Original Article
Quantitative assessment of amyotrophic lateral sclerosis with diffusion tensor imaging in 3.0T magnetic resonance

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Abstract: Diffusion tensor imaging (DTI) was used to measure the fractional anisotropy (FA) and apparent diffusion coefficient (ADC) value in amyotrophic lateral sclerosis (ALS) patients to determine their diagnostic value. 69 ALS patients and 23 healthy controls were scanned with DTI sequence in 3.0T MR, and FA and ADC values in 18 regions were evaluated. Compared with the controls, the ADC values of patients in bilateral centrum semiovale, deep frontal and parietal white matter were significantly increased ($P < 0.05$), while the FA values in cerebral peduncle, posterior limb of internal capsule (PLIC), corona radiata, centrumsemiovale, bilateral deep frontal white matter, the genu and the splenium of the corpus callosum were significantly decreased ($P < 0.05$). When the FA cut-off value was set to ≤ 0.6860 for the cerebral peduncle, sensitivity (SE) and specificity (SP) of ALS were 95.7% and 83.9% respectively. When the cut-off value was set to ≤ 0.7085 for PLIC, SE and SP were 95.7% and 85.7% respectively. When the cut-off value was set to ≤ 0.6950 for corona radiata, SE and SP were 100.0% and 95.7%, respectively. DTI can be used to quantitatively evaluate injury in ALS patients.

Keywords: Amyotrophic lateral sclerosis, diffusion tensor imaging, fractional anisotropy, quantitative measurment

Introduction

Amyotrophic lateral sclerosis (ALS) is a chronic and progressive nervous system disease characterized by the upper and lower motor neuron degeneration. A series of types of cells, including anterior horn cells, medullary motor nuclei, pons, cortical pyramidal cells and pyramidal tracts are affected in ALS. ALS is always found in the populations at the age of 50 to 63 years with the male and female ratio of 1.5:l [1]. With an annual incidence of approximately 2.1/100,000 [1], 90% to 95% of the patients are sporadic and average survival time is about 3 to 5 years [2, 3]. Most of the patients ultimately died of paralysis of medulla oblongata and respiratory muscles, and respiratory failure due to lung infection. Therefore, early diagnosis and treatment are crucial for the ALS patients. Damage in lower motor neuron (LMN) can be diagnosed with electromyography (EMG), while evaluation of injury in upper motor neuron (UMN) is mainly relied on the physical examina-

tion of the nervous system [4]. Up to now, there is no efficient diagnostic test that can be used to identify UMN damage. The conventional MRI imaging detects the neuron degeneration indirectly using signals from the brain matter. However, only the patients at later ALS stage can be diagnosed with this approach [5].

DTI is a newly developed magnetic resonance imaging technology, which is currently the only noninvasive method for in vivo study of the morphology and structure of white matter fiber [6]. In recent years, diffusion tensor imaging (DTI) has been increasingly used for ALS diagnosis. The main measurements include fractional anisotropy (FA) and apparent diffusion coefficient (ADC). FA value is one of the major indicators measuring the anisotropy of white matter fiber and related to the integrity of myelin, the density and parallelism of brain fiber [7]. ADC values are used to measure the rate and distance of diffusion of water molecules. The damage of axons and/or myelins of white matter
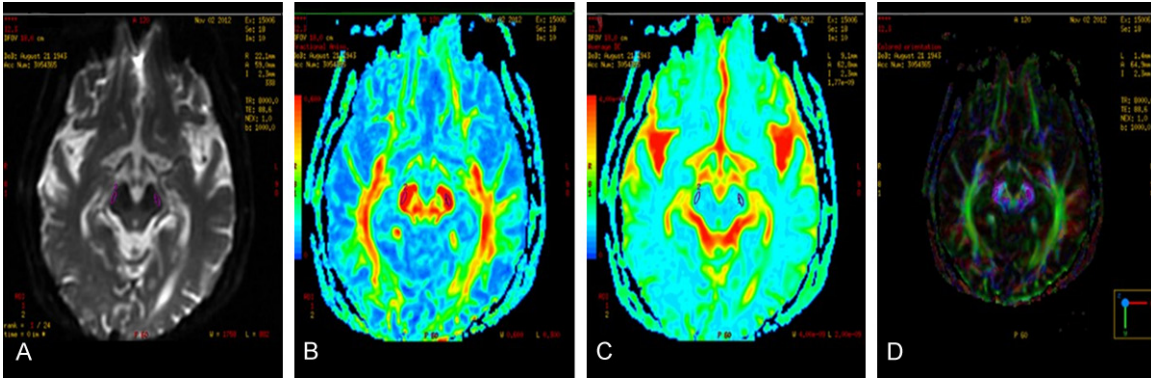
Figure 1. A-C: Were T2WI positioning images, color FA images, and color ADC images with the ROIs of cerebral peduncle (two, ROI 1 and 2).
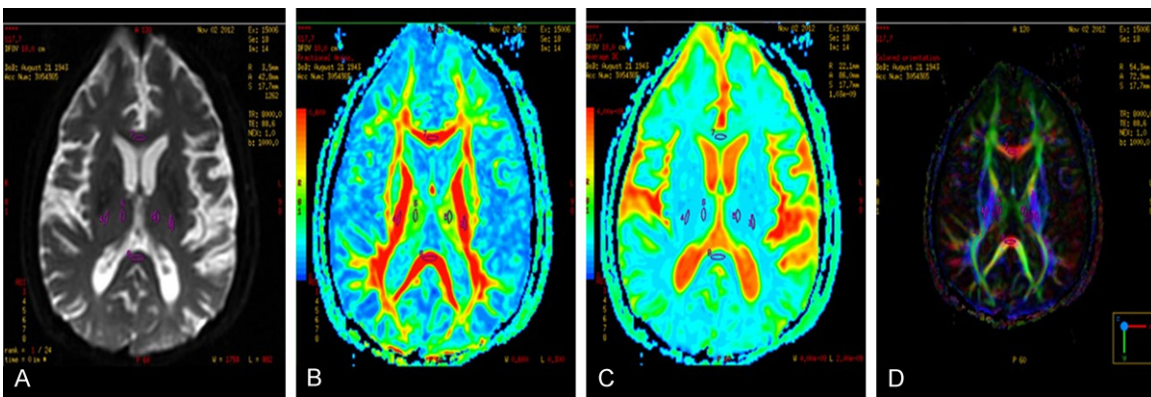

Figure 2. A-C: Were T2WI positioning images, color FA images, and color ADC images with the ROIs of 3/4 position behind PLIC (two, ROI 3 and 4), thalamus (two, ROI 5 and 6), the genu (one, ROI 7) and the splenium (one, ROI 8) of the corpus callosum.
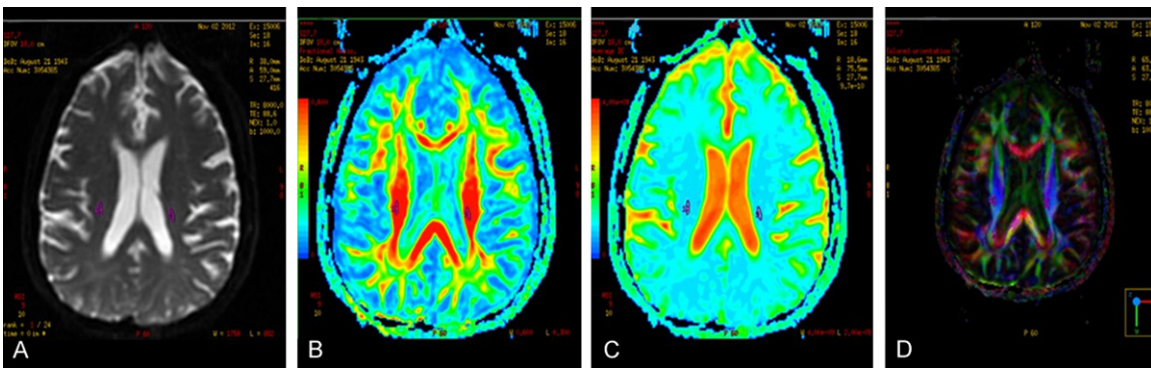

Figure 3. A-C: Were T2WI positioning images, color FA images, and color ADC images with the ROIs of bilateral corona radiata (two, ROI 9 and 10).

fiber tracts would decrease FA values but increase ADC values accordingly. Previously, Sage et al reported that changes of diffusion parameters including FA and averaged mean diffusivity (Dav) occurred throughout the brain, including frontal, temporal and parietal lobes [8]. Earlier Nelles et al [9] demonstrated that compared with healthy people, ALS patients had reduced FA values only in the central gyrus cortex, centrumsemiovale and posterior limb of internal capsule (PLIC). However, the exact relation of FA and ADC with sensitivity (SE) and
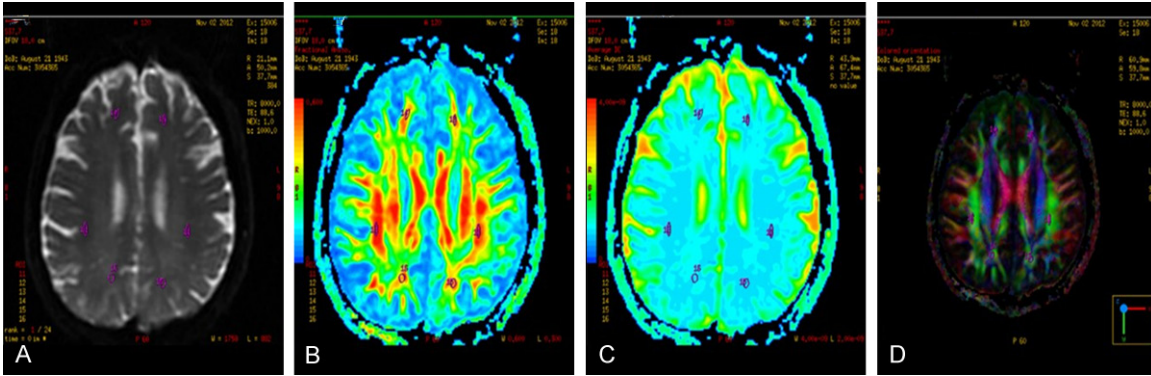
Figure 4. A-C: Were T2WI positioning images, color FA images, and color ADC images with the ROIs of bilateral centrum semiovale (two, ROI 11 and 12), bilateral deep white matter (two, ROI 13 and 14) of frontal lobe, bilateral deep white matter (two, ROI 15 and 16) of parietal lobe.
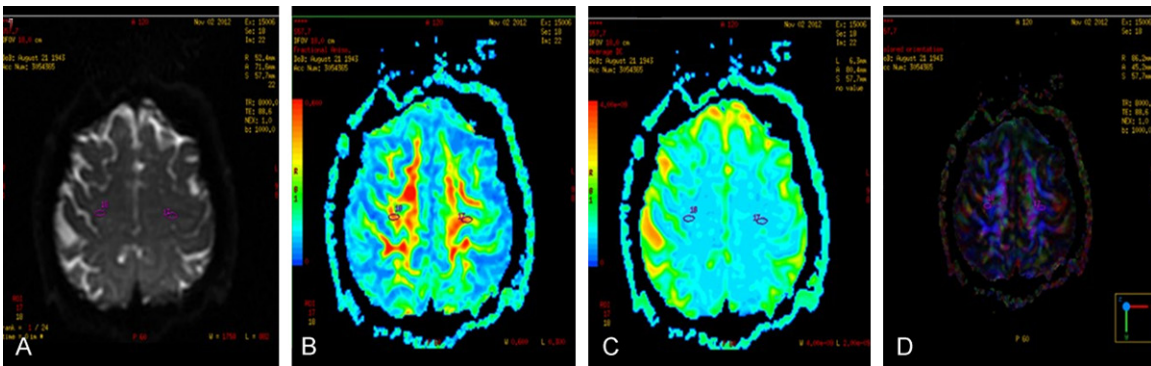

Figure 5. A-C: Were T2WI positioning images, color FA images, and color ADC images with the ROIs of bilateral subcortical white matter of central gyrus (two, ROI 17 and 18).

Table 1. Gender and age distributions of the patients and controls

Group	ALS	Control
Gender (male/female)	47/22	23/14
Average age	53.12±11.51	53.52±10.57

specificity (SP) of ALS are not disclosed. In this study, we attempted to use DTI imaging to determine the FA values and ADC values in and outside corticospinal tracts (CST) in ALS patients and healthy controls. These data were analyzed to identify specific quantitative indicators for early ALS diagnosis.

Materials and methods

Clinical data

69 ALS patients diagnosed according to the El Escorial criteria [10, 11] in Guangdong Provincial Chinese Medicine Hospital between

September 2008 and August 2013 were enrolled in this study. There were 38 males and 31 females, with the age from 34 to 69 years and disease duration of 8 to 29 months. Among them, 43 had limb weakness, 26 had drinking cough or swallowing difficulty and 15 had significant muscle atrophy. Patients were excluded if they had vascular disease, history of epileptic brain or other central nervous system disorders, history of brain surgeries or implanted pacemaker and severe kidney diseases.

23 healthy controls were recruited in the community and hospital with the gender and age matched (14 were male and 9 females, aged from 40 to 67 years). Informed written consents were obtained from all participants. The healthy participants did not have history of neurological diseases, and were diagnosed normally with neurological examination. The patients were free of any drugs which might affect the nervous system. This study was

Table 2. The FA values in 18 ROIs of 69 patients and 23 controls

ROI	Patient (n = 69)	Control (n = 23)	T value	P value
Subcortical white matter of central gyrus	0.453±0.064	0.465±0.068	0.178	0.674
Centrumsemiovale	0.428±0.033	0.546±0.043	13.576	0.000*
Deep white matter of frontal lobe	0.411±0.044	0.488±0.066	6.325	0.000*
Deep white matter of parietal lobe	0.400±0.052	0.434±0.060	2.883	0.005*
Corona radiata	0.624±0.042	0.728±0.018	11.71	0.001*
PLIC	0.663±0.050	0.737±0.024	9.837	0.002*
Thalamus	0.275±0.079	0.261±0.026	0.062	0.387
Genu of corpus callosum	0.624±0.039	0.687±0.023	7.417	0.000*
Splenium of corpus callosum	0.728±0.046	0.834±0.021	14.492	0.000*
Cerebral peduncle	0.620±0.072	0.740±0.034	6.697	0.011*

(Baseline α = 0.005, P < 0.05 was considered statistically significant, "*" indicates value of statistical difference).

Table 3. The ADC values in 18 ROIs of 69 patients and 23 controls

ROI	Patient (n = 69)	Control (n = 23)	T value	P value
Subcortical white matter of central gyrus	7.79±0.480	7.79±0.565	0.315	0.967
Centrumsemiovale	7.60±0.270	7.20±0.283	0.327	0.000*
Deep white matter of frontal lobe	7.68±0.412	7.46±0.353	1.716	0.026*
Deep white matter of parietal lobe	7.61±0.378	7.28±0.392	0.009	0.001*
Corona radiata	7.61±0.351	7.57±0.390	0.613	0.436
PLIC	7.59±0.313	7.14±0.406	0.483	0.489
Thalamus	7.56±0.297	7.59±0.322	0.372	0.711
Genu of corpus callosum	7.61±0.293	7.73±0.490	1.757	0.142
Splenium of corpus callosum	7.57±0.247	7.47±0.374	9.271	0.215
Cerebral peduncle	8.33±0.514	7.97±0.503	0.278	0.599

(Baseline α = 0.005, P < 0.05 was considered statistically significant, "*" indicates value of statistical difference).

approved by the Human Research Ethics Committee of Guangdong Provincial Chinese Medicine Hospital, Guangzhou China.

Equipment and scanning methods

3.0T Propeller HD MR Scanner (GE) with an ADW4.3 workstation was applied in this study. The coil was standard quadrature head coil. Scanning was performed at slice with thickness of 0.5 cm without gap on participants at supine position from the head. The axial scans included conventional MR scans (axial T1-FLAIR, T2-FRFSE and FLAIR imaging) and DTI scans covering the whole brain.

DTI imaging was conducted with single-shot spin-echo planar imaging (SE-EPI) sequence at TR of 8000 ms, TE of 88.6 ms. b value was 1000 s/mm^2 with a matrix of 128 × 128 and FOV of 24 cm × 24 cm. NEX was set to 1 with slice thickness of 0.5 cm without gap. The number of diffusion-sensitizing gradient direction

was set at 23. 26 images were captured for each layer, with the first one taken at b = 0 s/mm^2, which was equivalent to a T2WI image obtained with EPI. The remaining 25 images were obtained from 23 diffusion-sensitizing gradient directions with a b value of 1000 s/mm^2.

DTI image processing and data analysis

Captured images were uploaded to ADW 4.3 workstation, where the collected raw data were calibrated using Functool II software, including adjustment of the threshold range to include all brain tissue scanned. 18 regions of interest (ROI) at different slices were selected, including those in the cerebral peduncle (two), 3/4 position behind PLIC (two), thalamus (two), the genu (one) and the splenium (one) of the corpus callosum, bilateral corona radiata (two), bilateral centrumsemiovale (two), bilateral deep white matter (two) of frontal lobe, bilateral deep white

Table 4. SE and SP at different cut-off FA values of cerebral peduncle in the patient group for ALS diagnosis

FA value	SE	SP
FA ≤ 0.6525	100.0%	68.1%
FA ≤ 0.6545	95.7%	68.1%
FA ≤ 0.6645	95.7%	72.5%
FA ≤ 0.6815	95.7%	81.2%
FA ≤ 0.6860	95.7%	82.6%
FA ≤ 0.6890	91.3%	82.6%
FA ≤ 0.6935	91.3%	87.0%
FA ≤ 0.6945	87.0%	87.0%

Table 5. SE and SP at different cut-off FA values of PLIC in the patient group for ALS diagnosis

FA value	SE	SP
FA ≤ 0.6950	100.0%	71.0%
FA ≤ 0.6965	100.0%	72.5%
FA ≤ 0.6975	95.7%	73.9%
FA ≤ 0.6990	95.7%	76.8%
FA ≤ 0.7085	95.7%	88.4%
FA ≤ 0.7105	91.3%	88.4%
FA ≤ 0.7115	82.6%	88.4%
FA ≤ 0.7140	82.6%	89.9%

Table 6. SE and SP at different cut-off FA values of corona radiata in the patient group for ALS diagnosis

FA value	SE	SP
FA ≤ 0.6900	100.0%	94.2%
FA ≤ 0.6950	100.0%	95.7%
FA ≤ 0.6975	95.7%	95.7%
FA ≤ 0.7025	91.3%	95.7%
FA ≤ 0.7085	87.0%	95.7%
FA ≤ 0.7105	82.6%	95.7%
FA ≤ 0.7115	69.6%	95.7%

matter (two) of parietal lobe, bilateral frontal gyrus subcortical white matter (two). Three images were obtained on each slice with different information, including T2WI positioning, ADC image and color FA image. The FA values were quantified by coloration from large to small as the color changes from red to yellow, green and blue. FA has a value range from 0 to 1, where 0 is completely isotropic and 1 is completely anisotropic. The FA and ADC values of

the ROI were shown on the ADC and FA images. The size of ROI was between 15 and 30 mm^2. ROIs on one side were first drawn semi-automatically, and they were round or oval depending on the morphology of the slices selected. The ROIs on the other side were drawn based on symmetry, unless they were outside the tissue structures. Manual adjustments were made to move the ROIs within the positions corresponding to the other side. Attention was paid to the size and location of ROIs to avoid overlap with nearby tissue, and to ensure the same size of ROIs on both sides. The FA and ADC values for each ROI were measured three times, and average values were obtained.

Selection of 18 ROIs

From left to right, and top to bottom were T2WI positioning images, color FA images, and color ADC images. ROIs on each slice were selected based on the three images from the pons to brain, that is, from cerebral peduncle to central subcortical white matter (**Figures 1A-C** to **5A-C**).

Statistical analysis

The data was expressed as mean ± standard deviation. One way analysis of variance (ANOVA) was performed using SPSS17.0. Comparisons were carried out for differences in FA values and ADC values of ROIs between the patients and controls. Normality and homogeneity of variance of the data were tested. *P* value of < 0.05 was considered statistically significant. The relationship between the FA values in different locations and the diagnosis of ALS was analyzed using the ROC curves generated by SPSS 17.0. The best cut-off points were selected on the ROC curves to calculate the sensitivity (SE) and specificity (SP) of ALS, and their diagnosis values were evaluated based on the area under the curve.

Results

General clinical information

The composition of genders in the study was shown in **Table 1**. By chi-square test, there was no statistically significant difference in the gender composition between the two groups (χ^2 = 0.066, P = 0.631). The age distribution in the study was shown in **Table 1**. There was no sig-

nificantly different in age between the two groups (P = 0.882).

Comparison of FA and ADC values of ROIs in 69 patients and 23 controls

FA and ADC values in 18 ROIs of 69 patients and 23 controls were measured. There was no significant difference regarding these values between the two sides. Therefore, the data from both sides were pooled for average in subsequent analysis (**Table 2**).

As shown in **Table 2,** the FA values in patient groups were statistically lower than those in control group in the regions of Centrumsemiovale, Deep white matter of frontal lobe, Deep white matter of parietal lobe, Corona radiate, PLIC, Genu of corpus callosum, Splenium of corpus callosum and Cerebral peduncle. In other regions, including subcortical white matter of central gyrus and Thalamus, there was no significant difference regarding the FA value.

As shown in **Table 3**, ADC values were significantly higher in patient group than those in control group in centrumsemiovale, deep white matter of frontal lobe and deep white matter of parietal lobe.

SE and SP of the FA values on the patients in 6 ROIs over the corticospinal tract for ALS diagnosis

SE and SP at different cut-off FA values of cerebral peduncle in the patient group for ALS diagnosis are shown in **Table 4**. With the increase of FA values from 0 to 1, SE of ALS was decreased while SP was increased. When the cut-off value was set at ≤ 0.6860, the SE and SP values were 95.7% and 82.6% for confirmed ALS patients, respectively

SE and SP at different cut-off FA values of PLIC in the patient group for ALS diagnosis are shown in **Table 5.** With the increase of FA from 0 to 1, SE was decreased while SP was increased. When the cut-off value was set at ≤ 0.7085, the SE and SP values were 95.7% and 88.4% for confirmed ALS patients, respectively.

SE and SP at different cut-off FA values of corona radiata in the patient group for ALS diagnosis are shown in **Table 6**. With the increase of

FA values from 0 to 1, SE was decreased while SP was increased. When the cut-off value was set at ≤ 0.6950, the SE and SP values were 100.0% and 95.7% for confirmed ALS patients, respectively.

The ROC curve areas of the ROIs in the cerebral peduncle, PLIC and corona radiata over the CST were 0.943, 0.946 and 0.983, respectively. All of them were higher than 0.7, indicating that they are valuable diagnostic indicators. Among them, the area in corona radiata was the biggest with higher SE and SP.

Discussion

It is likely that the occurrence of ALS is resulted from interactions of multiple mechanisms that cause injury to motor neuron. The main pathological symptoms in ALS include degeneration of anterior horn cells, nuclei cells of brainstem motor nerves, cortical motor cells and pyramidal tract. In addition, some parts outside the motor system may also get involved. Microscopic observations showed that there was reduction in cell numbers on the fifth layer of motor cortex with active gliosis, shrinkage of remaining Betz cells and dendritic degeneration [12]. In this study, DTI imaging was used to determine the FA and ADC values in and outside corticospinal tracts (CST) in ALS patients and healthy controls.

Potential of ADC value and FA value for ALS diagnosis

In this study, 18 ROIs were selected in motor and non-motor areas and their differences in the FA and ADC values were compared between the patient and control groups. The values from both sides were comparable. This might be explained by that all the patients in the study had disease in both upper and lower extremities. Compared with the control group, the FA values were significantly lower in the patient group in cerebral peduncle, PLIC, corona radiata, centrum semiovale, the genu and splenium of corpus callosum, deep white matter of frontal lobe, and deep white matter of parietal lobe. Among them the lowest FA value was particularly remarkable in the genu and splenium of corpus callosum, centrumsemiovale and deep white matter of parietal lobe, followed by corona radiata and PLIC regions. The reductions of the FA values were observed in all ROIs of CST,

which is consistent with the pathology observations, i.e. myelin would be white in the region, once axon was degenerated [13]. Furthermore, the reductions of the FA values in the areas outside the CST, such as in deep white matter of frontal lobe, deep white matter of parietal lobe, the genu and splenium of corpus callosum, indicating that ALS could also have lesions in the neural pathways outside the CST. Our results showed that the apparent FA value decrease in the cerebral peduncle, PLIC and corona radiata, indicates that the FA values along the CST can be used as the first set of ROIs for clinical DTI diagnosis of suspected ALS.

The lesions of nerve fibers in the ALS patients was obvious in centrumsemiovale, frontal deep white matter and parietal deep white matter than in cerebral peduncle. These results support that ALS is resulted from the Wallerian degeneration of nerve fiber due to the degeneration and necrosis of the neurons in motor cortex neuron [14]. By contrast, the FA values of thalamus and central gyrus subcortical white matter were not significantly different between the patient and control groups. This might due to that the central gyrus cortex has fewer fiber tracts with lower anisotropy. Thalamus has high density of neurons and fewer fiber tracts, and lower anisotropy even in healthy people, which might explain the unremarkable decrease of FA values in the patients. Earlier study [9] demonstrated that compared with healthy people, ALS patients had reduced FA values only in the central gyrus cortex, centrumsemiovale and PLIC. In our study, the positive rates were much higher with larger and statistically significant ROIs. This might be attributed to smaller ROI areas used in the study, partially resulting in reduced impact from the volume effect. The heterogeneity of the disease in addition may also contribute to the difference.

The damage of cell membrane integrity increases the extracellular gaps, leading to the increase of ADC value. In this study, the increase of ADC value in the patients was remarkable in centrumsemiovale, frontal and parietal deep white matter. Particularly, the ADC value in centrumsemiovale was high, indicating that in ALS patients the diffusion of water molecules in these areas are restricted. ADC values were comparable in the patient and control groups in other areas such as cerebral peduncle, PLIC, corona radiata, central gyrus

subcortical white matter, the genu and splenium of corpus callosum, and thalamus. The lesions in white matter will lead to degeneration of axon [15], resulting in increased extracellular space and increased diffusion. Gliosis may also occur under this condition. The proliferated glial cells reduce the diffusion, leading to unchanged cell density at lesion sites. However, the lesions at different stages may also affect ADC values. This contradiction in pathogenesis may explain why the ADC values in most of the ROIs were not statistically different between the patient and control groups in our study. These data indicate that ADC values in most of ROIs are not beneficial for ALS diagnosis. In addition, axonal degeneration is sometimes accompanied by fibrous gliosis. The axonal structure will not be easily and completely restored. However, once the cell density extracellular space is not apparently affected, the change in the ADC values will be not evident. The results further suggest that for the diagnosis of motor neuron damage in ALS, FA values are of primary choice.

In this study, the pons and corticobulbar tract were not included, although they are critical in the ALS lesion of CST. However, due to the high sensitivity of our MR machine (3.0T) to magnetic field inhomogeneity, it is likely that the images will be deformed in areas that are susceptible to larger magnetic changes, such as the areas between the skull and pons. In addition, from the cerebral peduncle to pyramid, many fibers have left the pyramidal tract. Furthermore, the intersection of pyramids will interrupt the continuity of the fiber, resulting in highly variable FA and ADC values between the adjacent layers.

Analysis of SE and SP based on three layers of ROIs in CST for ALS diagnosis

It had been proved that there was CST damage in ALS patients in pathology [8]. In this study, we analyzed the SE and SP based on ROIs in three layers in CST, including cerebral peduncle, PLIC and corona radiata. SE and SP for determining the UMN injury were calculated based on receiver operating characteristic curve (ROC) using data from different ROIs in CST. A number of cut-off values are set out from the continuous variables in the diagnostic tests. A serials of SE and SP pairs are calculat-

ed to draw a curve with SE as the ordinate and 1-SP (misdiagnosis rate) as the abscissa. The statistical methods are useful to compare the results from two or more diagnostic tests. The advantage of ROC curve is simple, intuitive, and graphical and can be used to visually represent SE and SP. Area under the curve is an indicator of diagnostic value. If the area is greater than 0.7, it is considered to have diagnostic value.

In this study, the FA values in cerebral peduncle, PLIC and corona radiata were determined on both sides of CST. ROC curve analysis showed that with the increase of FA values in these three areas from 0 to 1, SE and SP of the FA values for ALS diagnosis were decreased and increased, respectively. Obviously, based on our results, FA value in the corona radiata presented the highest SE and SP. These results indicate that DTI is a sensitive and specific method to evaluate the CST UMN damage in ALS patients. The areas under the curve were 0.943, 0.946 and 0.983 for the cerebral peduncle, PLIC and corona radiata, respectively. They were all over 0.7, indicating that the FA values of the three layers are useful for ALS diagnosis.

In summary, our study demonstrate that FA values in the ROIs of cerebral peduncle, PLIC and corona radiate are critical for ALS diagnosis. These parameters can be used as quantitative indicators for ALS patients for their diagnosis and therapy. Nevertheless, the FA measurements are affected by a number of factors, such as models of instruments, cut-off values, and operators. Therefore, the standards have deviation and further studies are required for their clinical applications.

Acknowledgements

Disclosure of conflict of interest

None.

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