Research Report

Diffusion tensor imaging and MR spectroscopy of microstructural alterations and metabolite concentration changes in the auditory neural pathway of pediatric congenital sensorineural hearing loss patients

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A B S T R A C T

Purpose: Our objective was to evaluate age-dependent changes in microstructure and metabolism in the auditory neural pathway, of children with profound sensorineural hearing loss (SNHL), and to differentiate between good and poor surgical outcome cochlear implantation (CI) patients by using diffusion tensor imaging (DTI) and magnetic resonance spectroscopy (MRS).

Materials and Methods: Ninety-two SNHL children (49 males, 43 females; mean age, 4.9 years) were studied by conventional MR imaging, DTI and MRS. Patients were divided into three groups: Group A consisted of children ≤ 1 years old (n=20), Group B consisted of children 1–3 years old (n=31), and group C consisted of children 3–14 years old (n=41). Among the 31 patients (19 males and 12 females, 12 m- 14y ) with CI, 18 patients (mean age 4.8 ± 0.7 years) with a categories of auditory performance (CAP) score over five were classified into the good outcome group and 13 patients (mean age, 4.4 ± 0.7 years) with a CAP score below five were classified into the poor outcome group. Two DTI parameters, fractional anisotropy (FA) and apparent diffusion coefficient (ADC), were measured in the superior temporal gyrus (STG) and auditory radiation. Regions of interest for metabolic change measurements were located inside the STG. DTI values were measured based on region-of-interest analysis and MRS values for correlation analysis with CAP scores.

Results: Compared with healthy individuals, 92 SNHL patients displayed decreased FA values in the auditory radiation and STG (p<0.05). Only decreased FA values in the auditory radiation was observed in Group A. Decreased FA values in the auditory radiation and STG were both...
observed in B and C groups. However, in Group C, the N-acetyl aspartate/creatinine ratio in the STG was also significantly decreased (p<0.05). Correlation analyses at 12 months post-operation revealed strong correlations between the FA, in the auditory radiation, and CAP scores (r=0.793, p<0.01).

Conclusions: DTI and MRS can be used to evaluate microstructural alterations and metabolite concentration changes in the auditory neural pathway that are not detectable by conventional MR imaging. The observed changes in FA suggest that children with SNHL have a development delay in myelination in the auditory neural pathway, and it also display greater metabolite concentration changes in the auditory cortex in older children, suggest that early cochlear implantation might be more effective in restoring hearing in children with SNHL.

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1. Introduction

High-resolution CT is the primary imaging modality used in the initial workup of patients with profound hearing loss. However, high-resolution CT is not sufficient to evaluate the soft tissue structures of the inner ear, such as the membranous labyrinth and the vestibulocochlear nerve. Magnetic resonance imaging (MRI) can directly assess these soft tissue structures responsible for hearing (Moon et al., 2012). Imaging modalities, such as CT and MRI, can accurately and objectively evaluate sensorineural hearing loss due to the morphological abnormalities present (Jonas et al., 2012). In addition, pre-operative magnetic resonance imaging (MRI) can yield valuable information regarding the status of the inner ear in pediatric cochlear implant (CI) candidates, and can improve the success rate of cochlear implants (Hong et al., 2010; Migirov and Wolf, 2013). MRI anomalies in CI patients provide clinically significant information that may be related to poor postoperative outcomes (Moon et al., 2012; Hong et al., 2010). However, a portion of patients with no MRI anomalies have poor post-operative outcomes, indicating a need to identify functional changes or dysfunction of the central auditory pathway itself, which cannot currently be evaluated by conventional imaging (Chang et al., 2004). To further investigate the clinical significance of preoperative brain MRI in pediatric cochlear implant recipients, we investigated whether microstructural changes of white matter tracts and proton metabolites in the auditory cortex could be detected in SNHL patients.

Recent advances in MR imaging provide in vivo tools for studying the microstructure of the CNS. DTI allows one to quantify the integrity of densely packed fiber bundles, such as axonal tracts (Chang et al., 2004). Neural reorganization occurs when the inputs to the sensory system change. Similarly, the pattern of auditory cortical activation becomes altered when the inputs to the auditory system change as a result of peripheral hearing loss. In healthy white tracts, the anisotropy (limited directionality of diffusion) is higher than in less-organized gray matter, enabling myelin development to be detectable by DTI (Chang et al., 2004). In addition, MR spectroscopic studies of hearing loss disorders would be important in understanding how the metabolites listed above are affected in the auditory pathways of the brain during neuronal activity (Nikutun et al., 2006). In this study, to provide a basis for improving the effectiveness of cochlear implants, we use DTI and MRS to investigate the age-dependent neural integrity of subcortical auditory projections, central to the cochlear nerve, in SNHL children without inner ear malformations.

2. Analysis

Diffusion tensor data was processed and analyzed using DTI-Studio software and an Advantage workstation for Windows (AW4.5, GE Healthcare). After correction for movement and EPI-induced distortion artifacts, the diffusion tensor was calculated for each voxel. The final DTI dataset was fed into Functool software, which automatically computes the FA and ADC maps. The region of interest was about 25 mm² and was traced on the superior temporal gyrus and auditory radiation in the original DTI. FA and ADC values for the regions of interest of the control group. Three repeats were acquired and averaged to improve the signal-to-noise ratio. The average value of the data was measured by two experienced radiologists.

For proton MR spectroscopic imaging, a region of interest (ROI) was positioned in the center of an axial T2-weighted scout image positioned in the superior temporal gyrus. Spectroscopic data was processed using SAGE (spectroscopy analysis, GE Medical Systems) for automatic zero-order auto-phasing and two-dimensional discrete Fourier transformation. No spectral or spatial apodization was used to present unmodified raw data to LCModel. A SAGE macro was used to estimate metabolite concentrations using LCModel, a fitting algorithm using a linear combination of model spectra. All spectra were visually inspected to ensure a good fit. Peak area ratios of N-acetyl aspartate (NAA), total choline (Cho), combined glutamate and glutamine and myo-inositol were obtained relative to total creatine (Cr) for each voxel. Since chemical shift artifacts cause a displacement of the excited volume for different metabolites, LCModel concentrations for each voxel were corrected for this effect with an in-house program using information on the RF pulse profiles, the dimensions and position of the ROI, and the resonance frequency of each metabolite. Metabolite ratios calculated...
from the corrected concentrations for each voxel were exported to a spreadsheet for each subject.

Statistical analysis: ROIs along the subcortical auditory pathway were drawn bilaterally for evaluation; the auditory radiation and superior temporal gyrus were both evaluated bilaterally by a neuroradiologist. The mean and standard errors of all indices were computed from each region of interest for all subjects. For comparisons within each group and between groups, we used Student’s t-test. A two-tailed P-value < 0.05 was taken as statistically significant. We used Student’s t-test to analyze the DTI and MRS values between good outcome and poor outcome. We used Pearson correlation analyses to determine correlations among DTI values, MRS values and CAP score. The correlations between CAP scores were also determined. SPSS ver. 17 (SPSS, Chicago, IL, USA) was used for all statistical analyses.

3. Results

CT and conventional MRI showed no anatomical abnormalities of the brain or inner ear (cochlear aplasia, common cavity deformity, cochlear hypoplasia, Mondini deformity), and no cochlear nerve deficiency in any individual. The two diffusion indices at the STG and auditory radiation for the SNHL and control groups are summarized in Table 1. DTI showed that FA values in the auditory radiation were decreased (p < 0.05) in the SNHL group (n=92) compared to the controls. However, in Group A (≤ 1 years-old, n=20), only the FA values in the auditory radiation reached statistical significance (p < 0.05). In Group B (1-3 years-old, n=31) and Group C (3-14 years-old, n=41) a reduction of FA values in auditory radiation and STG were observed (p < 0.05) (Fig. 1).

The MRS values of sensorineural hearing loss in SNHL individuals and controls are depicted in Table 2. The NAA/Cr ratio in the STG was decreased in Group C (p < 0.05). However, in Group A and B, the difference in all parameters among SNHL individuals and controls did not reach statistical significance (p > 0.05). Normal MR spectroscopic values and DTI values at the STG and auditory radiation for good outcome and poor outcome by the CAP scores are presented in Table 3. The correlations between the MR spectroscopy and DTI values with CAP scores are displayed graphically in Fig. 2. When FA in the auditory radiation and CAP scores were evaluated at 12 months post-operation, strong correlations between FA and CAP score were observed, with higher FA values being found in the auditory radiation in good outcome subjects compared to poor outcome subjects (p < 0.05).

4. Discussion

Inner ear structure, integrity of the central auditory pathway and auditory and language centers, and the time of cochlear implantation influence the success of cochlear implantation. The time of cochlear implantation can affect cochlear nerve (CN) growth. Long-term injury of a peripheral hearing organ, such as the CN, can result in axonal loss and/or demyelination (Wu et al., 2009b). Previous studies have shown that bilateral damage of peripheral hearing organs can result in disruption of projections and decreased neuronal activity (Lin et al., 2008). Auditory development has a sensitive period. The central auditory system retains great plasticity upon lack of normal stimulation. Under conditions of normal hearing development, myelinated fibers extend projections to the auditory cortex, which continues from 1 to 12 years of age, with the formation of auditory cortical myelination being dependent on stimulation by a variety of sounds (Zwolan et al., 2004). The time of cochlear implantation plays an important role in the development of auditory cortical myelination in children with SNHL (Pulsifer et al., 2003).

White matter fiber myelination will be lost after blockage of the auditory afferents. Deaf participants have larger gray-white matter ratios bilaterally in the Heschl’s and superior temporal gyri than normal hearing participants. Emmorey et al. found that auditory deprivation results in less myelination and fewer fibers projecting to and from the auditory cortices (Emmorey et al., 2000). Tucci et al. (1999) found that, in animals, both unilateral conductive and sensorineural hearing loss (SNHL) markedly decrease metabolic activity in the ascending central auditory pathway (Tucci et al., 1999).

Table 1 – Summary of the diffusion indices (mean and SEM) at the STG and auditory radiation in SNHL and control groups.

<table>
<thead>
<tr>
<th></th>
<th>SNHL (SEM)</th>
<th>CONTROL (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td>FA in STG</td>
<td>0.24 (0.012)</td>
<td>0.23 (0.004)*</td>
</tr>
<tr>
<td>ADC in STG</td>
<td>9.84 (0.179)</td>
<td>9.34 (0.061)</td>
</tr>
<tr>
<td>FA in auditory radiation</td>
<td>0.30 (0.016)*</td>
<td>0.31 (0.005)*</td>
</tr>
<tr>
<td>ADC in the auditory radiation</td>
<td>10.11 (0.154)</td>
<td>9.44 (0.066)</td>
</tr>
</tbody>
</table>

Group A: SNHL: 0-1 years old. Group B: SNHL: 1-3 years-old. Group C: SNHL: 3-14 years-old
Although there is no evidence from animal studies to support that long-term SNHL will result in neural fiber degeneration of the central auditory tract, Emmorey et al. (2000) have performed morphometric analyses of auditory brain regions in congenitally deaf adults (Emmorey et al., 2000). MRI and CT can depict the macroscopic anatomy of the central nervous system, but cannot evaluate the cellular functions and disease processes at the microscopic level. All the patients in our studies were with normal brain manifestation based on conventional MRI. The main challenge to magnetic resonance imaging (MRI) studies of the subcortical auditory pathway is the spatial resolution, which limits the ability to probe these tiny structures. The diffusion anisotropy indices used only provide speculation, rather than a clear understanding of the pathophysiology of the auditory tract. The integrity of the subcortical auditory nerve bundles in patients with long-term bilateral hearing loss remains unclear.

DTI appears quite sensitive to functional changes of the white matter tract and has been introduced to study the microstructure of white matter of the CNS in vivo to investigate functional changes or dysfunction of the corticospinal tract in stroke (Chang et al., 2012; Wu et al., 2009a). A preliminary study by Chang et al. (2004) used DTI to suggest that a reduction of FA occurs in regions along the auditory

![Image of the ROIs (bilaterally) of the STG and auditory radiation are depicted in the picture (1.2), respectively.](image1)

### Table 2 – MR spectroscopic values (mean and SEM) at the STG for the SNHL and control groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>A (SEM)</th>
<th>B (SEM)</th>
<th>C (SEM)</th>
<th>Total (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA</td>
<td>12.26 (0.438)</td>
<td>14.86 (0.219)</td>
<td>16.10 (0.239)</td>
<td>14.46 (0.220)</td>
</tr>
<tr>
<td>NAA/CR</td>
<td>2.10 (0.051)</td>
<td>2.13 (0.020)</td>
<td>1.95 (0.018)</td>
<td>2.11 (0.016)</td>
</tr>
<tr>
<td>CHO</td>
<td>2.32 (0.045)</td>
<td>2.30 (0.032)</td>
<td>2.37 (0.031)</td>
<td>2.34 (0.030)</td>
</tr>
<tr>
<td>CHO/CR</td>
<td>0.40 (0.009)</td>
<td>0.33 (0.005)</td>
<td>0.32 (0.005)</td>
<td>0.36 (0.007)</td>
</tr>
<tr>
<td>Group A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>13.04 (0.479)</td>
<td>14.37 (0.397)</td>
<td>16.63 (0.474)</td>
<td>14.76 (0.346)</td>
</tr>
</tbody>
</table>

Group A: SNHL: 0-1 years old. Group B: SNHL: 1-3 years-old. Group C: SNHL: 3-14 years-old. Cho - Choline; Cr - Creatine; NAA - N-acetylaspartate; (* P < 0.05 compared with controls).

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pathway in participants with SNHL (Chang et al., 2004). We further evaluate the integrity of the auditory neural pathway by DTI in sensorineural hearing loss in different age groups and show that FA values are decreased in the auditory radiation in SNLH patients. FA values in the STG are also decreased in children 1-3 years of age, suggesting the presence of a delay in myelin development.

This is also evidence demonstrating that the central pathway undergoes developmental changes that occur, at least partially, in parallel with peripheral development (Paydar et al., 2014). The development of the central auditory system is associated with the age (Saksena et al., 2008).

MRS can reveal changes of proton metabolism in the auditory pathway under conditions of sensorineural hearing loss. Neuronal metabolite ratios derived from MRS sequences can objectively reflect the degree of nerve damage and progression of brain damage. Evidence of neuronal or axonal loss and damage can be obtained from measurements of NAA and CHO, markers of neuronal quality and myelin integrity, respectively. A statistically significant reduction in the NAA/Cr ratio in our study is found in the STG of SNHL individuals over three-years old compared with healthy controls. The decrease in NAA/Cr in the STG may suggest the presence of axonal injury indicative of nerve damage occurring in the auditory neuronal cortex. Therefore, our results show that the development of neurons and myelin in the STG and auditory radiation is related to the duration of deafness.

There is now abundant evidence that early implantation in children is advantageous (Kileny et al., 2001; Novak et al., 2000; Kral et al., 2001). Data from Kileny et al. (2001) implant program clearly demonstrate that children having cochlear implants between the ages of 12 and 36 months outperform children having implants between the ages of 37 and 60 months (Kileny et al., 2001). In addition, we show that good outcome subjects show higher FA values in the auditory radiation compared to poor outcome subjects. This suggests that the functional integrity of brain areas associated with language and auditory functions is an important consideration factor for CI. As of 2000, the FDA has approved a device for implantation for patients 12 months and older (Cheng et al., 1999). We suggest that early implantation might prevent the myelin development delay and facilitate improved development of listening perception skills in profoundly deaf children. Moreover, early implantation might be more favorable for the recovery of hearing.

Finally, the limitations of the current study should be noted. Because our cases were of profound bilateral sensorineural hearing loss, we could not separate SNHL patients into different groups according to their severity of hearing loss. The widespread of distribution of fractional anisotropy values among patients seems to reflect a correlation between severity of hearing loss and the degree of reduction in fractional anisotropy. However, this important question will remain until a larger population study is studied. Other possible potential limitations of our study merit consideration. First, we focused on DTI changes in the STG and auditory radiation only. These regions were chosen because they are more distinguishable along the auditory pathway. Moreover, the auditory nerve

| Table 3 – MR spectroscopic and DTI values (mean and SEM) at the STG and auditory radiation for good and poor outcome. |
|---------------------------------|--------------|--------------|--------------|
| CAP Scores                      | Good outcome | Poor outcome | P-value      |
| (n = 18)                        | (n = 13)     |              |              |
| FA in the STG                   | 0.22 (0.009) | 0.230 (0.006)| P > 0.05     |
| FA in the auditory radiation    | 0.31 (0.012) | 0.29 (0.012) | P < 0.05*    |
| ADC in the STG                  | 9.07 (0.143) | 9.12 (0.171) | P > 0.05     |
| ADC in the auditory radiation   | 9.21 (0.200) | 8.99 (0.159) | P > 0.05     |
| NAA                            | 15.66 (0.693)| 15.70 (0.638)| P > 0.05     |
| NAA/CR                         | 2.09 (0.039) | 2.19 (0.068) | P > 0.05     |
| CHO                            | 2.31 (0.077) | 2.28 (0.095) | P > 0.05     |
| CHO/CR                         | 0.31 (0.009) | 0.32 (0.016) | P > 0.05     |

CAP, categories of auditory performance.

Fig. 2 – Correlations between FA values and categories of auditory performance (CAP) scores in the auditory radiation. $r = 0.793$ p < 0.01.

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fibers here being chiefly vertically oriented, facilitating ROI designation with the use of color-encoded FA maps. We are aware that a hearing defect might occur at any place along the auditory pathway. However, other areas (including the cochlear nuclei, superior olivary body, trapezoid body, and medial geniculate body) are less distinguishable and contain a plait of longitudinal, transverse, and oblique fibers. We thus posit that reconstructed images in other areas might have poor spatial resolution and signal-to-noise ratio. Finally, in the language and speech perception performed after surgery, there was some possibility that the better recovery of neural integrity in the good outcome group after surgery could be due to unknown confounding factors, such as the child level of education.

5. Conclusions

DTI and MRS can evaluate microstructural changes of white matter tracts that are not detectable by conventional MR imaging. Auditory radiation and superior temporal gyrus neurons, with myelin developmental delay occur in the congenitally deaf, which is more evident in children three years of age and older. Good outcome subjects showed better neural integrity in brain areas associated with language and auditory functions, suggesting that the conservation of microstructural integrity of these brain areas is important. We suggest that early cochlear implantation might be more effective in restoring hearing in these children.

6. Experimental Procedures

Subjects: We recruited 92 SNHL patients (43 females, 49 males, 1–14 years of age; mean age 4.9 years), none of whom had previous otological surgery or systemic ototoxic drug therapy affecting the CNS. SNHL patients had bilateral profound sensorineural hearing loss prelingually, and all of them used hearing aids before imaging evaluation. Hearing level was measured in a sound proof booth using a calibrated pure tone audiometer (GSI 10, USA). The degree of hearing loss was severe and all participants had bilateral SNHL greater than 80 dB HL (averaged threshold of 500 Hz, 1 kHz, 2–4 kHz; pure tone average, PTA). Patients had no anatomical abnormalities of the inner ear (cochlear aplasia, common cavity deformity, cochlear hypoplasia, Mondini deformity), as determined by CT and conventional MRI. All 92 patients received their cochlear implant at a single implant facility. Among them, we determined the CAP score of 31 patients at 12 months postoperatively. At one year following CI, 18 patients (mean age, 4.8 ± 0.7 years) had a CAP score over 5, and were classified into the good outcome group, and 13 patients (mean age, 4.4 ± 0.7 years) with CAP score below five were classified into the poor outcome group (Table 4).

The 92 SNHL patients were divided into three groups according their ages: Group A was comprised of children ≤ 1 year old (n = 20), Group B was comprised of 1–3 year olds (n = 31), and Group C was comprised of children 3–14 years old (n = 41). Forty-six age-matched normal hearing subjects (27 females, 19 males, 1–10 years of age; mean age 3.7 years) were also divided into three groups (Group A’, Group B’, Group C’) included in this study. Two DTI parameters, FA and ADC, were measured in the superior temporal gyrus (STG) and auditory radiation. Regions of interest for metabolic change measurements were located inside the STG. DTI values that were measured by using the region-of-interest based analysis and MRS values for correlation analysis with CAP scores. Two-sample t-test evaluations between good and poor outcome subjects were performed for DTI and MRS values. All subjects gave written informed consent prior to the study. An independent institutional ethical committee approved the protocol used in this study.

Conventional MR Image Acquisition MRI, DTI and MRS were performed on all subjects. MRI images were acquired on a 1.5 T MR imaging system (Signa; GE Healthcare, Mobile EchoSpeed Plus) equipped with an 8-channel phased-array head coil.

Before the DTI and MRS scan, we performed conventional magnetic resonance imaging in order to ensure that there were no visible hemorrhagic or other lesions, and excluded anatomical abnormalities of the inner ear. The conventional MR series included T1WI, T2WI, T2-flair and DWI. After conventional MRI acquisition, we performed DTI with the following parameters: T1-weighted whole-head structural imaging was performed by using sagittal three-dimensional magnetization - prepared rapid acquisition gradient echo imaging (repetition time msec/echo time msec, 1800/24; field of view, 240 × 240 mm2; matrix, 320 × 256; slice thickness, 5 mm; section gap, 1 mm, 20 axial slices) parallel to a line passing through the anterior-posterior commissure to cover nearly the entire cerebrum. DTI was performed by using a single-shot spin-echo echo-planar pulse sequence with the following parameters: TR, 8000 ms; TE, 99.3 ms; NEX=1; section thickness, 5 mm; section gap, 0 mm; matrix=128 × 128; FOV=240 × 240 mm; diffusion tensor, 15 directions; minimum b-value of 0 s/mm2; maximum b-value of 1000 s/mm2.

For proton MR spectroscopic imaging, a region of interest (ROI) of approximately was positioned in the center of an axial T2-weighted scout image positioned above the STG. Chemical shift imaging was performed using a GE PROBE-SV Press sequence with a 24 × 24cm field of view, 20 mm slice thickness, matrix size 1 × 1, TR/TE 1500/144, one average.

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**Table 4 – Demographic and clinical data of patients with CI (n=31).**

<table>
<thead>
<tr>
<th>Demographic data</th>
<th>Good outcome (n=18)</th>
<th>Poor outcome (n=13)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age Clinical data</td>
<td>4.8±0.7</td>
<td>4.4±0.7</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>CI (side)</td>
<td>Right (1 l), left (7)</td>
<td>Right (5), left (8)</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Age at time of CI</td>
<td>3.5±1.3</td>
<td>3.7±1.1</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Duration of hearing aid</td>
<td>2.7 ± 0.6</td>
<td>2.5 ± 0.5</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>ABR (pre CI)</td>
<td>No response</td>
<td>No response</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>CAP score</td>
<td>5.6 ± 0.31</td>
<td>4.0 ± 0.09</td>
<td>P &lt; 0.05*</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD.

CI, cochlear implantation; ABR, auditory brainstem response; CAP, categories of auditory performance.

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All MRS parameters were set up by using PROBE-SV Press autoprescan.

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References


