Glaucoma

White Matter Abnormalities and Correlation With Severity in Normal Tension Glaucoma: A Whole Brain Atlas-Based Diffusion Tensor Study

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PURPOSE. To detect injury of whole brain white matter (WM) in normal tension glaucoma (NTG) patients by using diffusion tensor imaging (DTI) and to analyze the correlations between DTI parameters and glaucoma indices.

METHODS. Twenty mild, 17 moderate, and 18 severe NTG patients as well as 25 normal subjects were enrolled in this study. Atlas-based diffusion tensor analysis was performed to measure the fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD). One-way analyses of variance were used for comparisons of DTI parameters between NTG subgroups and normal control (NC) group. The relationships between DTI parameters and glaucoma indices were also assessed by Pearson’s correlation and “broken-stick” analyses.

RESULTS. As compared with NC subjects, significantly decreased FA and AD and increased MD and RD were observed in the bilateral posterior thalamic radiation, bilateral sagittal stratum, bilateral cingulum-hippocampus, and bilateral fornix/stria terminalis in NTG patients. The DTI parameters of these WM regions correlated with the mean deviation of visual field (MDVF) and retinal nerve fiber layer (RNFL) thickness. Additionally, there was a tipping point between MDVF and DTI parameters as well as between MDVF and RNFL thickness.

CONCLUSIONS. Atlas-based DTI analysis was capable of indicating WM damage in the four regions associated with visual and visual-related functions in NTG patients, and it could also be used for investigating disease progression and pathologic changes. In addition, WM impairment and RNFL thinning occurred before patients showed detectable visual field loss.

Keywords: normal tension glaucoma, diffusion tensor imaging, atlas-based analysis
(OCT), respectively, with a sensitivity that reflects the degree of glaucoma. Previous studies have demonstrated that DTI parameters of the visual pathway are correlated with MDVF and RNFL thickness via ROI analysis. However, to the best of our knowledge, this is the first study to explore the abnormalities of whole brain WM regions with DTI parameters in NTG patients by using the ABA method, and the correlations between them and MDVF and RNFL thickness.

**MATERIALS AND METHODS**

**Subjects**

The study was approved by the Institutional Review Board of our hospital and was conducted in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants.

The prospective observational study included patients with NTG as study group and a control group of healthy subjects. Fifty-five patients with bilateral symmetrical disease from NTG and 25 healthy controls who were age and sex matched were enrolled in this study from July 2016 to March 2017. All patients and healthy controls were examined by a specialized ophthalmologist with 10 years’ experience in glaucoma. The subjects underwent comprehensive ophthalmologic examinations, including optic intraocular pressure, slit-lamp microscopy, standard automated perimetry using the 30-2 program of the Humphrey Visual Field Analyzer (Carl Zeiss Meditec, Dublin, CA, USA) and RNFL thickness using the SD-OCT (Carl Zeiss Meditec). The diagnostic criteria for NTG patients...
Table 1. Clinical Characteristics of the Participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>NC, n = 25</th>
<th>NTG, n = 20</th>
<th>mi-NTG, n = 17</th>
<th>mo-NTG, n = 17</th>
<th>sc-NTG, n = 18</th>
<th>NC vs. NTG</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>58.7 ± 9.6</td>
<td>62.9 ± 9.4</td>
<td>63.4 ± 5.2</td>
<td>61.2 ± 8.3</td>
<td>64.1 ± 6.2</td>
<td>0.64</td>
<td>0.75</td>
</tr>
<tr>
<td>Male/female</td>
<td>13/12</td>
<td>29/26</td>
<td>10/10</td>
<td>10/8</td>
<td>0.85</td>
<td>0.86</td>
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<tr>
<td>MDVF, dB</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Left</td>
<td>−1.12 ± 1.71</td>
<td>−9.26 ± 5.84</td>
<td>−3.51 ± 1.02*</td>
<td>−8.25 ± 1.14†</td>
<td>−17.81 ± 2.97‡</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>Right</td>
<td>−0.71 ± 1.28</td>
<td>10.53 ± 6.19</td>
<td>−3.26 ± 1.16*</td>
<td>−8.35 ± 1.08†</td>
<td>−19.37 ± 3.14‡</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>Mean bilateral eyes</td>
<td>−0.92 ± 1.53</td>
<td>−9.88 ± 6.76</td>
<td>−3.38 ± 1.20*</td>
<td>−8.30 ± 1.24†</td>
<td>−18.60 ± 3.06‡</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>RNFL thickness, µm</td>
<td></td>
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<tr>
<td>Left</td>
<td>102.44 ± 9.36</td>
<td>72.81 ± 14.43</td>
<td>81.52 ± 10.83*</td>
<td>70.51 ± 11.42†</td>
<td>57.39 ± 9.33‡</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Right</td>
<td>108.81 ± 10.23</td>
<td>68.67 ± 15.52</td>
<td>83.93 ± 13.51*</td>
<td>67.64 ± 10.81†</td>
<td>50.75 ± 10.27‡</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean bilateral eyes</td>
<td>104.64 ± 8.86</td>
<td>70.89 ± 16.38</td>
<td>82.69 ± 12.37*</td>
<td>68.54 ± 10.38†</td>
<td>54.11 ± 10.08‡</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The values are expressed as the mean ± standard deviation, except sex; P values are calculated by using Student’s t test for NC versus NTG group and 1-way ANOVA for NC versus NTG subgroups (Dunnett’s post hoc test is further used for MDVF and RNFL thickness).

* mi-NTG versus NC.
† mo-NTG versus NC.
‡ sc-NTG versus NC.

 included an untreated peak intraocular pressure ≤ 21 mm Hg in 24-hour intraocular pressure profiles, an open anterior chamber, typical glaucomatous optic disc damage with nerve fiber bundle defects, and the exclusion of other causes of optic neuropathy. Then, NTG patients were further divided into three subgroups (mild, moderate, and severe NTG) by MDVF. Mild NTG is characterized by an MDVF of −0.01 to −6.00 dB. Moderate NTG is characterized by an MDVF of −6.01 to −12.00 dB. Severe NTG is defined with MDVF greater than −12 dB. The exclusion criteria were as follows: (1) age <18 years; (2) secondary glaucoma; (3) any history or suspicion of diseases that could affect the visual field; (4) central nervous system (CNS) diseases found on the initial MRI; and (5) any contraindications to the MRI examinations.

MRI Acquisition
Magnetic resonance imaging was performed on a 3.0-Tesla scanner (Verio; Siemens, Erlangen, Germany) equipped with a 32-channel head coil. DTI was performed with a single-shot spin-echo planar imaging (SE-PI) sequence with the following parameters: repetition time = 7425 ms, echo time = 84 ms, matrix size = 110 × 110, field of view = 220 × 220 mm, slice number = 50 slices, and slice thickness = 2.5 mm. Diffusion-weighted images were acquired along 30 noncollinear directions with a b value of 1000 s/mm². Additionally, a volume without diffusion weighting (b = 0 s/mm²) was acquired.

Table 2. Group Comparisons of the Mean DTI Parameters in Normal Controls As Well As The NTG Group and Subgroups

<table>
<thead>
<tr>
<th>Region</th>
<th>DTI Parameters</th>
<th>NC vs. NTG</th>
<th>mi-NTG vs. mo-NTG</th>
<th>sc-NTG vs. mo-NTG</th>
<th>P Value</th>
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All values are expressed as the mean ± standard deviation; MD, AD, and RD × 10⁻³ mm²/s. P values (FDR-correction) are calculated by using Student’s t test for NC versus NTG group (P < 0.05) and 1-way ANOVA with Dunnett’s post hoc test for NC versus NTG subgroups (P < 0.0167); **P < 0.01, ***P < 0.001.
DTI Data Processing

All DTI data were processed by using a pipeline toolbox for analysing brain diffusion images (PANDA). The main procedures of PANDA are described below. First, DICOM files were converted into NIfTI images. Second, the brain mask was estimated. Third, the raw images were cropped to reduce the image size. Fourth, the eddy current was corrected by coregistering each diffusion-weighted image to the $b_0$ image. Fifth, DTI parameters (FA, MD, AD, and RD) were calculated for each subject. Then, the DTI parameters were normalized into the MNI space and the average values within each region of the ICBM DTI-81 atlas were calculated. This version of the atlas did not suffer from the flaws pointed out by Rohlfing. Because the hippocampus is not included in the ICBM-DTI-81 labels, DTI parameters of bilateral hippocampus are additionally evaluated on the basis of the Automated Anatomical Labeling (AAL) atlas. Then DTI parameters of this region were also evaluated for each subject.

The WM regions with differences in DTI parameters between NC and NTG groups were shown with MRicron (http://www.nitrc.org/projects/mricron; provided in the public domain) and BrainNet Viewer (http://www.nitrc.org/projects/bnv; provided in the public domain), respectively.

Statistical Analysis

All statistical analyses were conducted by using IBM SPSS software (version 24; Chicago, IL, USA). The demographic characteristics, ophthalmologic examinations, and DTI parameters were compared between the NTG and NC groups by using the independent two-sample Student’s $t$-test for continuous variables, and the $\chi^2$ test was used for the sex proportion. The significant statistical threshold was set at a two-tailed $P < 0.05$. For DTI parameters, the false discovery rate (FDR) method was used to correct for multiple comparisons, and statistical significance was set at $P$ (FDR-corrected) $< 0.05$. The WM regions were considered to be abnormal when both FA and MD values were statistically different after FDR correction between NTG and NC groups. In addition, all quantitative data were further tested by 1-way analyses of variance (ANOVA) with Dunnett’s post hoc tests for comparisons between the NTG subgroups and NC group. After Bonferroni correction, $P$ (FDR-corrected) < 0.0167 ($0.05/3$, because intergroup comparisons were performed among three groups) was considered statistically significant. Finally, Pearson’s correlation analyses adjusted for age were used to explore the associations between the mean MDVF and RNFL thickness for both eyes as well as the mean FA, MD, AD, and RD values of the WM regions for all patients.

We also compared MDVF with DTI parameters of the four WM regions and RNFL thickness by using a “broken-stick” model. Davies’ test was used to calculate the tipping point. This analysis was conducted by using R Language and Environment for Statistical Computing program and the segmented R package. $P < 0.05$ was considered statistically significant.

RESULTS

Demographic and Ophthalmologic Measurements

The demographic characteristics and ophthalmologic examinations are presented in Table 1. The normal controls (NCs), mild (mi-NTG), moderate (mo-NTG), and severe (se-NTG) NTG...
patients did not differ significantly regarding age or sex. Compared with the NC group, all of the NTG subgroups had a significantly lower RNFL thickness ($P < 0.001$, using Dunnett’s post hoc test) and MDVF ($P < 0.001$, using Dunnett’s post hoc test).

**FA and MD Comparisons**

Significant differences in DTI parameters were found in the four WM regions that were linked to visual and visual-related functions between NTG patients and normal subjects (Fig 1); they are bilateral posterior thalamic radiation (B.PTR, with visual function), bilateral sagittal stratum (B.SS, with visual function), bilateral cingulum-hippocampus (B.CgH, with visual memory), and bilateral fornix/stria terminalis (B.FX/ST, with visual discrimination). In addition, DTI parameters of bilateral hippocampus (B.Hip) were also found to be different between the two groups.

The means and standard deviations of the FA and MD values and the statistical results across the groups are presented in Figure 2 and Table 2. Relative to the NC subjects, the NTG subjects showed significantly lower FA values and higher MD values in B.PTR, B.SS, B.CgH, B.FX/ST, and B.Hip.

Compared with the NC group, the mi-NTG subgroup demonstrated decreased FA and increased MD in B.PTR and B.SS. The mo-NTG subgroup showed lower FA and higher MD in B.PTR, B.SS, B.CgH, and B.FX/ST than the NC group. Moreover, the se-NTG subgroup had significantly lower FA and higher MD values than the NC group in B.PTR, B.SS, B.CgH, B.FX/ST, and B.Hip.

**AD and RD Comparisons**

The AD and RD values of these regions are shown in Figure 2 and Table 2. Compared to the controls, the mi-NTG subgroup had decreased AD in B.PTR and B.SS, and increased RD in B.PTR. The mo-NTG subgroup had decreased AD in B.PTR and B.SS, and increased RD in B.PTR and B.FX/ST. Furthermore, the se-NTG subgroup had lower AD than the NC group in B.PTR, B.SS, B.CgH, and B.Hip, with higher RD in B.PTR, B.SS, B.CgH, B.FX/ST, and B.Hip.

As shown in Figures 3 and 4, the FA and AD values in B.PTR, B.SS, B.CgH, and B.FX/ST were positively correlated with the MDVF ($P < 0.05$) and RNFL thickness ($P < 0.05$). The MD and RD values in these four regions were negatively correlated with MDVF ($P < 0.05$) and RNFL thickness ($P < 0.01$).

Relationships between visual field function and DTI parameters of the four WM regions were described by a “broken-stick” model (Fig. 5). The tipping point was existent at 0.496 (95% confidence interval [CI], 0.476–0.516), 0.457 (95% CI, 0.438–0.477), 0.389 (95% CI, 0.371–0.407), and 0.603 (95% CI, 0.585–0.621) for FA values; 0.928 (95% CI, 0.820–1.035), 0.946 (95% CI, 0.816–1.017), 0.905 (95% CI, 0.803–1.002), and 0.822 (95% CI, 0.752–0.892) for MD values; 1.242 (95% CI, 1.060–1.424), 1.261 (95% CI, 1.198–1.323), 1.333 (95% CI, 1.165–1.501), and 1.148 (95% CI, 0.918–1.379) for AD values; and 0.585 (95% CI, 0.454–0.716), 0.621 (95% CI, 0.555–0.687), 0.633 (95% CI, 0.545–0.721), and 0.710 (95% CI, 0.624–0.796) for RD values in the B.PTR, B.SS, B.CgH, and B.FX/ST, respectively. In addition, relationship between visual field function and retinal structure was also estimated at the tipping point of 68.39 μm (95% CI, 62.10–75.67).
DISCUSSION

Our preliminary study showed that WM changes caused by NTG can be found not only in the visual pathway, but also in the visual-related areas via the ABA method, as compared with previous ROI-based DTI studies on glaucoma. WM abnormalities were demonstrated in the four regions associated with visual (B.PTR, B.SS) and visual-related function (B.CgH, B.FX/ST) in NTG patients, as evaluated by lower FA and AD and higher MD and RD values. Further subgroup (mi-, mo-, and se-NTG) analyses revealed that these WM regions were damaged at different disease stages. In addition, DTI parameters of these four WM areas were well correlated with glaucoma indices (MDVF, RNFL thickness).

Few studies have assessed the microstructural integrity of the PTR or SS in glaucoma. Zikou et al. have observed decreased FA values in the PTR of POAG patients when using voxel-based DTI. In the current study, we found lower FA and AD and increased MD and RD values in the PTR and SS in NTG patients compared with NC subjects. Decreased FA and increased MD in our study suggested that the WM integrity of PTR and SS was damaged in NTG. This outcome probably occurred because both the PTR and SS primarily contain optic radiation. Optic radiation is one of the major components of the visual pathway, and previous studies have demonstrated that neurodegeneration of the optic radiation is a critical pathologic change in glaucoma. Subgroup analyses showed that significantly decreased AD and increased RD in the PTR and SS were present in the mi-NTG subgroup compared with normal group, and this effect became greater in the mo-NTG and se-NTG subgroups. Decreased AD and increased RD can reflect axonal degeneration and demyelination, inflammation or gliosis, respectively, and therefore we can further infer that these pathologic changes may occur in the PTR and SS during the early period of NTG and they would become more obvious as the disease progresses.

In this study, we found that FA decreased and MD increased in the B.CgH in NTG patients by using the ABA method. The CgH is a WM tract connection between cingulum and hippocampus, so the damage of this tract may impede the transmission of memory information from cingulum to hippocampus. The hippocampus is part of the Papez circuit and its main function is to integrate visual memory. To determine whether it is impaired in NTG patients, we additionally measured the DTI parameters of the bilateral hippocampus in our study. Interestingly, we also found significant differences in FA, MD, AD, and RD values in the B.Hip between NTG and NC groups, implying that the hippocampus was damaged in NTG patients. Therefore, an injury to the hippocampus may lead to visual memory deficit in NTG patients, which was consistent with a previous finding that patients with glaucoma may have memory impairments. Moreover, further subgroup analyses showed that the significant differences in FA, MD, AD, and RD values only appeared in the se-NTG subgroup. Our results indicated that the impairment of bilateral hippocampus occurred in the late stage of NTG, which may be related to axonal damage, demyelination, inflammation, gliosis, or a combination of different pathologic events. However, Wang et al. have not found significant structural alterations of the hippocampus in POAG patients compared with healthy controls. This different finding compared to our results may be due to the following reasons. On the one hand, the type of glaucoma studied by Wang et al. (POAG) is different from that in our study (NTG); thereby,
(a) Visual field function vs. Four white matter regions damage (alterations of FA, MD, AD and RD values)

(b) Visual field function vs. Retinal Structures

**Figure 5.** Relationships between visual field function, DTI parameters of these four WM regions (a), and retinal structures (b) in NTG and NC groups. Using the “broken-stick” model to determine the tipping points. *P < 0.05, **P < 0.01, ***P < 0.001.
different mechanisms may contribute to different brain structural changes. On the other hand, our results revealed that the hippocampus was damaged only in the se-NTG patients, but patients with different stages of POAG were analyzed together in the study of Wang and colleagues. Additionally, the presence of mild and moderate POAG patients enrolled in their study could reduce the differences in the hippocampus. In future studies, it would be worthwhile to study the microstructural changes in the hippocampus in patients with glaucoma of different types and clinical severities.

There has been no prior report that has assessed damage to the integrity of the FX/ST in glaucoma patients. However, in our study, we found significant decreased FA and increased MD in the FX/ST in both mo-NTG and se-NTG patients compared with NCs, indicating damage of FX/ST in the middle and late stage of NTG. The FX/ST is part of the limbic system, which is primarily responsible for visual discrimination. Thus, we suggested that NTG patients may have reduced function of visual discrimination, which was consistent with the study of Adams et al. Furthermore, RD was significantly increased in mo-NTG and se-NTG subgroups compared with normal subjects, while there was no significant difference in AD. The results indicated potential pathologic changes, such as demyelination, inflammation, or gliosis, occurred in the FX/ST in the middle and late stage of NTG, while the integrity of the axon was not destroyed. Some studies have demonstrated that the disruption of the FX/ST is associated with Alzheimer’s disease. Our results supported the notion that glaucoma and Alzheimer’s disease may share similar underlying mechanisms.

In addition, the current study also analyzed the relationship between the DTI parameters of these four WM regions (B.PTR, B.SS, B.CgH, and B.FX/ST) and ophthalmologic examinations (MDVF and RNFL thickness). We found that the FA and AD values in these four WM regions were moderately to strongly (\( r = 0.325, 0.702 \)) positively correlated with the MDVF and RNFL thickness, whereas the MD and RD values were moderately (\( r = -0.365, -0.544 \)) negatively correlated with them, respectively. The MDVF and RNFL thickness are sensitive parameters that reflect the severity of glaucoma. Therefore, the DTI parameters of these four WM regions, which were extracted from the ABA method, could serve as potential markers for assessing the WM (visual and visual-related functions) microstructural changes of early, middle, and late stages of NTG, and that may be helpful for monitoring the progression of NTG. Furthermore, for NTG patients with different severities, DTI parameters can indicate the pathologic changes in these WM regions, such as integrity damage, axonal degeneration, demyelination, inflammation, or gliosis, which cannot be acquired by MDVF or RNFL thickness.

To further determine the relationships between visual field function, WM regions, and retinal structures, we compared MDVF with DTI parameters of the four abnormal WM regions and RNFL thickness by using the “broken-stick” model. We detected tipping points between visual field function and FA, MD, AD, and RD values of the four abnormal WM regions. These results suggested that visual field function remained stable before DTI parameters of these four WM regions reached the tipping points, but visual field loss was detectable when the DTI parameters advanced beyond a tipping point. Additionally, a tipping point was also found between MDVF and RNFL thickness, which was in accordance with previous studies, indicating that RNFL thinning occurred before significant visual field loss. Thus, we observed that both retinal structure and WM regions were altered before detectable visual field loss in NTG patients, which was similar to a previous finding reported by Murphy et al. Our study had some limitations. First, some brain abnormalities could affect the quality of template matching. Therefore, we excluded patients with obvious encephalatrophy to improve the accuracy of the ABA method. Second, the sample size of NTG patients was relatively small because the patients were further divided into three subgroups. Therefore, a larger sample size will be needed in our further studies.

In conclusion, atlas-based DTI analysis demonstrated that significant WM abnormalities were found in the regions associated with visual and visual-related functions in NTG patients. In addition, the injury severity in these WM regions correlated with the MDVF and RNFL thickness, which can help elucidate the potential pathologic changes in these regions during NTG progression by the alterations of DTI parameters. Furthermore, deteriorations may be already present in these WM regions and retinal structure before clinical visual field loss.

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