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Article

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Diffusion Tensor Imaging of Auditory Pathway Fiber Bundles in Children with Hearing Impairment from Bilirubin Encephalopathy: Alterations in Fractional Anisotropy and Diffusion Coefficient

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Abstract

Objective: To investigate changes in diffusion tensor imaging (DTI) parameters of auditory pathway fiber bundles in children with hearing impairment caused by bilirubin encephalopathy.

Methods: A total of 36 children with bilirubin encephalopathy and 36 control children were examined using a 3.0T superconducting magnetic resonance scanner (Siemens 3.0T Skyra, Siemens, Germany). DTI parameters, including fractional anisotropy (FA) and radial diffusivity (RD), were measured in the lateral lemniscus and inferior colliculus along the auditory pathway. These data were analyzed using independent samples t-test, and linear regression.

Results: Compared to the control group, children with hearing impairment demonstrated a significant decrease in FA ($P < 0.0001$) and a significant increase in RD ($P < 0.0001$). Additionally, a linear negative correlation was observed between FA values ($R^2 = 0.7582$, $p < 0.0001$) and auditory threshold in the inferior colliculus. However, no linear correlation was found between auditory threshold and other DTI parameters.

Conclusion: DTI-based fiber bundle imaging provides direct insight into the structure of white matter fiber bundles in the brain. The observed significant changes in DTI parameters among children with hearing impairment suggest damage or underdevelopment of the myelin sheath in white matter fiber bundles, although the overall integrity of fiber bundles and axons appears to remain intact.

What is Known:

- In neonates, excessive unconjugated bilirubin readily penetrates the underdeveloped blood-brain barrier and accumulates within the basal ganglia, thereby inducing acute bilirubin encephalopathy, which may leave permanent sequelae of kernicterus in later life.
- The auditory tract is highly vulnerable to bilirubin neurotoxicity during acute bilirubin encephalopathy, resulting in sensorineural hearing impairment.

What is New:

- Markedly decreased FA and increased RD were detected within the brainstem auditory tracts (lateral lemniscus and inferior colliculus) among newborns complicated with hearing loss secondary to bilirubin encephalopathy, which suggests demyelinating injury of auditory nerve bundles.
- There existed a linear inverse association between FA metrics and infant auditory thresholds; DTI quantitative indices could serve as quantitative tools to assess bilirubin-mediated auditory neurotoxicity.

Keywords: diffusion tensor imaging, bilirubin encephalopathy, hearing loss, fractional anisotropy

Introduction

Hearing is an essential sense that enables communication between individuals and their external environment. The human auditory pathway comprises the auditory nerve, brainstem, thalamus, and auditory cortex. Profound or non-serviceable hearing loss is a devastating handicap significantly impacting the quality of life of children and adults. It also affects the development and integrity of the central auditory pathways and can lead to widespread functional and cognitive disabilities [1-2].

Diffusion tensor imaging (DTI) is a versatile MRI modality that can acquire such information about anatomical pathways noninvasively. It is based on the diffusion properties of water molecules. Processing of the raw DTI data gives scalar DTI parameters denoting the magnitude and directionality of diffusion at a given region of interest (ROI) [3-4]. The microstructural integrity and orientation of white matter tracts in the brain can be evaluated by diffusion tensor imaging (DTI). The entire length of white matter structures of interest can be visualized three-dimensionally, and their functional integrity can be evaluated.

The auditory pathway has been studied by several authors in sensorineural hearing loss and cerebellopontine angle lesions like vestibular schwannomas [5–9]. These studies have demonstrated the capability of DTI to explore changes in white matter fiber bundles, supporting its application in auditory research. This study aims to apply DTI to children with hearing impairment caused by bilirubin encephalopathy, focusing on the morphological alterations in the auditory conduction pathway that occur in this condition.

Methods

Clinical data

The case group consisted of 36 patients diagnosed with bilirubin encephalopathy at Changde First People's Hospital's neonatal department between March 2025 and December 2025. Based on clinical hearing assessments, 18 patients were

diagnosed with bilateral SNHL of varying degrees, and 18 patients were determined to have unilateral SNHL. The control group included 36 children with normal hearing (The control group of pediatric patients required a comprehensive head magnetic resonance imaging (MRI) examination due to cutaneous hemangiomas).

The inclusion criteria of the study were as follows (1) The clinical diagnostic criteria for acute bilirubin encephalopathy (ABE) were defined as follows: 1. presence of severe unconjugated hyperbilirubinemia; 2. occurrence of characteristic central nervous system manifestations, including lethargy, opisthotonus, high-pitched cry, convulsions, and upward gaze palsy. (2) patients with bilirubin encephalopathy and neonates who failed the hearing screening on both the first and 28th days after birth; the results of the auditory brainstem response and distortion product otoacoustic emissions tests indicate varying degrees of sensorineural hearing loss, (3) no history of ear surgery or exposure to ototoxic medications. The exclusion criteria of this study included patients with central nervous system lesions such as neuronal migration abnormalities, white matter dysplasia, tuberous sclerosis, and heterotopic gray matter.

Scanning method

Pre-examination preparation

Before the scanning, each child was administered oral 10% chloral hydrate at a dose adjusted to body weight to ensure they were in a deep sleep during the procedure.

Imaging equipment

All scans were performed using a 3.0T superconducting magnetic resonance scanner (Siemens 3.0T Skyra, Siemens, Germany) with a 12-channel head coil.

Scanning protocol

The imaging protocol included transverse axial T1-weighted imaging (T1WI), T2-weighted imaging (T2WI), coronal fluid-attenuated inversion recovery (FLAIR), and DTI. The DTI scans were performed using single-shot spin-echo imaging with the following parameters: repetition time (TR) = 5300 ms, echo time (TE) = 73.0 ms, 64 diffusion-sensitized gradient directions, a slice thickness of 3 mm, no interslice gap, field of view (FOV) = 230×230 mm, and an imaging matrix of $1.8 \times 1.8 \times 3.0$ mm. A single acquisition was performed with a total scanning time of 6 minutes and 13 seconds.

Image processing and data measurement

The DTI data were processed using the Syngo software, specifically the DTI analysis module, and the following parameters were extracted from the images: (1) Fractional Anisotropy (FA), (2) Axial Diffusivity (AD), (3) Radial Diffusivity (RD), and (4) Mean Diffusivity (MD).

Regions of interest (ROIs)

The lateral lemniscus and the inferior colliculus were selected as regions of interest along the auditory conduction pathway. These anatomical locations were determined using the directionally encoded color map, and the size of each region of interest (ROI) was approximately 12 mm². A representative ROI selection is illustrated in Figure 1.

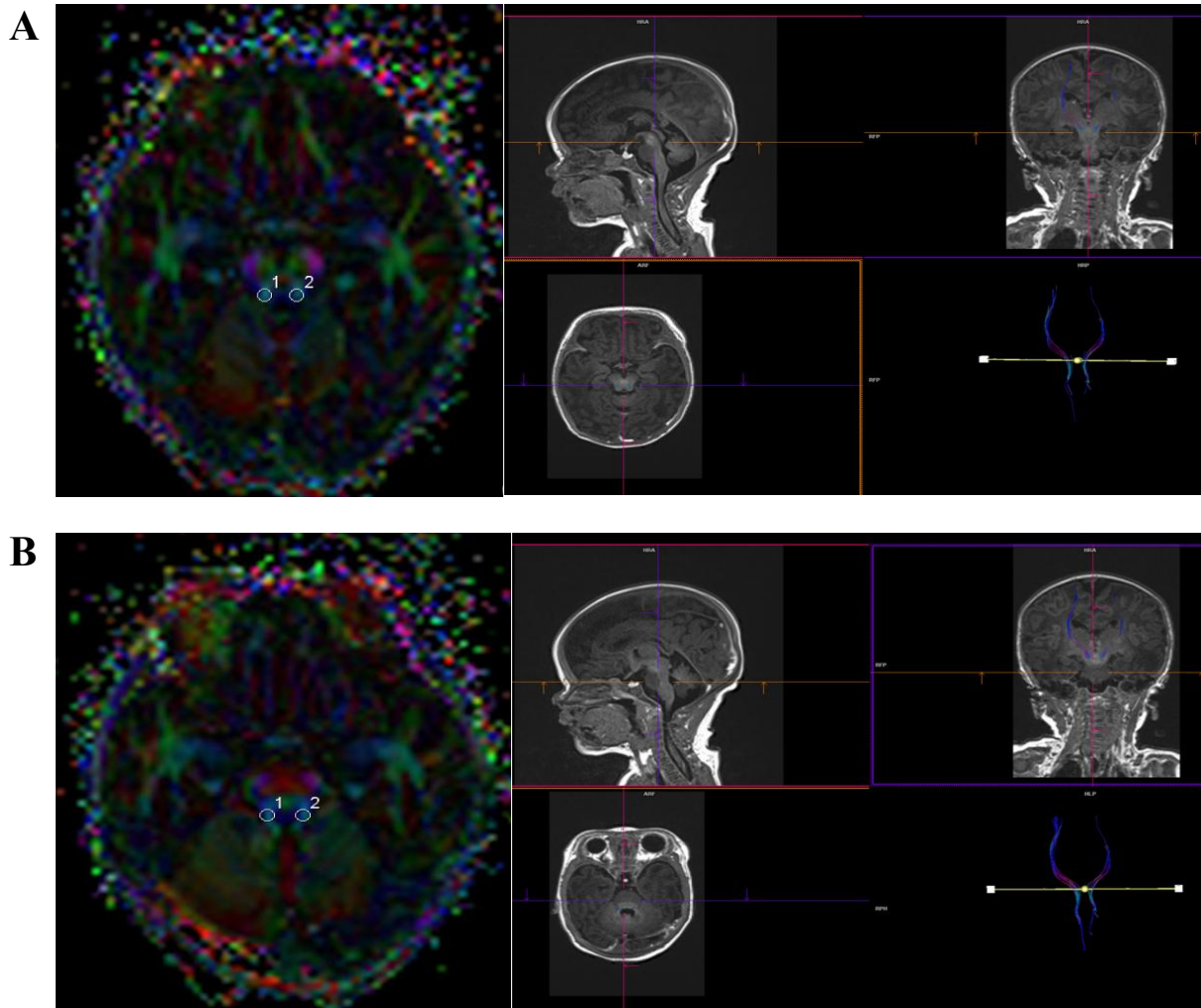


Fig. 1: Mapping of regions of interest (ROIs) in a child with bilateral hearing impairment. The lateral lemniscus (A) and inferior colliculus (B) were identified on the pseudo-color map. Circles with a diameter of 12 mm² were drawn on both the left and right sides of the image. Using fiber tracing technology, the corresponding fiber bundles were obtained, providing a clear visualization of the auditory conduction pathway.

Statistical analysis

All data were processed and analyzed using IBM SPSS Statistics 27 and Graphpad Prism 10.1.2 software. Normally distributed data are expressed as mean $\pm$ standard deviation (SD). An independent -test was used to compare measurement results between the two groups. Linear regression and correlation analyses were performed between the hearing threshold values (obtained from auditory brainstem response examinations) as independent variables and the corresponding DTI

parameter values. A significance level of $\alpha = 0.05$ was applied, with $p < 0.05$ indicating statistical significance and $p < 0.0001$ indicating highly significant differences.

Results

A total of 72 neonates were enrolled in this study and categorized into three groups based on the presence of acute bilirubin encephalopathy and auditory function: bilateral hearing impairment group (18 cases), unilateral hearing impairment group (18 cases), and normal hearing group (36 cases). comprising 18 males and 18 females, with ages ranging from 1 to 27 days, and a median age of 14 days. No statistically significant intergroup differences were found in gestational age, birth weight, gender distribution, and peak indirect bilirubin levels (all $P > 0.05$), indicating balanced baseline characteristics across the three groups. However, the auditory thresholds were markedly higher in the two hearing-impaired groups relative to the normal hearing group ($P < 0.001$).

Independent samples t-test revealed significantly decreased fractional anisotropy (FA, Figure C) and increased radial diffusivity (RD, Figure D) in the inferior colliculus and lateral lemniscus in the study subgroups, including the impaired side of the unilateral hearing impairment group versus the normal hearing group, and the bilateral hearing impairment group versus the uninjured side of the unilateral hearing impairment group (all $P < 0.0001$ for both inferior colliculus and lateral lemniscus). Additionally, axial diffusivity (AD, Figure E) values in the lateral lemniscus were significantly higher in the bilateral hearing impairment group than in the unaffected side of the unilateral hearing impairment group ($p = 0.0129$ for lateral lemniscus). The detailed results are shown in Table 1.

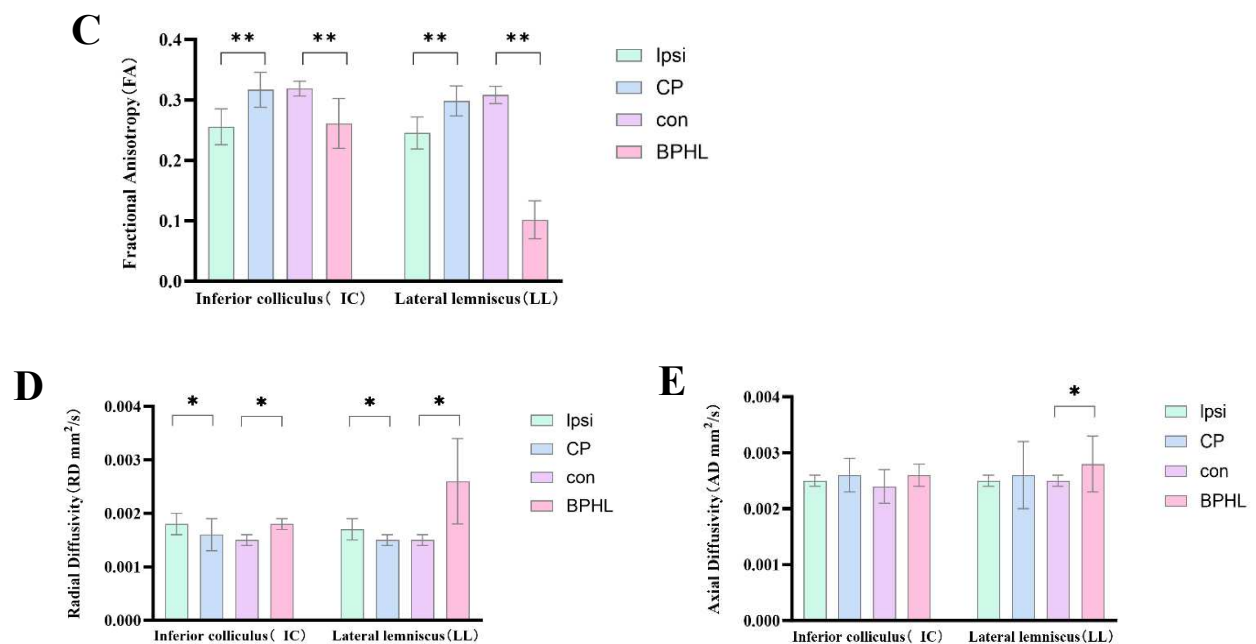


Fig. 2: Independent samples t-tests were performed for comparisons between the case group and the control group (IBM

SPSS Statistics 27) , Measurement data are expressed as the mean $\pm$ standard deviation ($\bar{x} \pm s$). Statistically significant differences were observed in FA(Fig. C),RD(Fig. D),and AD(Fig. E), with $p < 0.05$.

Table 1. Comparison of the results of different DTI values in the inferior colliculus (IC) and lateral lemniscus (LL) regions in the hearing impaired group ($x \pm s$)

DTI	IC					
	lpsi	CP	<i>p</i>	con	BPHL	<i>p</i>
FA	0.2557 $\pm$ 0.0296	0.3169 $\pm$ 0.0291	<0.0001**	0.3189 $\pm$ 0.0123	0.2612 $\pm$ 0.0411	<0.0001**
MD	0.0033 $\pm$ 0.0002	0.0033 $\pm$ 0.0003	0.8026	0.0033 $\pm$ 0.0002	0.0033 $\pm$ 0.0003	0.7631
RD	0.0018 $\pm$ 0.0002	0.0016 $\pm$ 0.0003	0.0019*	0.0015 $\pm$ 0.0001	0.0018 $\pm$ 0.0001	<0.001*
AD	0.0025 $\pm$ 0.0001	0.0026 $\pm$ 0.0003	0.3961	0.0024 $\pm$ 0.0003	0.0026 $\pm$ 0.0002	0.1832

DTI	LL					
	lpsi	CP	<i>p</i>	con	BPHL	<i>p</i>
FA	0.2455 $\pm$ 0.0265	0.2984 $\pm$ 0.0249	<0.0001**	0.3083 $\pm$ 0.0143	0.1017 $\pm$ 0.0313	<0.0001**
MD	0.0032 $\pm$ 0.0002	0.0032 $\pm$ 0.0004	0.6710	0.0032 $\pm$ 0.0001	0.0031 $\pm$ 0.0004	0.3401
RD	0.0017 $\pm$ 0.0002	0.0015 $\pm$ 0.0001	<0.001*	0.0015 $\pm$ 0.0001	0.0026 $\pm$ 0.0008	<0.001*
AD	0.0025 $\pm$ 0.0001	0.0026 $\pm$ 0.0006	0.3117	0.0025 $\pm$ 0.0001	0.0028 $\pm$ 0.0005	0.0129*

Abbreviations: DTI: Diffusion Tensor Imaging; Ipsi group: Impaired side in the unilateral hearing-impaired group; CP group: Normal control group; Con group: Uninjured side in the unilateral hearing-impaired group; BPHL group: Bilateral hearing loss group. * Indicates a statistically significant difference.

Linear regression and correlation analyses between hearing threshold,and DTI parameter values in all hearing-impaired children (unilateral and bilateral impairment groups). FA values(Figure F) in the inferior colliculus showed a linear negative correlation with hearing loss severity, with FA values decreasing as the hearing threshold increased (inferior colliculus: $R^2 = 0.7582$, $p < 0.0001$). A negative

correlation was also found between MD values(Figure G) in the lateral lemniscus and hearing threshold (lateral lemniscus: $R^2 = 0.1164$, $p = 0.0417$). No significant linear relationship was found between other diffusion coefficients and hearing thresholds. Detailed results are shown in Figure 3.

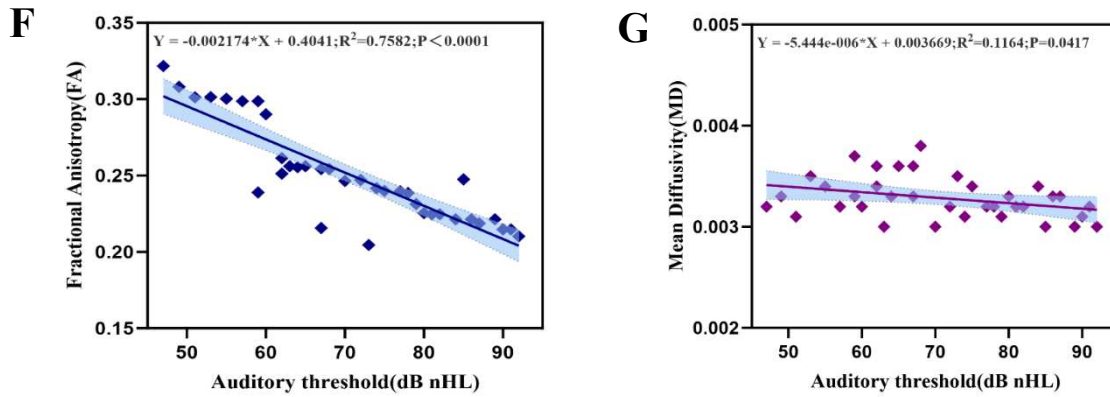


Fig. 3: Linear regression was conducted to assess correlations between auditory thresholds and DTI parameters(Graphpad Prism 10.1.2). Statistically significant differences were observed in FA(Fig.D),and MD(Fig. F) , with $p < 0.05$.

Discussion

Bilirubin encephalopathy is a major complication of hyperbilirubinemia, resulting from the toxic effects of unconjugated bilirubin on nerve cells. This type of toxicity primarily affects the basal ganglia, cochlea, and oculomotor nucleus. Thus, the auditory system is particularly sensitive to unconjugated bilirubin, although the precise mechanism by which it damages nerve cells remains unclear. Some researchers [10] suggest that bilirubin interferes with cell membrane permeability and function, disrupting cellular energy metabolism, which leads to an increase in intracellular calcium (Ca^{2+}), ultimately causing cell apoptosis and necrosis.

The auditory conduction pathway includes the cochlear nerve, brainstem, thalamus, and cortex. Auditory impulses originate in the cochlea, where external sound stimulation is converted into energy within cochlear hair cells. The impulses travel via the cochlear nerve through the internal auditory canal. Some of these impulses cross at the dorsal and ventral pons to the contralateral side, while others ascend ipsilaterally. Fibers cross the dorsal pontine tegmentum and ascend to the medial thalamus, forming a trapezoid body. Other fibers bypass this crossing and ascend via the lateral lemniscus, which extends along the lateral edge of the dorsal tegmentum of the midbrain. Most fibers terminate in the inferior colliculus, which projects to the medial geniculate body and eventually forms auditory radiation. The auditory signals then project to the transverse temporal gyrus (the auditory cortex) through the posterior limb of the internal capsule [11]. Based on the importance of these anatomical structures, the lateral lemniscus and inferior colliculus were selected as ROIs for this study.

FA is used to measure the directional movement of water molecules and is sensitive to microstructural changes in tissue. It has been shown to be related to the integrity of white matter fibers but lacks specificity [12-13], with a decrease in FA indicating axonal membrane and myelin sheath degradation, abnormalities in axonal myelination, or a reduction in the density of myelinated nerve fibers. Mean diffusivity (MD) reflects the average diffusion capacity of water molecules and can be categorized into eigenvectors parallel and perpendicular to the orientation of white matter fibers. It has been shown to be associated with cell density and extracellular space, and an increase in MD may indicate decreased cell or fiber density and expanded extracellular space. Axial diffusivity (AD) is related to axonal integrity, while radial diffusivity (RD) reflects the continuous process of myelination in white matter fibers. Previous studies have consistently reported that FA decreases in various disease conditions, while MD values have shown variability, with some studies noting an increase, a decrease, or no change [14-16]. Alterations in FA are thought to arise from changes in AD, RD, or both [17]. When these alterations reach measurable levels, MD values can reflect these underlying microstructural changes [18-21]. In this study, DTI was used to assess key regions of the auditory pathway in children with hearing impairment caused by bilirubin encephalopathy. The findings revealed that DTI is highly sensitive to detecting subtle white matter lesions that are undetectable by conventional MRI, highlighting its potential to identify early white matter abnormalities in affected children.

The results of our present study show that, in the comparison between the bilateral hearing-impaired group and the normal control group (Figure 2), the FA values in the lateral lemniscus and inferior colliculus were significantly lower in the hearing-impaired group. This reduction in FA values (Figure C) could be attributed to an increase in the transneuronal fiber diffusion coefficient, indicating impaired neuronal information transmission. The observed increase in RD values (Figure D) in the lateral lemniscus and inferior colliculus may be explained by myelin degeneration in these regions, leading to decreased FA values [22]. The decrease in FA values indicates damage to the subcortical auditory conduction bundles and loss of myelin. The increase in RD values reflects the destruction of nerve fiber integrity following demyelination, which weakens the barrier to water molecule diffusion. Notably, the FA and RD values in the inferior colliculus were more significantly altered than those in the lateral lemniscus, which might be due to the inferior colliculus being located at the terminus of the auditory pathway's nerve fiber bundle, making it more sensitive to damage than the lateral thalamus. In addition, mildly elevated radial diffusivity (RD) values (Figure E) in the lateral lemniscus were detected in some infants with hearing impairment, which may reflect axonal injury or inflammatory changes of neurons within the auditory pathway.

Similarly, linear correlation analysis between hearing thresholds and DTI parameters (Figure 3) showed that FA values in the inferior colliculus decreased as hearing thresholds increased, with a strong negative correlation in the region. These findings suggest that the most significant parameter differences were observed in the inferior colliculus, further confirming that myelin sheath degeneration in the inferior colliculus, a key auditory nucleus, was more severe than in the lateral lemniscus in children with hearing impairments.

This study had some limitations. First, the use of manual ROI drawing may have introduced variability in anatomical positioning, which could affect the precision of our measurements. Additionally, this study focused only on the lateral and inferior colliculi due to their ease of identification, while other key regions of the auditory pathway, such as the medial geniculate body, auditory radiation, and transverse temporal gyrus, were not included. These areas are more challenging to identify and delineate accurately because they contain complex fiber arrangements, including transverse, longitudinal, and oblique fibers, which current imaging software struggles to resolve. Future studies could address this by employing more advanced imaging techniques, such as voxel-based morphometry (VBM) [23], to more comprehensively analyze the morphology of nerve fiber bundles and assess DTI parameter changes across the entire auditory pathway.

Conclusion

In summary, this study demonstrates that children with hearing impairment exhibit varying degrees of myelin damage or loss of myelin phospholipids in the auditory pathway fiber bundles. Despite this, the axonal integrity of these fiber bundles remains intact. Additionally, the findings show a significant correlation between the decrease in FA values and the severity of hearing impairment, suggesting that FA could be a useful clinical marker for assessing the extent of the condition. These results provide valuable insights into the structural changes in the auditory pathways of children with hearing impairment caused by bilirubin encephalopathy. Further research is needed to determine whether these findings can assist in guiding rehabilitation strategies and evaluating treatment efficacy.

Additional Information

Informed consent was obtained from the families or legal guardians of all child participants involved in this study. The study protocol was approved by the Clinical Medical Research Ethics Committee of the First People's Hospital of Changde City (Approval No. 2025-077-01), This retrospective study was approved by the Institutional Ethics Committee, and all procedures were performed in accordance with the Declaration of Helsinki. Author contributions: Min Deng and Chao Zheng designed the study; Weian Wei and Ting Yi performed experiments; Zhangyong Deng analyzed data; all authors wrote and approved the final manuscript. Data are available from the authors upon reasonable request. Conflict of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and materials: The minimal anonymized datasets generated and analysed during the current study are available within the paper and its Supplementary Information. Raw MRI DICOM images cannot be publicly released due to patient-privacy and ethical restrictions imposed by our institutional review board.

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