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Evaluation of the Choroidal Thickness Using High Penetration Optical Coherence Tomography with Long Wavelength in Highly Myopic Normal Tension Glaucoma

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Short title: Choroidal Thickness and Highly Myopic NTG
Abstract

**Purpose:** To evaluate the choroidal thickness by high-penetration optical coherence tomography (OCT) using long wavelength in highly-myopic normal tension glaucoma (NTG).

**Design:** Cross-sectional retrospective study.

**Methods:** Setting: Institutional. Participants: Twelve eyes from 8 patients diagnosed as NTG without any other ocular diseases under 45 years old, spherical equivalent refractive error between –6 and –12 diopters and axial length greater than 26.5mm, and 12 eyes of matched healthy eyes. Intervention: Choroid was imaged with prototype high-penetration OCT and its thickness was measured. Main Outcome Measurements: Choroidal thickness at the fovea and 5 locations; 2mm superior, temporal, inferior to the center of the optic nerve head, and 2mm superior (supero-temporal) and 2mm inferior (infero-temporal) to the temporal location.

**Results:** In overall cases, the choroidal thickness in NTG group was approximately 50% than that in control. Mean choroidal thickness in NTG group was significantly thinner in control group at the fovea (166 vs.276 microns, P<0.001), at the superior (172 vs. 241 microns, P<0.05), at the supero-temporal (161 vs.244 microns, P<0.01), at the temporal (110 vs.161 microns, P<0.01), at the infero-temporal (115 vs. 159 microns, P<0.05) to the optic nerve head. Stepwise analysis disclosed that the foveal choroidal thicknesses the most influential factor on the occurrence of NTG (P < 0.0001, R²=0.4).

**Conclusions:** Choroidal thickness in highly myopic NTG is significantly thinner than in controls at least in some specific locations. Choroidal thinning is somehow related with highly myopic NTG and may be useful diagnostic parameters for myopic NTG.
It is well known that normal tension glaucoma (NTG) is augmented by typical glaucomatous optic neuropathy within the range of normal intraocular pressure (IOP). Myopia is one of the high-risk factors for development of NTG,\textsuperscript{1,2} and patients with highly myopic glaucoma sometimes have greater progression of visual field loss and severe loss of central visual function despite an IOP in the normal range.\textsuperscript{3,4} Therefore, myopic NTG is vision-threatening, whereas its pathogenesis is poorly understood.

Several studies have reported that blood flow disruption including low blood pressure, nocturnal hypotension, and fluctuation of the mean ocular perfusion pressure, are possible reasons for progression of NTG.\textsuperscript{5} Sung et al. reported that fluctuation of the 24-hour mean ocular perfusion pressure was the most consistent prognostic factor for NTG.\textsuperscript{6} Duijm et al. reported that choroidal circulation in NTG is slower than in control subjects.\textsuperscript{7} Previous histologic studies suggested that the choroidal thickness in glaucoma was significantly thinner than in normal subjects.\textsuperscript{8,9}

Recently, the results of several morphologic studies conducted in living human glaucomatous eyes using new non-invasive imaging techniques have been reported. A confocal scanning laser ophthalmoscope (Heidelberg Retina Tomograph [HRT], Heidelberg Engineering, Dossenheim, Germany) provides quantitative measurements of various parameters to evaluate the shape of the optic disc on three-dimensional topographic images. In a population-based, cross-sectional study, the sensitivity of the HRTII glaucoma classification program, version 3.0, was poor, whereas the specificity was satisfactory.\textsuperscript{10} GDx scanning laser polarimetry (Carl Zeiss Meditec, Inc., Dublin, CA) allows evaluation of the retinal nerve fiber layer in glaucomatous eyes.\textsuperscript{11} Optical coherence tomography (OCT) is also a noninvasive technique that provides high-resolution, cross-sectional retinal images. Spectral-domain OCT, the newest commercially available OCT model, provides differentiation of the retinal layers correlated with histology and allows evaluation of the optic disc and the retinal nerve fiber layer morphology with high reproducibility.\textsuperscript{12} RTVue-100 (Optovue Inc., Fremont, CA) facilitates evaluation of the macular ganglion cell complex.\textsuperscript{13}

As reported previously, the choroid is thought to be a target in the pathogenesis of glaucoma\textsuperscript{7}; however, in vivo choroidal imaging was unsatisfactory because of difficulties in visualizing the choroid with conventional OCT technologies using the 840-nm wavelength as a light source due to high scattering and consequent choroidal signal attenuation at the retinal pigment epithelium (RPE). The use of a long wavelength such as the 1-micron band allows penetration of the signal through the RPE and Bruch’s membrane, enabling visualization of the deep ocular tissues such as the choroid or even the sclera.\textsuperscript{14-17} To evaluate the choroid in living human eyes, we used a prototype
high-penetration OCT instrument.\textsuperscript{18,19} High-penetration OCT using a 1,060-nm light source allowed us to clearly measure the choroidal thickness. This new technology has shown that the choroidal thickness is affected by age, refractive error, and axial length.\textsuperscript{19} Unlike the histologic studies, high-penetration OCT technology enables in vivo evaluation of the choroidal morphologic abnormalities.

In the current study, we focused on morphologic changes in the choroid in highly myopic NTG. The choroidal thicknesses around the optic disc and fovea in patients with highly myopic NTG were measured and compared with those in myopic volunteers without glaucoma.

\section*{METHODS}

\section*{Patients}
Twelve consecutive eyes of eight patients younger than 45 years of age and diagnosed with NTG who met the following criteria in the glaucoma clinic and high myopia clinic of Osaka University Hospital were enrolled. The inclusion criteria were intraocular pressure (IOP) below 21 mmHg, spherical equivalent refractive error between $-6$ and $-12$ diopters (D) to exclude extremely high myopia, and axial length exceeding 26.5 mm. The exclusion criteria included macular abnormalities such as choroidal neovascularization or whitish myopic atrophy; systemic abnormalities such as vascular disease, hypertension, or diabetes mellitus; and a history of intraocular surgery. Three glaucoma specialists (S.U., A.M., and K.M.) independently evaluated the stereo-color fundus photographs and automated visual field analyzer to differentiate glaucomatous from normal eyes. Data also were collected from the normal healthy volunteer database in high-penetration OCT.\textsuperscript{19} Normal myopic volunteers without glaucoma were selected randomly from this database with matching of age, refractive error, and axial length.

The investigational review board of Osaka University Hospital approved the use of the prototypical high-penetration OCT and this retrospective study. The research adhered to the tenets of the Declaration of Helsinki.

\section*{Examination}
A technician masked to the clinical diagnosis of the patient performed all examinations of all patients that included measurement of the spherical equivalent refractive error, axial length, central corneal thickness, and corneal refraction and evaluation with the Humphrey visual field analyzer. The spherical equivalent refractive error and corneal refraction were measured by autorefractometry (ARK-700A, Nidek, Gamagori, Japan).

\textit{Usui et al., American Journal of Ophthalmology 153, 10-16 (2012).}
The axial length was measured by partial optical coherence interferometry (IOLMaster, Carl Zeiss Meditec, La Jolla, CA). The central corneal thickness was measured by specular microscopy (AP3000P, Topcon, Tokyo, Japan). The visual field examination was performed with Humphrey automated perimetry with the 30-2 program (Carl Zeiss Meditec, Inc.). The mean deviation and pattern standard deviation values were obtained from the software in the visual field analyzer.

**High-Penetration Optical Coherence Tomography and Measurement of Choroidal Morphologic Parameters**

The detailed profile of our prototype high-penetration OCT was described previously. This OCT is swept-source instrument with a scan speed of 50,000 A-scan/second. A 6x6-mm retinal region was scanned by a horizontal fast raster protocol, and the A-scan density was 512 lines (horizontal) x 255 lines (vertical). The scan time was 2.7 seconds. The center wavelength of the probe beam was 1,060 nm, and bandwidth was ≥ 80 nm. The axial resolution was 11 microns in tissue. This long wavelength probe enables deep penetration to the choroid.

The choroidal thicknesses were measured from the images obtained by high-penetration OCT at the fovea and five locations (2 mm superior, temporal, and inferior to the center of the optic nerve head and 2 mm superior (superotemporally) and 2 mm inferior (inferotemporally) to the temporal location (Figure 1). The choroidal thickness was measured according to the method previously described. The choroidal thickness was defined as the distance between the hyper-scattering line of the RPE and that of the choriocapillaris interface. The RPE and choriocapillaris interface were clearly identified in all cases.

**Statistical Analysis**

The data were analyzed using the unpaired t-test and multiple stepwise regression analysis (JMP statistical software package, version 8.0; SAS Institute Inc., Cary, NC). The receiver operating characteristic (ROC) curve was drawn using JMP software. The cut-off value was calculated from this curve. In any analysis, \( P < 0.05 \) was considered to be statistically significant.

**RESULTS**

**Patient Demographic Data**

The patient demographic data are shown in Table 1. The mean age, spherical equivalent refractive error, axial length, central corneal thickness, and corneal refraction were
similar between the highly myopic NTG and myopic volunteers without glaucoma groups.

The glaucoma parameters also were compared between the groups (Table 2). The average mean deviation value was significantly lower \((P<0.05)\) and the pattern standard deviation value was significantly higher \((P<0.001)\) in the highly myopic NTG group. The IOP did not differ significantly between the two groups \((P=0.63)\).

**Choroidal Thickness**

Figure 2 shows representative choroidal images in the myopic NTG group (left) and myopic volunteers without glaucoma group (right) with similar refractive errors and axial lengths. The choroidal thickness in the eyes with myopic NTG was thinner at the fovea and temporal to the optic disc than in the myopic eyes without glaucoma.

Table 3 shows the mean choroidal thickness at the fovea and 2 mm superior, 2 mm temporal, and 2 mm inferior to the optic disc and 2 mm superior (superotemporally) and 2 mm inferior (inferotemporally) to the temporal location in the highly myopic NTG and myopic volunteers without glaucoma groups measured by high-penetration OCT. The mean choroidal thickness in the NTG group was significantly thinner at the superior \((P<0.05)\), superotemporal \((P<0.01)\), temporal \((P<0.01)\), and inferotemporal \((P<0.05)\) locations around the optic disc and at the fovea \((P<0.001)\) compared with the myopic volunteers without glaucoma group.

**Stepwise Multiple Regression Analysis**

Stepwise multiple regression analysis was performed to determine the parameter with the greatest effect among age, spherical equivalent refractive error, axial length, central corneal thickness, corneal refraction, and the choroidal thicknesses at various locations. The choroidal thickness at the fovea was the parameter that had the highest correlation with the development of highly myopic NTG.

**Receiver Operating Characteristic Curve**

Because the subfoveal choroidal thickness was the factor that had the highest correlation with the development of myopic NTG in normal myopic eyes, we drew the ROC curve to evaluate the usefulness of this parameter and the cut-off value in a clinical setting. The area under the ROC curve was 0.88, a favorable indicator, and the cut-off value determined by the ROC curve was 227 microns (Figure 3, Table 3).

**DISCUSSION**

In the Tajimi Study, a population-based eye study of Japanese subjects, the prevalence of open-angle glaucoma (OAG) was 3.9% among patients aged 40 years of age or older. The IOP in 92% of these subjects was under 21 mmHg, resulting in an overall prevalence of NTG of 3.6%. The Japanese population is about 100 million individuals; therefore, about 3 to 4 million people have NTG. Furthermore, myopia was a significant risk factor for development of OAG in that survey. The number of patients with myopic NTG is increasing; thus, an understanding the mechanisms or disease processes of highly myopic NTG is critical.

There is increasing evidence of ocular blood flow abnormalities in the pathogenesis of NTG. Flammer et al. reviewed blood studies in glaucoma and forwarded the following hypothesis. Hemodynamic alterations may be partially responsible in patients with glaucoma because of vascular dysregulation, not artherosclerosis, which causes both low perfusion pressure and insufficient autoregulation. This leads to unstable ocular perfusion pressure, and reduced ocular blood flow often precedes damage to the retinal nerve fibers. The investigators concluded that the vast majority of studies have reported reduced ocular perfusion in patients with glaucoma.

Because the choroid accounts for 85% of the total ocular blood flow, we evaluated in vivo the choroidal thickness in patients with glaucoma using high-penetration OCT, which enabled detection of the choriocapillaris and allowed measurement of the full choroidal thickness around the optic disc and at the fovea.

The choroidal thickness was significantly thinner in the eyes with myopic NTG than in the eyes of normal myopic volunteers without glaucoma. This raised the question about why the choroid is thinner in eyes with NTG? One explanation is that choroidal thinning leads to reduced choroidal circulation, which in turn may cause a circulatory problem in the prelamellar region, because the prelamella is supplied mainly by branches from recurrent choroidal arterioles and short posterior ciliary arteries. Furthermore, the perfusion to the lamina cribrosa may decrease because the major direct blood flow also is supplied by the posterior ciliary arteries, a branch of the ophthalmic arterial circle of Zinn-Haller, which originates from the short posterior ciliary arteries. A possible reason for the lower choroidal circulation may be narrowing of the posterior ciliary arteries by axial length elongation in myopic NTG, because the choroid has little autoregulation and changes in the perfusion pressure affect the blood flow. Thus, reduced circulation to the lamina cribrosa may underlie choroidal thinning. However, it is uncertain how the anatomic choroidal thinning and blood flow are related. Doppler OCT technology is a future modality to measure the blood flow, and this type of study is needed to confirm if the choroidal blood flow actually

decreases in myopic NTG.

Another possibility is that mechanical stretching affects the optic nerve and lamina cribrosa. It is well known that the incidence of NTG increases in myopic eyes, indicating that the IOP level may not be solely responsible for development of glaucoma. Extreme choroidal thinning may indicate a dynamic effect on the optic neurons by the stretched sclera, which may somehow mechanically affect and consequently damage the lamina cribrosa. The thicknesses of the lamina cribrosa and peripapillary sclera decreased significantly with axial length and glaucoma.31 The lamina cribrosa also becomes thinner with progression of glaucoma.32,33 IOP is also an important factor because the fragile lamina cribrosa is easily exposed to severe damage by the IOP, even IOP that is within the normal range.34 Thus, not only circulation but also mechanical stress may be related to choroidal thinning in myopic NTG.

The mean choroidal thickness at the fovea is 354 ± 111 microns in healthy Japanese volunteers without high myopia.19 In the current study, the mean choroidal thickness at the fovea in highly myopic eyes without glaucoma was 276.1 ± 74.1 microns and in highly myopic NTG the mean choroidal thickness was 166.7 ± 40.9 microns. In the current study, patients under 45 years of age with an IOP under 21 mmHg, a spherical equivalent refractive error between −6 and −12 D, and axial length exceeding 26.5 mm were enrolled to exclude as many factors as possible that could affect the results. Healthy subjects matched for age-, spherical equivalent refractive error, and axial length were compared with patients with glaucoma, because previous reports had suggested that the central choroidal thickness was associated with these factors.35-37 The number of patients enrolled was limited; however, we compared two groups with highly myopic NTG and myopic without glaucoma that were matched for age, spherical equivalent refractive error, axial length, central corneal thickness, corneal refraction, and IOP.

Stepwise multiple regression analysis showed that the choroidal thickness at the fovea was more important than the other parameters. In the current study, the area under the ROC curve was relatively high even with an average mean deviation of -4.2 decibels in relatively early glaucoma. The choroidal thickness in advanced highly myopic glaucoma remains unknown, whereas choroidal thinning might be an important risk factor in highly myopic glaucoma that is progressing. The ROC curve indicated that the subfoveal choroidal thickness can help differentiate NTG from myopia without glaucoma. However, conventional automatic perimetry and observation of the optic nerve head are also important for diagnosing myopic NTG. In addition, this study included only cases of early disease, which suggested that the conclusion drawn from
this study may be limited to early cases and may not apply to intermediate or advanced glaucoma.

In summary, the choroidal thickness measurement, which is typically done by conventional methods, is a supplemental examination to help establish the diagnosis of highly myopic NTG.
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C. Contributions to Authors in each of these areas: Involved in design and conduct of study (Y.I.); collection of data (S.U., K.M., A.M.); management (Y.I.), analysis (S.U.), and interpretation of the data (S.U.); and preparation (S.U., Y.I.), review (S.U., Y.I., K.M., A.M., and K.N.), provision of high-penetration OCT (Y.Y.), or approval of manuscript (Y.I).

D. Statement about Conformity with Author information: This study has been approved by the investigational review board of Osaka University Hospital. All patients/subjects provided informed consent for performing this prototypical OCT investigation and for the use of his/her data in this study.

E. Other Acknowledgements: None
REFERENCES


FIGURE LEGENDS

FIGURE 1. The six locations of the choroidal thickness measurements (indicated by x). Five locations are around the optic disc: 2 mm superior, temporal, or inferior to the center of the optic nerve head and 2 mm superior (superotemporally) and 2 mm inferior (inferotemporally) to the temporallocation and the fovea.

FIGURE 2. Representative choroidal images of highly myopic normal tension glaucoma (NTG) and control eyes (myopic eyes without glaucoma) by high-penetration optical coherence tomography (OCT). Various examination results from eyes with highly myopic NTG (left) and myopic volunteers without glaucoma (right). Color fundus photographs from a patient with myopic NTG (top left) and a fundus from a myopic volunteer without glaucoma (top right). The results of the C-30-2 Humphrey visual field analyzer show superior asymmetrical visual field abnormalities in an eye with myopic NTG (top left) and a normal field from a myopic volunteer without glaucoma (top right). High-penetration OCT images of the macula in an eye with myopic NTG (middle left) and an eye of a myopic volunteer without glaucoma (middle right) show a full-thickness choroid in both cases. The black arrowheads indicate the position of the inner and outer border of the choroid and the location at which the choroidal thickness was measured. The choroid is somewhat thicker in the eye of a myopic volunteer without glaucoma. The high-penetration OCT image temporal to the optic nerve disc in an eye from a patient with myopic NTG (bottom left) and an eye of a myopic volunteer without glaucoma (bottom right) shows a full-thickness choroid in both cases. The black arrowheads indicate the position of the inner and outer border of the choroid and the location at which the choroidal thickness was measured. The choroid and subfoveal choroid are somewhat thicker in an eye of a myopic volunteer without glaucoma.

FIGURE 3. The receiver operating characteristic (ROC) curve for discriminating highly myopic NTG from healthy high myopia based on the subfoveal choroidal thickness. The area under the ROC curve of the choroidal thickness at the fovea is 0.88.
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Table 1.
Comparison of patient background parameters of eyes with normal tension glaucoma and control
Who underwent high-penetration optical coherence tomography for choroidal thickness measurement

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Highly-Myopic NTG (mean ± SD)</th>
<th>Control (mean ± SD)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (eyes)</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>33.6 ± 6.4</td>
<td>31.2 ± 4.1</td>
<td>0.33</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>(8/4)</td>
<td>(3/9)</td>
<td>0.10**</td>
</tr>
<tr>
<td>Spherical Equivalent Refractive Error (diopters)</td>
<td>-9.3 ± 1.2</td>
<td>-8.8 ± 1.6</td>
<td>0.34</td>
</tr>
<tr>
<td>Axial Length (mm)</td>
<td>27.6 ± 0.5</td>
<td>27.2 ± 0.5</td>
<td>0.08</td>
</tr>
<tr>
<td>Central Corneal Thickness (mm)</td>
<td>0.48 ± 0.04</td>
<td>0.49 ± 0.01</td>
<td>0.36</td>
</tr>
<tr>
<td>Corneal Refraction (diopter)</td>
<td>43.9 ± 0.56</td>
<td>43.7 ± 0.92</td>
<td>0.55</td>
</tr>
</tbody>
</table>

NTG= normal tension glaucoma., SD=standard deviation; *=by unpaired t-test, **=by Fisher's exact test

Table 2.
Comparison of glaucoma parameters of eyes with normal tension glaucoma and control
Who underwent high-penetration optical coherence tomography for choroidal thickness measurement

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Highly-Myopic NTG (mean ± SD)</th>
<th>Control (mean ± SD)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD (db)</td>
<td>-4.2 ± 3.7</td>
<td>-1.2 ± 0.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PSD (db)</td>
<td>5.8 ± 3.8</td>
<td>1.5 ± 0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IOP (mmHg)</td>
<td>13.7 ± 2.6</td>
<td>14.2 ± 2.4</td>
<td>0.63</td>
</tr>
</tbody>
</table>

NTG= normal tension glaucoma. SD=standard deviation; MD=mean deviation, PSD=pattern standard deviation, IOP=intraocular pressure,
*by unpaired t-test
Table 3.
Location of choroidal thickness measurement and the receiver operation curve (ROC) area

<table>
<thead>
<tr>
<th>Location</th>
<th>Choroidal Thickness (μm)</th>
<th>P-value*</th>
<th>ROC Curve Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Highly-Myopic NTG (mean ± SD)</td>
<td>Control (mean ± SD)</td>
<td></td>
</tr>
<tr>
<td>Fovea</td>
<td>166.7 ± 40.9</td>
<td>276.1 ± 74.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Disc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior</td>
<td>172.3 ± 77.7</td>
<td>241.5 ± 62.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Supero-Temporal</td>
<td>161.1 ± 71.9</td>
<td>244.8 ± 61.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Temporal</td>
<td>110.9 ± 40.1</td>
<td>161.5 ± 45.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Infero-Temporal</td>
<td>115.4 ± 36.1</td>
<td>159.9 ± 41.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Inferior</td>
<td>123.4 ± 44.3</td>
<td>162.1 ± 47.6</td>
<td>0.051</td>
</tr>
</tbody>
</table>

NTG=Normal tension glaucoma, SD=standard deviation; *=by unpaired t-test