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Brain diffusion tensor imaging in children with tuberous sclerosis



M.S. Dogan^{a,*}, K. Gumus^b, G. Koc^a, S. Doganay^a,
H. Per^c, S.B. Gorkem^a, M. Canpolat^c, A.K. Bayram^c,
A. Coskun^a

^a Erciyes University, Faculty of Medicine, Department of Radiology, Kayseri, Turkey

^b Erciyes University, Faculty of Medicine, Department of Biophysics, Kayseri, Turkey

^c Erciyes University, Faculty of Medicine, Department of Pediatric Neurology, Kayseri, Turkey

KEYWORDS

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Abstract

Purpose: To evaluate diffusion characteristics of tubers and white matter lesions in children with tuberous sclerosis (TS) using diffusion tensor imaging (DTI).

Materials and methods: Eighteen children (11 male, 7 female; mean age 9.3 years, age range 1–16 years) with a definite diagnosis of TS were recruited in this study. Apparent diffusion coefficient (ADC), fractional anisotropy (FA), radial diffusivity (RD), and axial diffusivity (AD) values in 89 tubers and 37 white matter lesions were measured and compared with those of contralateral normal regions.

Results: ADC, AD, and RD values were significantly higher and FA values were lower in lesions, than the ones measured in contralateral normal regions for tubers ($P < 0.001$). Similarly RD values were significantly higher and FA values were lower in white matter lesions ($P < 0.05$). ADC and AD measures were detected to increase in white matter lesions, however no statistically significant difference was observed. The increase in the mean values of RD was significantly greater than the increase in the AD values for tubers and white matter lesions ($P < 0.05$).

Conclusion: DTI can provide valuable information about the cytoarchitectural changes in TS lesions beyond morphologic MRI findings alone.

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* Corresponding author. Erciyes University Faculty of Medicine, Radiology, Kayseri 38039, Turkey.
E-mail addresses: msaitdogan@hotmail.com (M.S. Dogan), kzgumus@gmail.com (K. Gumus), ggulkoc@gmail.com (G. Koc), selimdoganay@gmail.com (S. Doganay), huseyinper@yahoo.com (H. Per), drburcugorkem@gmail.com (S.B. Gorkem), drmetcanpolat@gmail.com (M. Canpolat), draysebayram@gmail.com (A.K. Bayram), coskunah@yahoo.com (A. Coskun).

Tuberous sclerosis (TS) complex, the second most common neurocutaneous syndrome after neurofibromatosis type 1, is characterized by multiple benign hamartomatous lesions involving skin, brain, kidneys, eyes, and heart [1–3]. It occurs in 1 of 6000 newborns and 1.5 million people are estimated to suffer worldwide [1]. This autosomal dominant phakomatosis is caused by a mutation in either one of two tumor suppressor genes called *TSC1* and *TSC2* [1–3]. The typical appearance of TS is epilepsy, mental retardation, and facial angiofibromas; named as “Vogt’s triad”. However, this triad is seen in only 30–40% of patients. The clinical expression of TS has great variability [1,2]. Several neurological symptoms, such as autism, behavioral problems, mental retardation, and seizures may be seen in TS [1,2].

Typical intracranial lesions of TS are tubers, subependymal nodules, subependymal giant cell astrocytomas (all of which are the major features for the diagnosis of TS) and white matter abnormalities [1–3]. Magnetic resonance imaging (MRI) is the main modality to display all these intracranial lesions. Tubers [1,3], which constitute the hallmark of the disease, are hamartomatous cortical-subcortical lesions that are seen in 90% of patients. Tubers have low signal on T1-weighted (T1-W) images and high signal on both T2-weighted (T2-W) and FLAIR images unless they are calcified. The white matter lesions of TS are also hypointense on T1-W images and hyperintense on T2-W and FLAIR images [1,3]. Radial glial bands, also called radial migration lines and periventricular cyst-like lesions are defined as white matter lesions of TS. Radial glial bands are linear or wedge shaped lesions, which may reach from ventricular ependymal surface to the cortex, occasionally terminating at tubers. They are thought to represent heterotopic neuronal and glial elements that arrested during cortical migration [1,3].

Although conventional MR sequences are routinely used for detecting intracranial lesions, they are not able to reveal the subtle microstructural characteristics of TS. Diffusion tensor imaging (DTI) makes investigation of the three dimensional microanatomical structure of brain parenchyma possible [4–6]. This technique evaluates the direction and magnitude of water diffusion in tissues [4–7]. Protons diffuse freely in all directions in an isotropic diffusion (as in cerebrospinal fluid), whereas diffusion is restricted in some directions in an anisotropic diffusion (as in organized biological tissues like cerebral white matter). Diffusion tensor is a second-order matrix that represents direction and magnitude of diffusion at each voxel on the image [5,6]. It is characterized by three eigenvalues (λ_1 , λ_2 , and λ_3) and three eigenvectors (ε_1 , ε_2 , and ε_3), which represent the magnitude and direction of diffusion respectively [6,8]. Major eigenvalue (λ_1) also called “axial diffusivity” (AD) represents the highest diffusivity parallel to the axon. Intermediate and minor eigenvalues (λ_2 and λ_3 respectively) define diffusivities perpendicular to the axon and the “radial diffusivity” (RD) is the arithmetic average of λ_2 and λ_3 [9]. The apparent diffusion coefficient (ADC) evaluates the overall magnitude of water diffusion in the tissue and is equal to the average of the 3 eigenvalues. Another main DTI index fractional anisotropy (FA), which measures the degree of anisotropy and is scaled from 0 (completely isotropic) to 1 (completely anisotropic) [5,7].

In recent years, DTI has been used for investigating several pediatric neurological diseases, like malformations, periventricular leukomalacia, tumors, multiple sclerosis, epilepsy, and phacomatosis [7]. There are few studies described DTI findings of TS up to date. Most of these studies investigated either ADC [10] or ADC and FA both [11–13]. Firat et al. [10] reported higher ADC values in tubers than normal appearing white matter in controls and other studies [11–13] which investigated ADC and FA, found lower values of FA and higher values of ADC in tuberous sclerosis lesions than normal appearing corresponding areas. However the directional diffusivity indices like AD and RD can give additional beneficial information on the microstructure of tubers and white matter lesions [14]. Therefore, in this work, we aimed to characterize water diffusion and its directionality in TS lesions by using DTI method investigating axial and radial diffusivities in addition to apparent diffusion and anisotropy measures.

Materials and methods

Patients

This study was approved by the local ethics committee of our institution. The sample of our study consisted of 18 pediatric patients (11 male, 7 female; age range 2–16 years with mean age 9.3 and standard deviation 5 years) with a definite diagnosis of tuberous sclerosis who were regularly followed up in our pediatric neurology outpatient clinic. Presence of tubers on MRI was identified as selection criteria. All 18 patients suffered from epilepsy and mental retardation. Other clinical manifestations included hypopigmented macules in all patients, facial angiofibromas in 12 patients, shagreen patches and renal angiomyolipomas in 11 patients, cardiac rhabdomyomas in three patients.

Magnetic resonance imaging procedure

Routine cranial MRI and DTI were performed on a 1.5 Tesla MRI device (Siemens Aera; Siemens Medical Systems, Germany). The routine cranial MR imaging protocol consisted of 3-D MP-RAGE T1-weighted imaging (TR = 1900 ms, TE = 2.86 ms, slice thickness (ST) = 1 mm, FOV: 250 × 250 mm, Resolution: 256 × 256), coronal and axial T2-weighted imaging (TR = 4000 ms, TE = 96 ms, Number of slice (NS) = 23, ST = 5 mm, FOV: 230 × 230 mm, resolution: 320 × 320), axial FLAIR (TR = 7000 ms, TE = 84 ms, NS = 23, ST = 5 mm, FOV: 260 × 260 mm, resolution: 256 × 256), and susceptibility weighted imaging (SWI) (TR = 49 ms, TE = 40 ms, ST = 3 mm, FOV: 260 × 260 mm, resolution: 256 × 256). Additionally DTI images with following parameters were acquired by using a single-shot echo-planar pulse sequence: TR = 3500 ms, TE = 83 ms, Resolution = 128 × 128, FOV: 230 × 230 mm, ST = 5 mm and 3 averages. Two *b* values (0, 1000 s/mm²) were applied in 12 non-collinear directions. The total acquisition time for DTI sequence was approximately two and a half minutes.

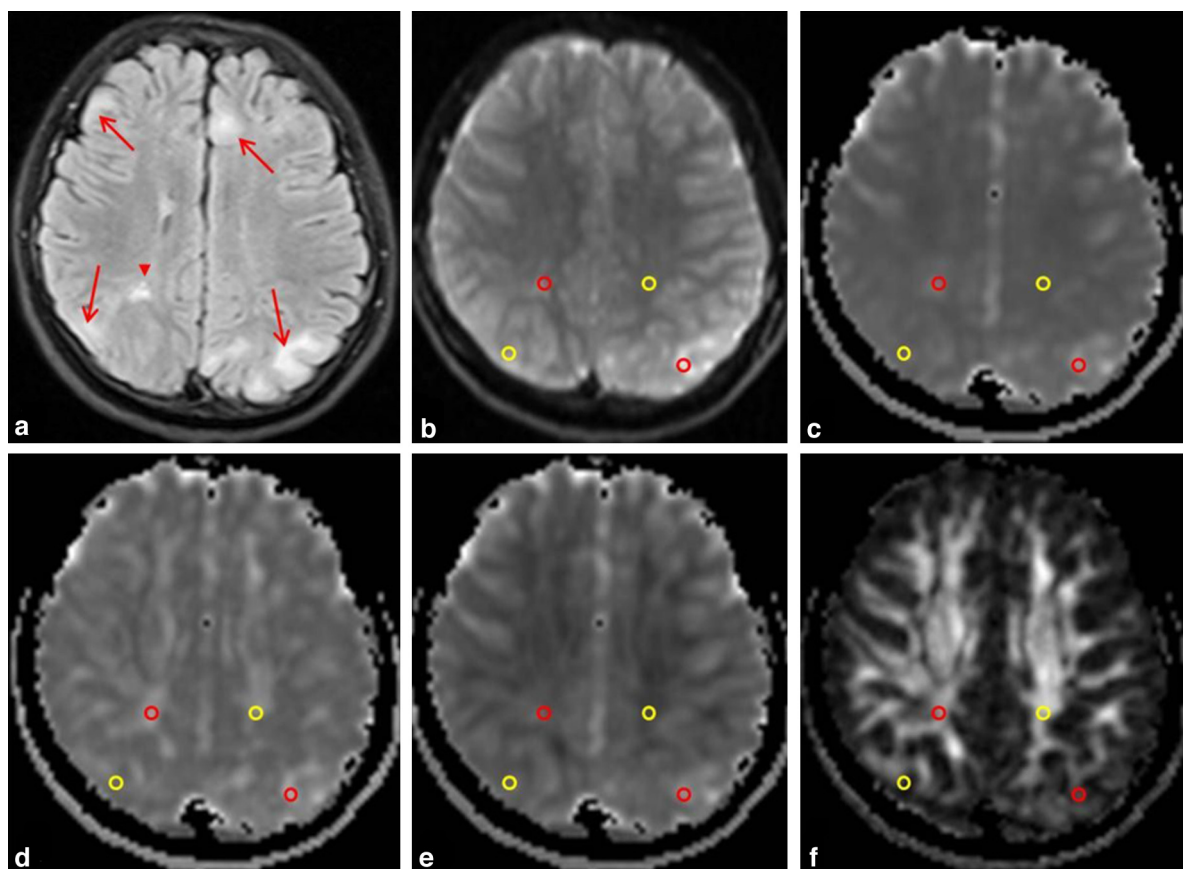


Figure 1. An 11-year-old female patient with tuberous sclerosis. Axial FLAIR image (a) demonstrates a white matter lesion (arrowhead) in the right parietal lobe and multiple tubers (arrows) on both hemispheres. Placement of the ROIs within a tuber (red circle), a white matter lesion (red circle), and corresponding contralateral normal regions (yellow circles) are shown on $b=0$ (b), ADC (c), AD (d), RD (e), and FA (f) maps.

Image analysis

Following image acquisition, an image processing software called “DtiStudio” [15] was used for generating ADC, AD, RD, and FA maps and region-of-interest (ROI) measurements of DTI indices in lesions and contralateral normal regions. The areas of similar location in the contralateral cerebral hemisphere without any signal intensity abnormalities or volume changes in routine MR images are assumed as contralateral normal regions. After detecting lesions on FLAIR images, corresponding ROIs were drawn within the cortical tubers and white matter lesions on the $b=0$ images manually in DtiStudio software. The drawn ROIs on the $b=0$ image were automatically placed on the generated ADC, AD, RD, and FA maps by DtiStudio. ROIs were also manually located in the corresponding contralateral normal regions. The mean and standard deviation of the ADC, AD, RD, and FA values of the lesions and contralateral normal regions were measured. We used two dimensional ROIs which was centered on lesions and ROIs size ranged from 4–16 pixels based on the size of the lesions (Fig. 1). DTI parameters were not calculated in lesions smaller than 5 mm in diameter to prevent contamination from adjacent normal appearing brain parenchyma. Calcified tubers were detected on SWI images and not included in the study. The values of DTI indices in lesions were compared with the ones

of contralateral normal regions in each patient. Statistical analysis was performed using the IBM SPSS Statistics 22.0, Armonk, New York, IBM Corp., 2013. Paired sample t -test was used for statistical analysis method and $P < 0.05$ value was considered as significant and patient age was used as a covariate.

Results

We observed a total of seven calcified tubers in two patients out of 18 patients using SWI images. After these were excluded, a total of 126 lesions and 126 contralateral normal regions consisting of 89 tubers and 37 white matter lesions were evaluated. Distribution of lesions is as follows: 57 frontal, 27 parietal, 27 occipital, and 15 temporal. Sixty-three lesions were on the right cerebral hemisphere and same number was on the left side. Average number of cortical tubers and white matter lesions per patient were five and two, respectively.

Fig. 1 shows a representative slice of an 11-year-old female patient with tuberous sclerosis. A white matter lesion (arrowhead) in the right parietal lobe, multiple tubers (arrows) and corresponding contralateral normal regions (yellow circles) are shown on FLAIR, $b=0$, ADC, AD, RD, and FA maps.

Table 1 Comparison of mean DTI indices values between tubers and white matter (WM) lesions and contralateral normal regions.

	Lesion	Contralateral normal region	P value
ADC ($\text{mm}^2/\text{s} \times 10^{-3}$)			
Tubers	3.53 ± 0.63	2.59 ± 0.21	< 0.001
WM lesions	3.34 ± 0.68	2.54 ± 0.29	0.092
FA			
Tubers	0.12 ± 0.05	0.19 ± 0.07	< 0.001
WM lesions	0.21 ± 0.08	0.34 ± 0.11	< 0.05
AD ($\text{mm}^2/\text{s} \times 10^{-3}$)			
Tubers	1.32 ± 0.22	1.04 ± 0.01	< 0.001
WM lesions	1.36 ± 0.25	1.17 ± 0.19	0.459
RD ($\text{mm}^2/\text{s} \times 10^{-3}$)			
Tubers	1.10 ± 0.22	0.77 ± 0.08	< 0.001
WM lesions	0.99 ± 0.23	0.69 ± 0.10	< 0.05

Variables were expressed as mean ± standard deviation (SD). ADC: apparent diffusion coefficient; FA: fractional anisotropy; AD: axial diffusivity; RD: radial diffusivity.

The measurements of the DTI indices of lesions and the contralateral normal regions in the patients with TS were presented in Table 1. The ADC, AD, and RD values were significantly higher and FA values were lower in tubers than the ones measured in contralateral normal regions ($P < 0.001$). Similarly RD values were significantly higher and FA values were lower in white matter lesions ($P < 0.05$). Although ADC and AD measures were detected to increase in white matter lesions, there was no statistically significant difference. ($P = 0.092$ and 0.459 for ADC and AD values, respectively). The increases in the mean values of RD were significantly greater than the increases in the AD values for tubers and white matter lesions ($P < 0.05$). RD increased by 42% and 43% for tubers and white matter lesions, respectively whereas AD increased by 27% and 16% for tubers and white matter lesions, respectively.

Discussion

Our results showed that measured DTI indices (ADC, AD, RD, and FA) were significantly different between cortical tubers and contralateral normal regions, besides there was significant difference for RD and FA values between white matter lesions and contralateral normal regions. When compared with the contralateral normal regions, statistically significant increase in ADC values, decrease in FA values for tubers and decrease in FA for white matter lesions were observed. The ADC values in white matter lesions were not significantly different from contralateral normal regions but they had an increasing trend. Previous studies which compared TS lesions and normal regions using DTI or DWI showed similar results for both ADC and FA values of tubers and FA values of white matter lesions but the results were different for ADC values in white matter lesions. Piao et al. found higher ADC values in cortical tubers, and lower FA and higher ADC of white matter lesions compared to contralateral regions [11]. Peng et al. reported lower FA, and higher ADC values of white matter lesions of tubers compared to contralateral brain regions

[12]. Karadag et al. have demonstrated not only higher ADC within tubers compared to normal controls, but also higher ADC and lower FA in white matter abnormalities and perilesional white matter compared to contralateral side and normal controls [13]. Firat et al. observed greater ADC values of tubers than normal appearing white matter in controls [10]. In our study, the reason for the non-significant increase in ADC values of white matter lesions may be relatively stable AD values.

Directional diffusivity indices, AD and RD may provide further information about the cytoarchitectural changes than the overall mean diffusion as measured by ADC. There are three conditions that could lead to decreased FA and increased ADC values: an increase in radial diffusivity (perpendicular to the axon), an increase in axial diffusivity (parallel to the axon) and the combination of both [5]. In this study, AD and RD values as directional diffusivity indices, were also significantly higher in cortical tubers and RD values were higher in white matter lesions than contralateral normal regions. There was no significant difference for AD values between white matter lesions and contralateral normal regions. Another crucial result of this study is that the increase in RD values was greater than the increase in AD values for tubers and white matter lesions. Peng et al. stated that λ_1 , λ_2 and λ_3 were significantly larger in the tubers than those in the contralateral normal appearing brain regions of patients with TS [12]. Makki et al. reported that the patients with TS had higher diffusivity in normal appearing white matter parallel and perpendicular to the axons compared with that of normal controls and the increase in diffusivity was more pronounced in directions orthogonal to the axons measured by λ_2 and λ_3 than by λ_1 [16]. Widjaja et al. have observed diminished FA and elevated RD values in subcortical white matter adjacent to tubers within epileptogenic zone compared to non-epileptogenic zone [17]. This limited number of studies suggests that the changes in mean diffusion in TS lesions indicate microstructural changes resulting in increased water diffusion in perpendicular direction of the axonal fibers.

Tubers are hamartomatous lesions that exhibit disorganized cortical lamination and contain neuronal and glial elements, such as maloriented neurons, atypical giant astrocytes and bizarre giant cells [1]. These changes may cause loss of structural barriers to water motion, leading to a significant decrease in FA and increase in ADC, and its components (AD and RD) compared to the contralateral normal regions for tubers. Radial glial bands as the white matter lesions of TS represent heterotopic neuronal and glial elements that arrested during cortical migration [1]. They are primarily located within the subcortical white matter [2]. In the subcortical white matter lesions, cytoarchitecture is usually loose due to usually depleted and disordered myelin sheaths in these lesions, and often accompanied with intense fibrillary gliotic reaction [12]. Although there are few studies describing directional diffusivity findings in patients with TS, in converse to our study, none of the studies statistically compared the alteration of both AD and RD values of tubers and white matter lesions [12,16,17]. The greater increase in RD values compared to AD values may be interpreted as hamartomatous proliferation of gray matter and dysmyelination of the axons caused by disordered myelin sheaths and intense fibrillary gliotic reaction of white matter. So that, aforementioned finding may further help shed light on the physiopathology of the TS. Furthermore, animal studies also have shown that elevated AD was related to axonal injury or disarray of axons while elevated RD was related to demyelination or abnormal myelin [5,14,18–20].

This study has some limitations. First, the lesions were detected on FLAIR images and manually drawn on $b=0$ images. DTI is intrinsically a low-resolution technique to minimize signal loss due to T2 effect. Thus, the acquisition matrix of DTI generally does not match with the ones of T1- and T2-weighted images. The manual placement of lesion on the $b=0$ image and the selection of contralateral ROIs might contain some error. One can suggest applying simple rigid transformation to co-register T2-weighted and DTI $b=0$ images. However since DTI is affected by image distortions, this approach would introduce additional errors into the analysis. That is why we preferred manual drawing of the lesions on the $b=0$ images. We believe that this error was minimized due to careful analysis by an almost ten-year experienced radiologist. Second, in this study, we compared diffusion characteristics of tubers and white matter lesions with contralateral normal appearing regions. However it was reported in literature that normal appearing white matter in TS patients might have different diffusion properties than the ones in healthy controls [21,22]. Our data shows that tubers and white matter lesions have significantly different diffusion characteristics than contralateral normal appearing regions. Comparison with healthy controls will be done on a larger population and contrasted with literature in future work. Third, our DTI protocol used 12 diffusion directions. Of note, the tensor calculation is more accurate with more diffusion directions. However, increasing diffusion direction increases scan time, which usually causes other problems such as motion artifacts particularly in scans of pediatric patients. Although more diffusion direction is preferred, we consider that 12 directions were sufficient enough to characterize lesions in TS.

Conclusion

In conclusion, we found significant increase in ADC, AD, RD and significant decrease in FA measures in tubers, higher RD values and lower FA values in white matter lesions in comparison to control regions using DTI. The increase in RD measures was significantly greater than the increase in AD measures. This was attributed to hamartomatous proliferation, depleted and disordered myelin sheaths. DTI can provide important information about the cytoarchitectural changes in TS lesions beyond morphologic MRI findings alone.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Ethical approval: This study was approved by the local ethics committee of our institution.

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