Optical diagnostic techniques in ophthalmology

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CHAPTER THREE

ANOMALOUS RELATION BETWEEN AXIAL LENGTH AND RETINAL THICKNESS IN AMBLYOPIC CHILDREN

Abstract

In healthy eyes, the retinal thickness (RT) is determined by several factors. Besides age and gender, one of these factors is axial length (AL) and it has been demonstrated that there is a significant correlation between a thin retina and a higher axial length in healthy subjects. Because amblyopic eyes tend to be hypermetropic (i.e. they have a shorter eye) axial length can be a confounder in studies measuring retinal thickness in these patients. In this study we investigate the relationship between the pericentral retinal thickness and the axial length, in amblyopic and fellow eyes and both eyes of healthy children. Thirty-six amblyopic patients and 30 healthy children were enrolled in this study. Amblyopic and healthy children underwent full ophthalmic and orthoptic examination, volume scanning of the macula with OCT and recording of axial length. Although the mean axial length of amblyopic and fellow eyes is shorter compared to healthy eyes, the retinal thickness is not larger. Furthermore, the linear correlation between axial length and retinal thickness found in healthy eyes seems to be lost in the eyes of amblyopic children.

This chapter has been submitted to: Graefe’s Archive for Clinical and Experimental Ophthalmology:

3.1 INTRODUCTION

Amblyopia is defined as a unilateral or bilateral decrease of visual acuity caused by pattern vision deprivation or abnormal binocular interaction during the critical period of visual development for which no optical or organic origin can be detected (1, 2). Despite much research, the pathophysiology of amblyopia has still not been fully understood. Although the visual cortex is said to be the primary site of amblyopia, involvement of processes in the retina in amblyopia is increasingly being investigated. However, due to contradicting outcomes over the years, involvement of retinal processes remains a controversial subject (3-6).

During the past decade optical coherence tomography (OCT) has been used to study macular retinal thickness (RT) in amblyopic eyes (7-16). OCT is a non-invasive, non-contact cross-sectional imaging technique and is found to be well tolerated and easy to undergo by children from the age of 3 (17). The OCT techniques currently used in clinical practice are time-domain OCT (TD-OCT) and spectral-domain OCT (SD-OCT) (18, 19), the latter being much faster, thus being in particular suited for the investigation of the retina of small children. OCT is often used to determine the retinal thickness (i.e. the thickness between the ILM and the RPE) and/or to determine the thickness of the RNFL. The retinal thickness is often mapped in three areas according to an ETDRS grid: the foveal area covers the central part of the retina with a diameter of approximately 1 mm. The pericentral area is outside the foveal area and has a diameter between 1 mm and 3 mm. The third and largest area is the peripheral area with a diameter between 3 mm and 6 mm.

Studies that used OCT to study the retinal thickness in amblyopic eyes differed in design and outcome, yet in most studies amblyopic eyes tended to have a thicker retina and/or retinal nerve fiber layer (RNFL) (10, 13, 15, 16). A possible explanation is given by Yen et al. They postulated that amblyopic eyes have a thicker retina due to the arrest of the physiological postnatal ganglion cell reduction (15, 20). The ganglion cell layer is thickest in the pericentral area of the macula. Therefore a hypothetical arrest of the normal post natal ganglion cell reduction is expected to lead to an abnormally thick retinal thickness and especially the pericentral retinal thickness (21).

In healthy eyes, the retinal thickness is determined by several factors. Besides age and gender, one of these factors is axial length (AL) and it has been demonstrated that there is a significant correlation between a thin retina and also the RNFL with greater axial length in healthy subjects (22-27). Because amblyopic eyes tend to be hypermetropic (i.e. they have a shorter eye) axial length can be a confounder in studies measuring retinal thickness in these patients.
In this study we investigate the relationship between the axial length and the retinal thickness, in amblyopic and fellow eyes and both eyes of healthy children. We measured the thickness of all three retinal areas, however, emphasis is put on the pericentral part of the retina since changes in the ganglion cells due to disease are most pronounced in this area of the retina.

### 3.2 METHODS

This observational, transversal study was approved by the ethics committee of the Academic Medical Center in Amsterdam. Informed consent was provided by all patients and their parents. Amblyopic patients that were seen at the orthoptic department of the Academic Medical Center (Amsterdam, the Netherlands) were asked to participate in this study. Children with a history of amblyopia but with a lesser difference than 2 lines in visual acuity between eyes at the time of testing were still assigned to the amblyopic group. Anisometropic amblyopic eyes needed to have at least a 2 diopter difference in refraction between both eyes present. Strabismic amblyopia was defined as a manifest ocular deviation or eccentric fixation with an acuity difference present or recorded in the past. If the amblyopia was caused by strabismus as well as by anisometropia these patients were included in a combination group.

Healthy children were recruited during the same period; these children were referred with a suspicion of a visual acuity disorder which proved to be absent or caused by a refraction error which was equal in both eyes. The healthy participants needed to have a straight eye position; phorias were allowed as long as there was a quick recovery. The participants had to be at least three years old and cooperative enough to sit through all examinations.

All included children underwent standard orthoptic examination and after signing informed consent, supplementary SD-OCT (3DOCT-1000, software version 3.01, TOPCON Corporation, Tokyo, Japan) examination and measurement of axial length with the IOL Master (Carl Zeiss Meditec). All patients also underwent additional ophthalmic examination, consisting of inspection of the ocular media and funduscropy to rule out intraocular pathology.

The orthoptic examination consisted of testing binocular single vision using the TNO or Titmus Fly binocular vision test, determining any ocular deviation by cover testing at near (30cm) and at distance (2.5 m and/or 5 m), testing best corrected visual acuity with Lea symbols or ETDRS chart (please note that an ETDRS chart is different from the ETDRS grid that is used to indicate the areas on the retina) and measurement of refractive error through cycloplegic retinoscopy. Best corrected visual acuity was converted into the logarithm of the minimum angle of resolution.
CHAPTER 3

(LogMAR) scale. After pupil dilatation, an experienced examiner performed volume scanning of the macula with SD-OCT. A fast volume scan of the macula with 32 b-scans consisting of 512 a-scans was chosen to overcome motion (28) and blinking artifacts in this group of young children. Due to incapacity of the built-in OCT software to correct for eccentric fixation using this faster scan protocol, the foveal retinal thickness, pericentral retinal thickness and peripheral retinal thickness were calculated using a custom made program (Matlab, the Mathworks, Inc.) after exporting the acquired thickness data from the SD-OCT system. The Carl Zeiss Meditec IOL Master was used to measure axial length; it is a non-contact, very accurate method of measuring axial length to within ± 0.02 mm (29, 30).

Statistical analysis was performed using SPSS 15.0 (SPSS Inc, Chicago, USA). An unpaired t-test was used to assess differences in mean age between the amblyopic group and healthy controls. Mean visual acuity and spherical equivalent were compared between amblyopic, fellow and control eyes using analysis of variance (ANOVA) followed by a Bonferroni post hoc analysis to correct for multiple comparisons and p-value <.01 considered as statistically significant.

The axial length and retinal thickness for amblyopic and healthy subjects were tested for normal distribution using a Shapiro-Wilk test. To compare means of the axial length and the thickness parameters (foveal retinal thickness, pericentral retinal thickness and peripheral retinal thickness) between groups parametric and non-parametric (paired and unpaired) testing was done using a t-test, with p<0.05 considered as statistically significant. The axial length and the pericentral retinal thickness of the amblyopic eye versus the fellow eye of the patient group, and of the right eye (OD) versus the left eye (OS) of the healthy group were fitted with a linear regression model and a correlation coefficient was calculated. To correlate retinal thickness with axial length a correlation coefficient was calculated for the healthy eyes, the fellow eyes and the amblyopic eyes.

3.3 RESULTS

Thirty-six children diagnosed with amblyopia caused by an anisometropia (n=17), strabismus (n=11) or a combination of these two (n=8) were enrolled in this study. Thirty healthy children were enrolled as a control group. There was no significant difference in age (p=0.12) and gender (p=0.35) between the amblyopic patients (mean age 7.7±1.8; gender M:F, 21:15) and controls (mean age 8.3±1.5; gender M:F, 14:16), see Table 3-1.

There was a significant difference in mean visual acuity between amblyopic eyes versus fellow eyes and control eyes (p-value<0.01 and p<0.001 respectively). Mean visual acuity (LogMAR ± SD) values were 0.20 ± 0.13 for amblyopic eyes, 0.04 ± 0.84
for fellow eyes and 0.09±0.12 for control eyes (Table 3-1). There was a significant difference in mean spherical equivalent between the amblyopic and the healthy control eyes (p<0.001), but no significant difference between the amblyopic and the fellow eye (p=0.14). Mean spherical equivalent values were respectively 3.1±3.2 in amblyopic eyes, 2.7±2.2 in fellow eyes, and -0.9±2.5 in the control eyes. The axial length for the fellow eye and the axial length and retinal thickness for the control group are distributed normally (Shapiro-Wilk test, p>0.05). The axial length for the amblyopic group is not distributed normally (p=0.02).

Table 3-1: Parameters of the patients and healthy subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Amblyopic eyes (N=36)</th>
<th>Fellow eyes (N=36)</th>
<th>Control eyes (N=30)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M:F)</td>
<td>21:15</td>
<td>21:15</td>
<td>14:16</td>
<td>0.35</td>
</tr>
<tr>
<td>Mean Age (yr)</td>
<td>7.6 (1.7)</td>
<td>7.7 (1.8)</td>
<td>8.3 (1.5)</td>
<td>0.12</td>
</tr>
<tr>
<td>Visual Acuity (Logmar)</td>
<td>0.20 (0.13)</td>
<td>0.04 (0.84)</td>
<td>0.09 (0.12)</td>
<td>&lt; 0.01*&lt;0.001#</td>
</tr>
<tr>
<td>Spherical Equivalent</td>
<td>3.1 (3.2)</td>
<td>2.7 (2.2)</td>
<td>-0.9 (2.5)</td>
<td>0.14*&lt;0.001#</td>
</tr>
</tbody>
</table>

*Amblyopic eyes versus fellow eyes
#Amblyopic eyes versus healthy eyes

### 3.3.1 AXIAL LENGTH AND RETINAL THICKNESS MEASUREMENTS

Figure 3-1 shows the axial length of the subject’s right eye (OD) versus the axial length of the subject’s left eye (OS) of the normal control group (black dots). The linear regression model gives a slope of 0.99±0.03 and a vertical-axis intercept of 0.18±0.79 mm. The axial length of OD is highly correlated with the axial length of OS (Pearson’s correlation coefficient R=0.98, p=0.0001). The mean axial length and the standard deviation of OD and OS are 23.28±1.38 mm and 23.29±1.40 mm, respectively.

The axial length of the amblyopic eye versus the axial length of their fellow eyes is also displayed in Figure 3-1(triangles). The slope of the linear regression is 0.79±0.15 and the vertical-axis intercept is 4.5±3.5 mm. The Spearman correlation coefficient of the linear regression model is R=0.77 (p=0.0001). The mean axial length and standard deviation of the amblyopic eye and the fellow eye are 22.00±1.14 mm and 22.18±0.96 mm, respectively.
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Figure 3-1: In dots the axial length of the right eyes of the healthy control group is displayed versus the axial length of the left eye. The slope of this linear regression model is 0.99 and a vertical-axis intercept of 0.18. The axial length of the amblyopic eye is displayed versus the axial length of the fellow eye for patients are displayed with triangles. The slope of the linear fit is 0.79 with a vertical-axis intercept of 4.5. The arrows indicate the mean value of the axial length of the eye; amblyopic (dashed line) eyes are significantly smaller than healthy eyes (solid).

Table 3-2: Comparison of axial length and retinal thickness (RT) parameters values between groups, displaying the mean value and the standard deviation between brackets.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Amblyopic eyes (N=36)</th>
<th>Fellow eyes (N=36)</th>
<th>p-value*</th>
<th>Healthy OD (N=30)</th>
<th>Healthy OS (N=30)</th>
<th>p-value*</th>
<th>p-value#</th>
<th>p-value&amp;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axial Length [mm]</td>
<td>22.00 (1.14)</td>
<td>22.18 (0.96)</td>
<td>0.23</td>
<td>23.28 (1.38)</td>
<td>23.30 (1.40)</td>
<td>0.76</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Foveal RT [µm]</td>
<td>229.7 (22.6)</td>
<td>231.0 (21.3)</td>
<td>0.41</td>
<td>231.3 (17.0)</td>
<td>231.0 (17.6)</td>
<td>0.89</td>
<td>0.71</td>
<td>0.92</td>
</tr>
<tr>
<td>Pericentral RT [µm]</td>
<td>299.1 (14.2)</td>
<td>299.2 (14.0)</td>
<td>0.96</td>
<td>294.7 (13.2)</td>
<td>295.5 (13.2)</td>
<td>0.38</td>
<td>0.19</td>
<td>0.18</td>
</tr>
<tr>
<td>Peripheral RT [µm]</td>
<td>261.8 (13.3)</td>
<td>260.2 (12.6)</td>
<td>0.35</td>
<td>258.5 (13.4)</td>
<td>258.4 (12.7)</td>
<td>0.86</td>
<td>0.37</td>
<td>0.48</td>
</tr>
</tbody>
</table>

* parametric paired t-test of respectively amblyopic eyes versus fellow eyes and right control eyes versus left control eyes
# parametric unpaired t-test amblyopic eyes versus right control eyes
& parametric unpaired t-test fellow eyes versus right control eyes

There is no significant difference in axial length of the amblyopic eyes and the fellow eyes and between the OS and OD of healthy subjects (p=0.23 and p=0.76, respectively), see Table 3-2. There is a statistically significant difference between the axial length of the amblyopic eyes compared to the axial length of healthy subjects (OD) and a statistically significant difference between the axial length of the fellow eyes and the axial length of healthy subjects (OD, p<0.01).

Figure 3-2 shows the pericentral retinal thickness of the subject’s right eye (OD) versus the pericentral retinal thickness of the subject’s left eye (OS) for the normal...
control group (displayed as dots). The slope of the linear fit is 0.92±0.07, with a vertical-axis intercept of 24.3±21.4 µm and a Pearson’s correlation coefficient of R=0.921 (p=0.0001). The mean retinal thickness of OD and OS are 294.6 and 295.6 µm, respectively. The pericentral retinal thicknesses of the patient’s amblyopic eyes are displayed as triangles versus the retinal thickness of the fellow eyes. The slope of the linear fit is 0.83±0.1, the vertical-axis intercept is 50.1±29.1 µm and the Pearson’s correlation coefficient is R=0.821 (p=0.0001). The mean pericentral retinal thickness for the amblyopic eye and the mean pericentral retinal thickness for the fellow eye (299.1 and 299.2 µm, respectively) are slightly thicker than the mean pericentral retinal thicknesses for the normal group, but not significantly different (p=0.19). The statistical analysis shows no difference in retinal thickness between the groups (Table 3-2).

**Figure 3-2:** In dots the retinal thickness of the right eyes of the healthy control group is displayed versus the retinal thickness of the left eye. The slope of this linear regression model is 0.83 and a vertical-axis intercept of 50.1µm (standard error=29.1). The retinal thickness of the amblyopic eye displayed versus the retinal thickness of the fellow eye for patients are displayed with triangles. The slope of the linear fit is 0.92 with a vertical-axis intercept of 24.3 µm (standard error=21.4). The arrows indicate the mean value of the axial length of the eye, no difference between healthy (solid) and amblyopic (dashed).

Lastly, we investigated the relation between retinal thickness and axial length for healthy, amblyopic, and fellow eyes. Figure 3-3 shows the retinal thickness versus axial length for normal controls (only OD is used in this analysis) in closed dots. A moderate linear correlation (R=0.41, p=0.02) can be observed.

The slope of the linear fit describing the relationship between retinal thickness and axial length is -4.09±1.64 [µm/mm]. The negative sign indicates that eyes with a short axial length tend to have a thicker retina. However, this relationship is not found in the amblyopic or in the fellow eyes of amblyopic patients: for amblyopic eyes the correlation coefficient is -0.04 (p=0.82).
For normal eyes a correlation was found between thinning of the pericentral ring thickness with increasing axial length (R=0.41). This relation could not be found in amblyopic eyes and their fellow eyes (R=-0.04 and R=0.05, respectively).

For fellow eyes a similar result is found: the correlation coefficient is 0.05 (p=0.78). After splitting the data of the amblyopic eyes based on their type of amblyopia, no differences could be demonstrated comparing the same parameters between the amblyopic and the fellow eye, data not shown.

3.4 Discussion

In this study we found no significant differences in foveal, peripheral, and most importantly pericentral retinal thickness between the amblyopic eye and the fellow eye of amblyopic children, although the axial length of the amblyopic eyes and fellow eyes are significantly shorter than the healthy eyes. We demonstrated that in amblyopic children, for the axial length and pericentral retinal thickness, a significant linear correlation exists between the amblyopic eye versus the fellow eye; comparable to the correlation between both eyes of healthy controls (Figure 3-1 and Figure 3-2, respectively).

The linear relation shown in Figure 3-1 between axial length OS and axial length OD indicates similar axial length development of the eyes of healthy subjects. The data for amblyopic eyes overlay the data for healthy subjects, albeit that the mean axial lengths are shifted to lower values indicating a reduced axial length; both for the amblyopic and the fellow eye. In contrast to the different distribution (i.e. the range of axial lengths are different) of axial length between the amblyopic group and healthy group a similar distribution (i.e. similar data range) is observed for the retinal thicknesses of amblyopic patients and healthy subjects, which is also expressed by the similar mean retinal thicknesses. Note that if a one-on-one relation would exist between axial length and retinal thickness as found in another study (27), Figure 3-2 would have shown the amblyopic eyes primarily at the extremes of the retinal thickness range. Figure 3-3 shows the relation between retinal thickness and axial length; where for healthy subjects a linear relation can
be found. However, as is also suggested by the data in Figure 3-2, no linear relation between retinal thickness and axial length is found for amblyopic patients. Consequently, the retinal thickness in amblyopic and fellow eyes cannot be predicted from the axial length.

Five other studies (7, 9-11, 16) described macular retinal thickness measurements in amblyopic eyes. Altintas et al. (7) reported no significant difference in macular thickness between the amblyopic and fellow eye in 14 unilateral strabismic amblyopic patients, similar to our results. Kee et al. (11) also did not find differences in foveal and RNFL thicknesses between normal children and children with amblyopia. However, strabismic amblyopic eyes showed a thicker foveal thickness but thinner RNFL thickness when compared to anisometropic children. The study by Yoon et al. (16) described a thicker RNFL thickness and, confirming our study results, also no differences in macular thickness when comparing amblyopic with their fellow eyes in 31 hyperopic anisometropic patients comparable to our results. Dickmann et al. (9) measured the foveal and mean macular thickness of strabismic amblyopic eyes and demonstrated that the foveal thickness and mean macular thickness are significantly higher than anisometropic children.

Just recently Al-Haddad et al. (26) described macular and RNFL thickness in amblyopia. They found a significantly increased central macular thickness, e.g. foveal thickness, in 31 anisometropic amblyopic patients (anisometropia was defined as an interocular spherical equivalent of 1.0 diopter or more, aged ≥6 years) compared to their fellow eyes using SD-OCT. No significant differences were noted between hyperopes and myopes. Anisometropia without amblyopia did not produce significant differences in central macular thickness. Huynh et al. (10) described retinal thickness differences resulting from the Sydney Childhood Eye Study in 53 amblyopic children compared to 3185 healthy controls. After adjusting for age, gender, ethnicity, height, axial length and cluster sampling, amblyopic eyes proved to have a statistically significant increased foveal retinal thickness and a thinner inner ring average thickness, e.g. pericentral retinal thickness. No explanation is given for the thinner inner ring average thickness (e.g. pericentral retinal thickness) found in amblyopic eyes. As an explanation for the increase in foveal retinal thickness, the authors of both studies mentioned that the physiological postnatal ganglion cell reduction could be inhibited in amblyopia, as hypothesized by Yen et al. (15). We could not demonstrate differences in foveal retinal thickness in the present study (see Table 3-2), which supports this hypothesis.

There are a few limitations to our study. The number of patients in this study is limited, but nevertheless in line with the number of patients used in literature (9,
The study included mostly patients with a relatively mild amblyopia. This may have affected our study results since the progression of the disease could influence the thickness; however, other studies have not shown significant differences in retinal thickness in amblyopic patients compared to healthy. We think this influence is negligible. Finally this study focuses on pericentral retinal thickness measurements and did not include RNFL thickness measurements. In respect of the literature RNFL measurements can be of additional value when investigating amblyopia.

In conclusion, the axial length and the pericentral retinal thickness show the same relationship between the amblyopic and the fellow eye as compared to the eyes of healthy controls. However, the axial length of amblyopic and their fellow eyes are significantly shorter, e.g. more hypermetropic compared to healthy eyes. Although there is a moderate correlation between axial length and pericentral retinal thickness in healthy eyes, this is not the case in amblyopic or in fellow eyes. This could be due to a disturbed development of both the amblyopic and fellow eye, something which should be further investigated.

3.5 References

AXIAL LENGTH AND RETINAL THICKNESS IN AMBLYOPIC EYES


