±5.4 years. There is no consensus of opinion in the literature about the effect of age on retinal measurements using OCT. Both Leung et al [27] and Turk et al [28] found no significant correlation between age and RNFL thickness. On the other hand Qian et al [29] found that RNFL thickness was positively correlated with age.

Another SD-OCT study demonstrated that the mean macular thickness was significantly increased in amblyopic eyes versus the fellow eye while the mean the RNFL thickness was similar [30]. In this study Al-Haddad et al[30] compared the RNFL and macular thickness between fellow eyes of patients with unilateral amblyopia using SD-OCT (Cirrus HD-OCT). They determined the retinal thickness from internal limiting membrane to the retinal pigment epithelium (RPE) as we did in our study. Our findings are in agreement with those reported by Kee et al[9] and Yoon et al[20]. However, they are in contrast with those reported by Park et al [26] and Al-Haddad et al [30]. Making a comparison between different OCT devices' results can be a mistake. Studies comparing the RNFL thickness measurements using TD-OCT and SD-OCT demonstrated that measurements between these two different devices cannot be comparable [31-33]. In addition, Leite et al[34] studied the agreement of RNFL thickness measurements among three SD-OCT instruments. They found that RNFL thickness measurements of different SD-OCT devices cannot be compatible [34]. To clarify whether the cause of this difference is due to difference in study design or difference in devices is required.

In our study, there was no significant difference in the thicknesses of the RNFL, macula, and GCC between amblyopic eyes of 17 children with strabismic amblyopia and 19 children with anisometropic amblyopia. However, Kee et al [9] found that the fovea was thicker in children with strabismic amblyopia and that the retinal nerve fiber layer was thicker in children with anisometropic amblyopia. Yen et al [11] reported that the RNFL thickness of amblyopic eyes in patients with anisometropia was thicker than that in fellow eyes, but not in patients with strabismus. Yoon et al[18] reported that the thickness of the RNFL was significantly greater in hyperopic anisometropic amblyopia. The differences between OCT devices, patients' ages, and the number of patients, in addition to measurement errors among

examiners, may explain the differences between the strength of the studies.

The strong point of this study is that we used a normal control group because the patients with amblyopia may have similar but less severe abnormalities in the fellow eye [33,34]. Also there was no statistically significant difference among the groups with respect to SE refractive error that may affect the measurements [28,35]. A limitation of our study was the small size of the amblyopia patient group.

In conclusion, our results suggest that amblyopia does not seem to have a profound effect on the RNFL, macula, and the GCC. Additional histopathological and instrumental studies with a greater number of patients are required to confirm these findings.

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