Altered Microstructure of White Matter Except the Corpus Callosum Is Independent of Prematurity

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Key Words
Diffusion tensor imaging • Fractional anisotropy • Preterm infants • Gestational age • Clinical variables

Abstract
Background: Diffusion tensor imaging (DTI) reflects the maturation of the brain microstructure. Although preterm infants are at significant risk for altered brain microstructure, it remains unclear whether this is affected by prematurity itself or other clinical factors. Objectives: To investigate DTI parameters in preterm infants at a term-equivalent age (TEA) compared with healthy term infants and to assess the associations between DTI parameters and clinical factors that may affect brain development. Methods: We studied 34 preterm infants without apparent brain lesions and 12 healthy term infants using tract-based spatial statistics. Region-of-interest analysis was performed in the posterior and anterior limbs of the internal capsule (PLIC and ALIC), corpus callosum (CC), optic radiation, and cerebral peduncle. Results: Preterm infants had significantly decreased fractional anisotropy (FA) in nearly the entire white matter (WM) compared with term infants ($p < 0.01$). Multiple regression analysis showed that FA in the PLIC, ALIC, optic radiation, and cerebral peduncle were positively associated with postmenstrual age (PMA) at imaging and that the apparent diffusion coefficient was negatively associated with PMA. Only FA in the CC was positively correlated with gestational age. Chronic lung disease (CLD) and postnatal infection were associated with decreased FA in the CC and PLIC, respectively. Conclusions: Preterm infants at TEA showed an altered microstructure of the WM compared with healthy term infants. The altered microstructure of the measured WM except the CC was independent of the degree of prematurity. Chronic lung disease and postnatal infection are related to localized WM alterations. Copyright © 2012 S. Karger AG, Basel

Introduction
Preterm infants have high rates of neurodevelopmental impairments, including major cognitive deficits, neurosensory impairments, and learning disabilities [1, 2]. The most important brain abnormality in preterm infants is periventricular leukomalacia (PVL), which is frequently accompanied by neuronal/axonal disease [1]. Diffusion tensor imaging (DTI) has been proposed as a useful tool for investigating the structure of white matter (WM) tracts [3, 4]. DTI enables the quantitative assess-
ment of brain maturation and tract organization by using diffusion parameters such as fractional anisotropy (FA) and apparent diffusion coefficient (ADC). In general, FA increases and ADC decreases with age, which is believed to reflect WM maturations such as fiber coherence, axonal density, and myelination [5].

Compared with term infants, decreases in FA and increases in ADC have been observed within the WM in preterm infants [4, 6]. These alterations are dependent on the degree of prematurity [7] and are linked to later neurodevelopmental outcomes [8]. Several studies suggested that postnatal complications, such as chronic lung disease (CLD) or infection, are associated with adverse neurodevelopmental outcomes in preterm infants [9–11]. However, it remains unclear whether the altered brain microstructure is affected by prematurity itself or by postnatal complications encountered during the hospital stay.

The aim of our study was to assess the WM microstructure in preterm infants without apparent brain lesions compared with healthy term infants using tract-based spatial statistics (TBSS). To investigate the effects of clinical risk factors on the WM microstructure, a significant correlation between any of the study variables and the DTI parameters was further explored with local region-of-interest (ROI) analysis.

Materials and Methods

This study was approved by the institutional review board at Gachon University Gil Hospital, and written informed consent was obtained from all parents or caregivers of the participating infants.

Table 1. Demographic data and clinical variables for the preterm and term groups

<table>
<thead>
<tr>
<th></th>
<th>Preterm group (n = 34)</th>
<th>Term group (n = 12)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>18 (52.9)</td>
<td>5 (41.7)</td>
<td>0.737</td>
</tr>
<tr>
<td>Gestational age, weeks</td>
<td>30 ± 1 (23–35)</td>
<td>38 ± 2 (37–40)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>1,396 ± 481 (550–2,800)</td>
<td>3,100 ± 572 (2,800–4,000)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Postmenstrual age at MRI, weeks</td>
<td>38 ± 2 (35–42)</td>
<td>40 ± 2 (38–42)</td>
<td>0.024</td>
</tr>
<tr>
<td>Duration of ventilator care, days</td>
<td>19.5 ± 24.8 (0–94)</td>
<td>0</td>
<td>0.01</td>
</tr>
<tr>
<td>Age at full enteral feeding, days</td>
<td>11.4 ± 5.93</td>
<td>4.5 ± 0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chronic lung disease</td>
<td>8 (23.5)</td>
<td>0</td>
<td>0.090</td>
</tr>
<tr>
<td>Patent ductus arteriosus</td>
<td>12 (35.3)</td>
<td>0</td>
<td>0.020</td>
</tr>
<tr>
<td>Postnatal infection1</td>
<td>7 (20.6)</td>
<td>0</td>
<td>0.165</td>
</tr>
<tr>
<td>Use of postnatal steroid</td>
<td>5 (14.7)</td>
<td>0</td>
<td>0.306</td>
</tr>
<tr>
<td>Use of inotropics</td>
<td>7 (20.6)</td>
<td>0</td>
<td>0.165</td>
</tr>
</tbody>
</table>

Values are means ± SD (range) or n (%).  
1 Included culture-proven sepsis (n = 4) and NEC (n = 3).

Infants

Magnetic resonance imaging (MRI) was performed in 48 preterm infants (≤35 gestational weeks at birth, preterm group) who were admitted to the neonatal intensive care unit at Gachon University Gil Hospital. MRI was conducted around the term-equivalent age (TEA) (postmenstrual age (PMA) 35–42 weeks). As the control group, 14 healthy term infants (≥37 gestational weeks at birth, term group) whose parents agreed to participate in the study were recruited, and MRI was conducted within 1 month of age (PMA 38–42 weeks) (table 1). Infants with brain abnormalities such as PVL, intraventricular hemorrhage grade III–IV (IVH), and ventriculomegaly (>8 mm measured at the level of the glomus of the choroid plexus) [12, 13], diagnosed by conventional MRI or cranial ultrasonography (cUSG) (in 4 preterm infants who failed to obtain conventional MRI), were excluded. Ten preterm infants were excluded because of brain abnormalities (3 PVL, 3 IVH, 2 post-hemorrhagic ventriculomegaly, 1 cerebellar hemorrhage, and 1 callosal agenesis). Among the remaining enrolled infants (38 preterm and 14 term infants), 4 preterm and 2 term infants were excluded in the DTI sequence because of motion artifacts. Finally, 34 preterm and 12 term infants were included.

In the preterm group, clinical variables were prospectively collected by two clinical nurses who were blinded to the study. In the term group, parents or caregivers completed questionnaires about their infants.

Image Acquisition

The infants were scanned during natural sleep after feeding, and they were monitored with a MR-compatible pulse oximeter. If feeding failed to induce natural sleep, a low dose of chloral hydrate (30 mg/kg) was given orally. Each infant received individually modulated earplugs (McKeon Products, Warren, Mich., USA) and a manually made ear cushion for damping down the noise. A physician stayed in the scanner room during the entire examination. We used a conventional 3.0-T MRI (Verio, Siemens) with a Siemens matrix coil. 3D MPRAGE imaging and T1- and T2-weighted imaging were obtained prior to DTI. The DTI sequence parameters were as follows: b = 0 and 700 s/mm²; TR/
**Image Analysis**

The diffusion-weighted images were processed with the FMRIB Software Library (FSL v4.1.4: www.fmrib.ox.ac.uk/fsl) [14]. The DTI data were first corrected for eddy-current-induced spatial distortion, and the images were brain extracted using the Brain Extraction Tool [15]. Individual FA and ADC maps and eigenvalue maps ($\lambda_1$, $\lambda_2$, and $\lambda_3$) were generated. Voxel-wise statistical analysis of FA (or ADC) was performed with TBSS for neonates [16]. A correction for multiple comparisons and cluster formation was conducted with threshold-free cluster enhancement (TFCE) [17]. Voxels with $p < 0.05$ (TFCE-corrected) were considered significantly different.

To investigate the relationship between the DTI parameters and clinical variables, we selected the five regions from a TBSS-generated FA map in each subject (fig. 1). Two researchers drew ROIs, and no significant differences were found either between observers ($p = 0.78$) or between test-retest ($p = 0.45$).

**Statistical Analysis**

For continuous variables, the mean, standard deviation, and range are reported. Individual t tests and Fisher’s exact test were used for comparisons between two groups. Stepwise multiple regression analysis was used to assess the clinical variables that are associated with the DTI parameters via the ROIs. The clinical variables included gestational age (GA) at birth, PMA at imaging, gender, duration of ventilator care, age at full enteral feeding (defined as the total volume of enteral feeding $\geq 100$ ml/kg/day), use of postnatal steroids or inotropics, presence of patent ductus arteriosus (PDA), diagnosis of CLD (defined as the need for supplemental oxygen at 36 weeks of PMA), and postnatal infection (including culture-proven sepsis and necrotizing enterocolitis (NEC) $\geq$ Bell’s stage IIa). Variables for which the $p$ value by univariate analysis was $< 0.2$ were included in the subsequent linear stepwise analysis. $p < 0.05$ was considered statistically significant for all analyses.

**Results**

**General Characteristics of the Study Population**

The clinical characteristics of the preterm ($n = 34$) and term groups ($n = 12$) are shown in table 1.

**Comparisons of the DTI Parameters between the Preterm and Term Groups**

The group-wise voxel-based comparison was conducted between the preterm ($n = 34$) and term groups ($n = 12$), and revealed significantly decreased FA values in the preterm group compared with the term group ($p < 0.01$; TFCE-corrected). This finding remained after the correction for PMA difference; for removing PMA difference between two groups, 17 preterm infants with $\geq 38$ weeks of PMA were selected and analyzed again. Their median (range) GA and PMA were $27^{+4}$ ($23^{+6}$–$34^{+6}$) weeks and $39^{+4}$ ($38^{+0}$–$42^{+2}$) weeks, respectively (fig. 2). The quantitative FA and ADC values of five ROIs are
shown in table 2. As there was no significant difference between the right and left hemispheres (p > 0.05), the values were averaged. In the preterm group, all FA values in the measured ROIs were lower compared with the term group. Conversely, all ADC values in the ROIs except for the corpus callosum (CC) were higher in the preterm group.

**Association between DTI Parameters and Clinical Variables**

The results of the multiple regression analysis between the DTI parameters and clinical variables (see statistical analysis) in five ROIs are shown in figures 3 and 4. The preterm and term groups were not separated for this analysis. The FA in the CC was found to be significantly correlated with GA at birth (fig. 3). In contrast, the FA and ADC in the posterior and anterior limb of internal capsules (PLIC and ALIC), optic radiation, and cerebral peduncle were not significantly associated with GA but were significantly correlated with PMA (fig. 4). In particular, the FA and ADC in the PLIC showed the strongest association with PMA. CLD and postnatal infection were not correlated with ADC but were significantly correlated with decreased FA in the CC (p = 0.023) and PLIC (p = 0.041), respectively. The FA and ADC in all ROIs were independent of other study variables.

**Discussion**

We demonstrated that preterm infants without apparent brain lesions at TEA showed an altered microstructure of the WM compared with healthy term infants. The advantage of using TBSS for the analysis of diffusion data is that it provides an objective, sensitive, and clearly interpretable method for multi-subject, whole-brain fusion data analysis [7]. A previous study using TBSS showed a reduction in FA in the CC, centrum semiovale, and frontal WM in preterm infants at term [7]. We found that the preterm group had a lower FA and higher ADC in nearly the entire WM compared with the term group. One possible reason for the more extensive reduction in FA in our study was that the median GA in preterm infants was younger than a previous study (27+4 vs. 28+6 weeks). However, our TBSS results did not consider other clinical factors that may influence brain maturation. Therefore, we investigated the relationship between the measured DTI parameters and clinical variables previously related to the risk of short- and long-term neurological outcomes [9–11, 13, 16, 18, 19].

**Table 2. Comparison of the mean FA and the mean ADC between the preterm and term groups**

<table>
<thead>
<tr>
<th>ROIs</th>
<th>Preterm group (n = 34)</th>
<th>Term group (n = 12)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corpus callosum</td>
<td>0.319 (0.028)</td>
<td>0.361 (0.030)</td>
<td>0.001</td>
</tr>
<tr>
<td>ALIC</td>
<td>0.266 (0.037)</td>
<td>0.325 (0.043)</td>
<td>0.001</td>
</tr>
<tr>
<td>PLIC</td>
<td>0.348 (0.028)</td>
<td>0.400 (0.036)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Optic radiation</td>
<td>0.322 (0.041)</td>
<td>0.378 (0.056)</td>
<td>0.001</td>
</tr>
<tr>
<td>Cerebral peduncle</td>
<td>0.387 (0.049)</td>
<td>0.480 (0.073)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ADC (10⁻³ mm²/s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corpus callosum</td>
<td>1.501 (0.101)</td>
<td>1.472 (0.104)</td>
<td>0.463</td>
</tr>
<tr>
<td>ALIC</td>
<td>2.583 (0.164)</td>
<td>2.260 (0.158)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PLIC</td>
<td>1.107 (0.045)</td>
<td>1.029 (0.041)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Optic radiation</td>
<td>3.232 (0.226)</td>
<td>2.951 (0.187)</td>
<td>0.001</td>
</tr>
<tr>
<td>Cerebral peduncle</td>
<td>3.071 (0.442)</td>
<td>2.582 (0.245)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are means (SD).

In our study, five ROIs were selected from a TBSS-generated skeleton FA slice. The CC is the main WM tract between the two hemispheres and plays an important role in neurodevelopment [11]. The PLIC is known to be sensitive to hypoxic insult, and the cerebral peduncle mediates the communication within the neuronal network [20]. The optic radiation is known to be related to visual function in preterm infants [21, 22]. Multiple regression anal-
Fig. 4. Significant correlation between the DTI parameters and PMA at imaging in ROIs. The DTI parameters in each ROI were independent of GA at birth. Hollow circles and black squares indicate preterm infants and term infants, respectively.
ysis showed that the FA in the CC increased linearly with the GA at birth. A similar finding was reported by Hasegawa et al. [23]; however, de Bruine et al. [24] found no significant maturation trends between the CC and GA at birth. At present, we cannot clearly explain why only the FA of the CC was dependent on GA but not on PMA. Previous studies reported that younger GA is related to smaller callosal size and altered callosal microstructure in preterm infants and that these are linked to poor neurological outcomes [7, 11, 23, 25]. Impairment of callosal growth is suggested as a potential indicator of cerebral axonal disturbance [1]. The CC appears more vulnerable in preterm infants with a younger GA at birth [11]. In other aspects, our result may just reflect delayed maturation of the CC. An evaluation of how the changes in the CC will affect neurodevelopment will be explored in a clinical follow-up. We used infants without apparent brain lesions and this may account for the lack of an ADC change in the CC because the ADC in the CC can be altered by WM injury [23].

Interestingly, the FA and ADC of the PLIC, ALIC, optic radiation, and cerebral peduncle were significantly associated with PMA at imaging. In these ROIs, we did not find any associations between the DTI parameters and GA at birth. Increases in the FA and decreases in the ADC reflect the myelination and maturation of the brain [5]. The maturation of the investigated WM, with the exception of the CC, appears to progress with PMA and is independent of the degree of prematurity. Until now, several studies have focused on the development of PLIC using DTI [20, 24, 26]. Most of these studies found that the FA of the PLIC increases with age and that lower values for the FA in the PLIC reflect a poorer neurological outcome. The myelination of the PLIC is suggested to be an important hallmark of WM maturity [26]. Our study supports this finding because the DTI parameters in the PLIC showed the strongest correlation with PMA. Previous studies showed a close relationship between the FA in the optic radiation and visual function in preterm infants [21, 22], and our study may reflect that this visual function progresses with PMA.

Bonifacio et al. [27] reported that comorbid conditions such as PDA, NEC, and the need for mechanical ventilation, rather than prematurity may be an important determinant of brain microstructure maturation. Their finding is in accord with our result that showed most measured regions except the CC are independent of GA. We demonstrated that CLD and postnatal infection were associated with reduced FA in the CC and PLIC, respectively. Ball et al. [19] reported the association between CLD and reduced FA in the inferior longitudinal fasciculus, CC, and centrum semiovale. Several studies found that postnatal infection in preterm infants is associated with WM injury [1, 10, 18]. One potential reason for our result is that the CC and PLIC may be particularly susceptible to hypoxic-ischemic injuries [20, 28] encountered during the disease course. Gender differences, postnatal steroid use, and the duration of ventilator care are known to be associated with brain injury [11, 19]. However, we did not find any correlations with DTI parameters.

A limitation of our study was that brain lesions were screened by cUSG in 4 of 48 preterm infants. However, the cUSG was performed serially several times by two experienced pediatric radiologists, and we think that apparent brain lesions could be successfully identified. We could not demonstrate any follow-up data associated with neurological outcome, but this will be included in a subsequent study.

In conclusion, our study demonstrated that preterm infants without apparent brain lesions at TEA showed altered microstructures of the WM compared with healthy term infants. The measured WM, including the PLIC, ALIC, optic radiation, and cerebral peduncle, showed ongoing maturation with PMA but was independent of GA at birth. CLD and postnatal infection were associated with altered microstructures in the CC and PLIC, respectively. Only the CC depended on the degree of prematurity. Longitudinal studies are required to assess neurodevelopmental outcomes linked to the regional brain development in our sample.

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References


Shim/Jeong/Son/Jeong/Oh/Park/Ryu/Kim/Cho


