Clinical Investigative Study

Altered Brain White Matter Integrity in Temporal Lobe Epilepsy: A TBSS Study

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ABSTRACT

BACKGROUND AND PURPOSE

The aim of this study is to explore the possible changed cerebral white matter regions in patients with temporal lobe epilepsy (TLE) using diffusion tensor imaging (DTI) and tract-based spatial statistics (TBSS).

METHODS

Twenty TLE patients and 22 age- and gender-matched normal controls were included in this study. Voxel-wise analyses of multiple diffusion metrics, including fractional anisotropy (FA) and mean diffusivity (MD) were performed with TBSS.

RESULTS

TLE patients exhibited significantly reduced FA in widespread white matter regions including bilateral limbic circuit, corpus callosum, thalamus, internal/external capsule, temporooccipital connections, frontotemporal connections; increase of MD was exhibited significantly almost in the left hemisphere. A significant decrease in global FA integrity was shown in epilepsy subjects compared to healthy controls. Furthermore, it exhibited a significant positive correlation between the disease duration and MD of whole brain.

CONCLUSIONS

TLE is associated with widespread abnormalities in cerebral white matter tracts and these changes may have important clinical consequences.

Keywords: Temporal lobe epilepsy, white matter integrity, tract based spatial statistics.

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Introduction

Temporal lobe epilepsy (TLE) is the most common form of focal epilepsy, with structural abnormalities both within and distant from the ictal onset zone.[1] Although the pathophysiology has been investigated for several decades, much remains unclear. In recent years, many advanced magnetic resonance imaging (MRI) approaches have been extensively used for the assessment of brain structural changes and their association with clinical characteristics in patients with TLE,[2–4] which are important for our understanding the neuropathological mechanisms of the disease.

Diffusion tensor imaging (DTI) is one of the most promising techniques with regard to TLE. It quantifies the amount of nonrandom water diffusion within tissues and provides unique *in vivo* information about the pathological processes that affect water diffusion as a result of brain microstructural damage. Fractional anisotropy (FA) and mean diffusivity (MD) are two important diffusion metrics for the analysis of DTI datum, FA reflects the degree of directionality of cellular structures within the fiber tracts by measuring anisotropic water diffusion, while MD represents the diffusion in the noncolinear direction of free diffusion.[5]

The tract-based spatial statistics (TBSS) method is a fully automated whole-brain analysis technique that uses voxel-wise statistics on diffusion metrics but simultaneous minimizes the effects of misalignment using a conventional voxel-based analysis method.[6] In this study, we applied this TBSS analysis to the DTI data collected from patients with TLE in order to describe the white matter change patterns across the whole brain in TLE patients, and explore multiple diffusion metrics (FA and MD) alteration related to clinical variables in TLE.

Materials and Methods

Participants

This study included 20 right-handed patients with clinically definite TLE (13 males; mean age 25.5 years, and SD 8.6) and 22 age-, gender-matched normal controls (NC) (13 males; mean age 25.2 years, and SD 6.6) with normal findings on neurological examination and without history of neurological

Table 1. Demographic and Clinical Characteristics of all Participants (mean ± SD)

Characteristics	TLE ($n = 20$)	Control ($n = 22$)	*P* Values
Age (years)	25.5 ± 8.6	25.2 ± 6.7	.892
Gender (male/female)	16/4	13/9	.143
Education (years)	9.9 ± 1.9	9.8 ± 1.7	.729
Age of seizure onset (years)	15.4 ± 7.2	NA	NA
Duration of illness (years)	10.9 ± 8.2	NA	NA
LTLE/RTLE/BTLE	12/6/2	NA	NA

TLE = temporal lobe epilepsy; LTLE = left temporal lobe epilepsy; RTLE = right temporal lobe epilepsy; BTLE = bilateral temporal lobe epilepsy.

dysfunction. Entry criteria for patients included ability to undergo MRI. The diagnosis of TLE was confirmed in all 20 patients using continuous video-EEG (electroencephalography) monitoring, demonstrating unilateral ictal temporal EEG discharges of theta frequency or higher within 30 seconds after onset, and thus were classified as definite TLE.[7] All patients had complex partial seizures, and parts of them were accompanied tonic-clonic seizures. All of them had one or more typical symptoms of TLE, such as automatisms, dystonic posturing of the limbs, and olfactory hallucinations, abnormal emotional experiences. In 8 out of 20 patients, the diagnosis with TLE was also supported by MRI evidence of mesial temporal sclerosis. None of the participating patients had been treated with related medications (eg, corticosteroids and immunosuppressants) within 3 months before MRI scanning.

Twenty-two right-handed healthy volunteers were recruited as controls (13 males; mean age 25.2 years, and SD 6.6). None of them had neurological or psychiatric disorders. All examinations were carried out under the guidance of the Declaration of Helsinki 1975. Written informed consent forms were obtained from all participants and this research protocol was approved by the institutional review board of Guangdong 999 Brain Hospital (Table 1).

Data Acquisition

All participants were imaged with a 1.5 T MR unit (Gyroscan Intera 1.5 T, Philips Medical Systems, Best, and Holland). The brain was imaged by using the following sequences with an identical field of view (FOV) = 144 × 144 mm, repetition time (TR)/echo time (TE) = 11000/62 milliseconds; number of excitation (NEX) = 1; number of axial slices (67), slice thickness (2.0 mm), covering the entire cerebrum and brainstem without gaps, 33 directions with $b = 800$ s/mm^2 and a b0 image. All patients were seizure-free per self-report for a minimum of 24 hours prior to the MRI scan.[2]

Data Preprocessing

Image files in Digital Imaging and Communications in Medicine (DICOM) format were transferred to a Linux workstation for further processing. Then, eddy current distortions and motion artifacts in the DTI data set were corrected by applying affine alignment of each diffusion-weighted image to the b0 image, using FMRIB's Diffusion Toolbox (FDT) (FSL 4.1.9; www.fmrib.ox.ac.uk/fsl). After this process, the first volume of the diffusion data without gradient applied (ie, b0 image) was used to generate a binary brain mask with the Brain Extraction Tool. Finally, DTIfit was used to independently fit diffusion tensor to each voxel. The output of DTIfit yielded voxelwise maps of FA and MD for each subject.

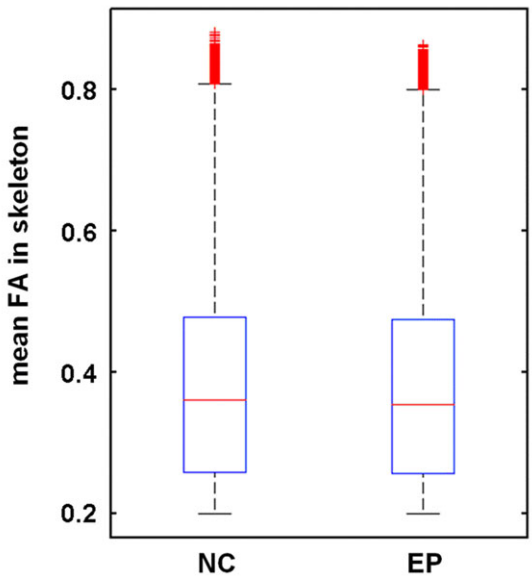

Fig 1. Box plot shows mean FA in skeleton per group, a significant decrease in global FA integrity for epilepsy patients (EP) is shown comparing to healthy controls (HC) ($t = 7.67$, $P = 1.699e-14$).

Tract-based Spatial Statistics (TBSS)

First, the mean diffusion metrics (FA and MD) in the white matter skeleton were extracted for each subject. TBSS of FA images was carried out using TBSS in the FMRIB software library (FSL 4.1.9; www.fmrib.ox.ac.uk/fsl; for detailed description of methods, see Smith et al.[6]).

Statistical Analyses

We first calculated the mean diffusion measures (FA and MD) in the whole-brain white matter skeleton for each subject, and performed two-sample *t*-tests to compare the mean diffusion measures between the TLE and NC groups. Age and gender were considered as covariates in the analyses. Second, voxel-wise statistics in TBSS were carried out using a permutation-based inference tool for nonparametric statistical thresholding ("randomize," part of FSL). In this study, voxel-wise group comparisons were performed using nonparametric, two-sample *t*-tests between the TLE and NC groups after controlling for the effect of age and gender. The mean FA skeleton was used as a mask (thresholded at a mean FA value of .2). The significance threshold for between-group differences was set at $P < .05$ (Familywise Error Rate [FWE] corrected for multiple comparisons) using the threshold-free cluster enhancement (TFCE) option in the "randomize" permutation-testing tool in FSL. Similarly, group comparisons of MD images were performed respectively.

Additionally, correlation analyses between FA, MD and disease durations were performed in a voxelwise manner with a

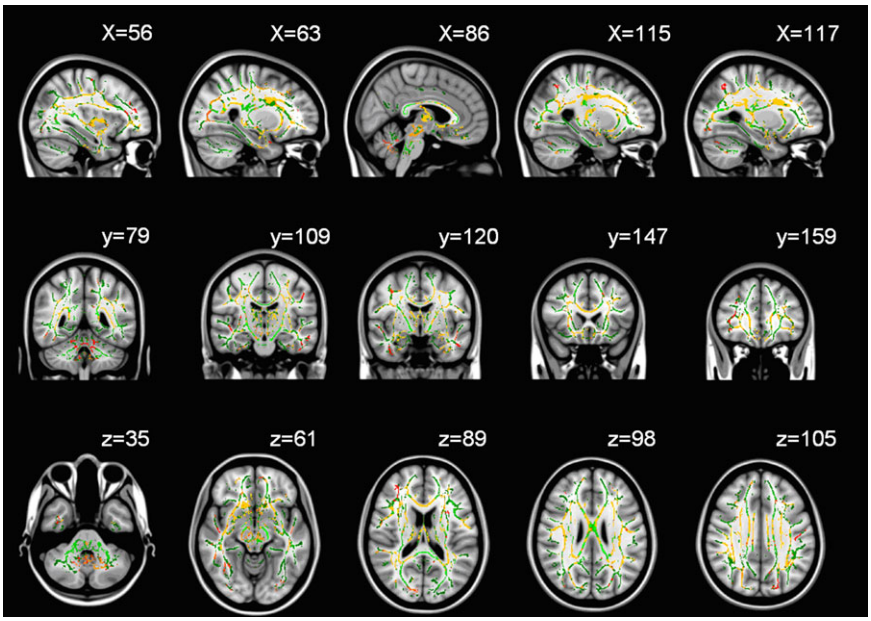

Fig 2. TBSS result of FA images between controls and TLE patients. Green represents mean FA skeleton of all participants; red and yellow represents regions with decreased FA in TLE patients ($P < .05$, FWE corrected for multiple comparisons).

mask of areas with group differences. We used a linear regression model to perform correlation analyses while treating age and gender as covariates of no interest, significant correlation was set at $P < .05$ (FWE corrected for multiple comparisons).

Results

Group Differences in FA and MD

Statistic analysis revealed significant group differences in all mean diffusion metrics of the white matter skeletons (for all measures: $P < 10^{-8}$). Compared with the NCs, TLE patients had significantly decreased FA (TLE [mean ± std]: .389 ± .007; NC [mean ± std]: .397 ± .006), increased MD (TLE [mean ± std]: [1.097 ± .0544] × 10^{-4}; NC [mean ± std]: [1.055 ± .044] × 10^{-4}). A significant decrease in global FA integrity for epilepsy patients is shown comparing to healthy controls ($t = 7.67$, $P = 1.699e - 14$; Fig 1).

Second, TBSS analyses revealed widespread white matter regions with abnormal diffusion changes decreased FA (Fig 2), including bilateral limbic circuit (fornix, insular lobe), intrahemispheric fiber tracts (corpus callosum), temporooccipital connections, frontotemporal connections, motor projection tracts, deutocerebrum, thalamus, internal/external capsule, and cerebellum. However, increase of MD was exhibited significantly almost in the left hemisphere, including left limbic circuit, left temporal lobe, left internal/external capsule, left temporooccipital connections, and left frontotemporal connections (Fig 3).

Correlations between Diffusion Metrics in the Whole-brain White Matter and Clinical Variables in TLE Patients

The FA of whole-brain white matter have negative correlations with the disease duration and seizure onset ($r = -.55$, $P = .01$; Fig 4), and significant positive correlations between the MD of whole-brain and the disease duration lesion volumes were identified across the whole-brain white matter ($r = .71$, $P < .01$). For diffusivity metrics, similar regions with clinical correlations were identified but to a much less extent.

Discussion

In this study, we determined global maps of the white matter changes in TLE patients by measuring FA and MD across the brain, offering a comprehensive view of the landscape of white matter damage in TLE patients. Compared with the NCs, the TLE patients exhibited significantly reduced FA in widespread white matter regions, which suggests that TLE is a disease that affects the brain globally. However, increase of MD was exhibited significantly almost in the left hemisphere. Furthermore, it exhibited a significant positive correlation between the disease duration and the mean MD of whole brain, and a significant negative correlation between the disease duration and the mean FA of whole brain. These correlations between white matter DTI metrics and clinical variables may suggest a role for DTI in monitoring disability and progression of the disease.

This study demonstrated that TLE was involved in diffusion changes of white matter regions corresponded to a widespread network of white matter tracts, not only in the bilateral limbic circuit (fornix, insular lobe), intrahemispheric fiber tracts (corpus callosum), but also in temporooccipital connections, frontotemporal connections, motor projection tracts, deutocerebrum, and cerebellum, which is consistent with the findings of previous TBSS studies.[8–10] However, this study showed a greater number of abnormal white matter clusters including thalamus and internal/external capsule, which is similar to early DTI studies of TLE used region of interest (ROI)-based

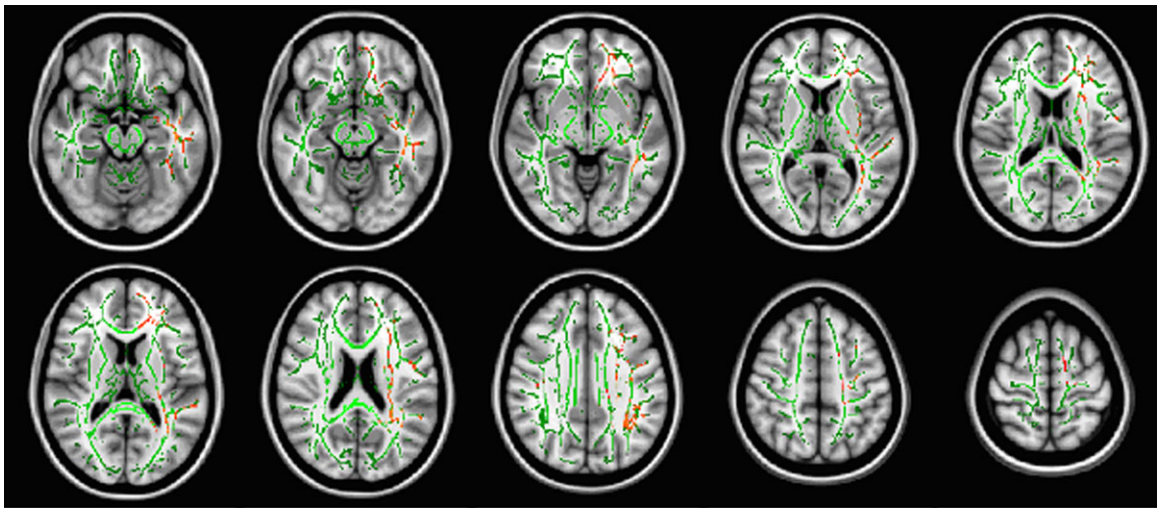

Fig 3. TBSS result of MD images between controls and TLE patients. Green represents mean MD skeleton of all participants; red and yellow represents regions with increased MD in TLE patients ($P < .05$, FWE corrected for multiple comparisons).

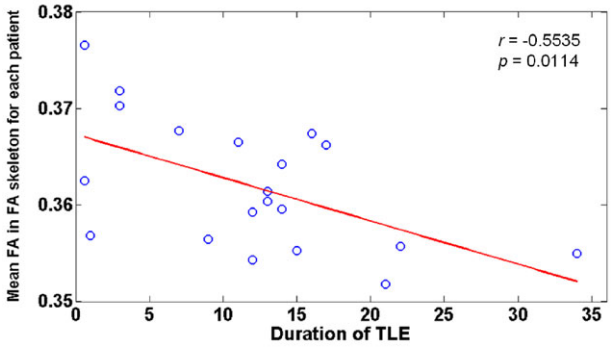

Fig 4. Relationship between the mean FA of each patient and epilepsy duration. Significant negative correlation ($r = -.5535$, $P = .0114$).

analysis[11,12] or VBM method.[4] In this study, we employed a more reproducible voxel-based approach TBSS to overcome the disadvantages of the ROI-based (low reproducibility) or conventional voxel-based methods (alignment and smoothing issues).[13]

In this study, we found that there was a significant positive correlation between the disease duration and the mean MD of whole brain, and significant negative correlation between the disease duration and the mean FA of whole brain. TLE patients had reduced FA across the whole white matter, which is consistent with the findings of previous DTI studies in TLE patients.[2,3,14] Different patterns of MD changes in different fiber tracts may indicate that various white matter pathological changes occur with TLE. The left frontal lobe, temporal lobe, parietal lobe, external capsule, posterior limb of internal capsule, splenium of the corpus callosum, temporooccipital connections, and left frontotemporal connections showed decreased FA, and increased MD. This diffusivity pattern has been associated with chronic changes related to ischemic stroke[12] and chronic white matter degeneration such as those seen after corpus callosotomy.[15] These diffusion abnormalities likely reflect a combination of axon and myelin loss, therefore leading to lower membrane density and higher extracellular volume.[16] However, the right frontal lobe, temporal lobe, parietal lobe, internal/external capsule, splenium of the corpus callosum, temporooccipital connections, frontotemporal connections, and bilateral thalamus, body of corpus callosum, deutocerebrum, and cerebellum showed decreased FA without significant changes in MD. This pattern may reflect subtle white matter fiber incoherence, such as minor fiber loss. This observation is similar to the previous studies.[3] In this study, we observed that patients with TLE showed a significant left-right asymmetry in their MD values, which mean asymmetry damage of white matter in brain hemispheres, and the left hemisphere showed greater degree of white matter abnormalities than contralateral ones. One possible explanation is that left hemisphere was more vulnerable to early-onset seizures, because the left hemisphere matures later and more slowly than the right hemisphere, it may be more vulnerable to early insults from febrile and early onset seizures for a longer period. Furthermore, there were 12 left TLE and 6 right TLE in current study, a number of studies have shown that patients with left TLE exhibit more abnormalities in ipsilateral and contralateral regions than those with right TLE,[8,9,17,18] and there was evidence that patients with left TLE exhibited a more widespread pattern of white matter atrophy.[19]

In addition, we found reductions of FA values in the cerebellum in patients with TLE, this may be related to a mixed impact of phenytoin using and seizures, it has been verified that phenytoin use can lead to cerebellar atrophy, and about 50% of the current patient population had exposure to this medication.[20] In our study, there were 16 TLE patients that had exposure to phenytoin. Similarly, white matter abnormalities in the cerebellum have been reported in previous studies.[20,21]

Several limitations should be addressed. First, the correspondence between axon and myelin damage and tensor diffusivity is still controversial. Thus, we cannot resolve the histopathological implications in TLE versus FA changes and the clinical

correlations in TLE. Second, the sample size of current study is small, we included both left TLE, right TLE and bilateral TLE patients in this study, a direct comparison between left TLE and right TLE would be interesting. However, the number of subjects included does not allow such a comparison. We also did not analyze the relationship between FA, MD, and other clinical information (such as frequency of seizures, number of antiepileptic drugs [AED] used) this time. Future studies enrolling larger populations should also focus on side-specific effects and the relationship between FA, MD, and other clinical information. Third, 16 TLE patients in this study had exposure to phenytoin, phenytoin use has been associated with cerebellar atrophy.

However, the impact of this medication use on cerebral white matter remains largely unclear. Further studies in larger well-matched patient samples will examine these issues.

Conclusions

In this study, we determined a global map of the white matter changes in TLE patients by means of TBSS method. Compared with NCs, the TLE patients showed significantly reduced FA and increased MD in distributed white matter regions. The diffusion changes and clinical correlations were mainly contributed by reduced FA and increased MD, implying the predominant role of DTI metrics in reflecting the subtle pathological changes in TLE patients. Furthermore, it will allow more precise monitoring of disability and progression in TLE.

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

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