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Cerebrospinal Fluid τ Protein Expression is Correlated with DTI Quantification in Leukoaraiosis Patients

Longjun Guo*, Jiaqi Teng, Juan Wang and Yukun Chen
Beijing Rehabilitation Hospital, Capital Medical University, Beijing, 100144, China

ABSTRACT

This study was aimed to investigate the relationship between diffusion tensor imaging (DTI) quantification and cerebrospinal fluid τ (Tau) protein expression in leukoaraiosis patients. Inpatients who attended the Department of Neurology of our hospital from January 2018 to December 2018 were selected as study subjects, with 58 patients with leukoarais as the experimental group, and 60 patients without leukoarais in the same period as the control group. ELISA was used to detect τ (Tau) protein expression level in the cerebrospinal fluid of the two groups. DTI technique was taken to compare DTI anisotropic diffusion coefficients of the two groups. According to our results there was a statistically significant difference in cerebrospinal fluid Tau protein concentration between the experimental group and the control group ($P<0.05$), and there was no statistically significant difference in CSF IgG index between the experimental group and the control group ($P>0.05$). DTI was used to process diffusion tensor data and visually and clearly display the distribution and metastasis of brain nerve fibers or white matter. In terms of leukoaraiosis degree, FA decreased. Where, DR increased, AMO decreased, Dave and DAx remained unchanged. Most FA reduction observed in white matter was observed in the corpus callosum. In the end we concluded τ protein expression in cerebrospinal fluid can enable identification of the occurrence of LA symptoms to a certain extent. Its combined use with DTI, an important tool for non-invasive analysis of the internal brain structure, is playing an increasingly important role in medical clinical and basic research.

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Authors' Contribution
LG put forward the research experiment. LG and JT analyzed the data and YC helped with the constructive discussion. LG, JT, and JW wrote the manuscript.

Key words
Leukoaraiosis, Diffusion tensor imaging, τ protein, Magnetic resonance imaging, Correlation.

INTRODUCTION

Although leukoaraiosis (LA) was first described by Hachinski 30 years ago, it is still an important and extensive research topic, especially its etiology and clinical relevance. Referring to abnormal neuroimaging of white matter, leukopenia is manifested as low-density or high-intensity areas around the ventricle, which often occurs in the elderly (Yuan *et al*., 2017). The term was originally coined by Hachinski in 1987 (leuko-white matter, araiosis-osteoporosis), which refers to ischemic abnormalities observed in the deep white matter of the brain. The appearance of LA depends on the imaging method used, but it usually manifests as multifocal or diffuse periventricular or subcortical lesions of varying sizes. These lesions have hypoxic-ischemic etiology, which undergo axonal atrophy and subsequent gliosis due to the interaction of many factors (Yuan *et al*., 2017; Lin *et al*., 2017). LA is caused by hypoxia-ischemia which is usually due to small vessel disease (usually the thalamic artery and other perforating arteries). However, there is controversy regarding the causes of such vascular stenosis or occlusion. With age, due to the accumulation of atherosclerotic plaque, amyloid and hyaluronic acid, the arteries have relaxed elasticity, which leads to ischemia and gliosis, and then subsequently neurotransmission disorders (Yuan *et al*., 2017; Mi *et al*., 2017). Hypoxia-ischemia plays a role in the etiology of LA. However, it is uncertain whether it is the primary or secondary cause, namely, whether the blood flow decrease is the cause or an influencing factor of nerve cell damage (Mi *et al*., 2017). Another hypothesis focuses on the destruction of blood-brain barrier and endothelial dysfunction (Yuan *et al*., 2017; Mi *et al*., 2017; Bian *et al*., 2019). Hemodynamic disorders and destruction of blood-brain barrier are considered as elements of endothelial dysfunction (Mi *et al*., 2017). The presence of body fluid proteins such as IgG, τ protein and fibrinogen in LA patients reflects the damage of blood-brain barrier. Therefore, it is believed that brain damage is due to the toxic effects of body fluid proteins on nerve cells (Mi *et al*., 2017). τ protein (Tau protein) as an important microtubule-related protein has a high content in nerve cells, which is mainly concentrated in nerve cell axons in the brain. Under normal physiological conditions, τ protein can promote polymerization to form microtubules after binding with tubulin; after further binding with the formed microtubules, it can maintain stability of the microtubules, reduce dissociation of tubulin molecules, and induce microtubule bundling, thereby maintaining the normal transport of neurotransmitters. If the neuron or its

* Corresponding author: dalong531@126.com
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axon is destroyed, the normal τ protein in the cytoplasm can free to the outside of the cell, thereby increasing τ protein content in the cerebrospinal fluid (CSF). No studies have yet revealed the diagnostic value of CSF τ protein concentration for LA. Diffusion tensor imaging (DTI) is a magnetic resonance imaging (MRI) technique used to measure the level of fluid molecules in biological tissues in water. The useful quantitative parameters provided by DTI include mean diffusivity (MD) which indicates the diffusivity of water molecules in the tissue, as well as fractional anisotropy (FA) that can quantify the degree of anisotropy in the tissue for that specific area. It has been proved that the decrease of FA is related to the increase of radial diffusion coefficient (DR) and average diffusion coefficient (Dave) as well as the decrease of anisotropic mode (AMO). DTI also allows reconstruction of cranial nerve fiber tracts by a method known as tractography. This study detects and investigates the correlation between τ protein expression in CSF and anisotropic diffusion coefficient of DTI in LA patients.

Table I.- Basic demographic information of the study subjects.

Clinical manifestation		Control group (n=60)	LC group (n=58)
Age (years, Mean±SD, range)		53.40±11.73 (40.98-63.06)	53.71±12.08 (41.68-62.11)
Body mass index (Mean ± SD, range)		24.15±1.43 (18.75-37.02)	24.22±1.37 (18.55-36.12)
Gender	Male (n, %)	41 (68.33)	42 (72.41)
	Female (n, %)	19 (31.67)	16 (27.59)
Disease status	Hypertension (n, %)	35 (58.33)	38 (65.52)
	Cerebral hemorrhage (n, %)	8 (13.33)	6 (10.34)
	Stroke (n, %)	2 (3.33)	2 (3.45)

MATERIALS AND METHODS

General information

Inpatients who attended the Department of Neurology of our hospital from January 2018 to December 2018 were selected as study subjects, with 58 patients with leukoarais as the experimental group, and 60 patients without leukoarais in the same period as the control group. The basic demographic information of the two groups is shown in Table I. There are no significant differences in age, body mass index, disease status, *etc*. between the two groups (P>0.05), and the test results are comparable. The study was approved by the ethics committee and institutional review committee of our hospital, and all patients or their families signed the informed consent form for the trial.

Diffusion weighted magnetic resonance imaging

Brain images of the LA patients were collected on the 11.1 Telsa Magnex Scientific MR scanner (Agilent 205/120HD with gradient setting: inner diameter gradient 120 mm, maximum gradient intensity 600 mT/m, and rise time 130 ms), under control by Agilent Technologies VnmrJ 3.1 control software. The internal 2.5cm×3.5cm orthogonal surface transmission and/or receiving coil was tuned to 470.7 MHz (^{1}H resonance) for B_1 excitation and signal detection (AMRIS Facility, Gainesville, FL, USA). First, anesthesia was induced at a concentration of 2.0%-2.5% isoflurane (0.1mL/min), delivered in 100% oxygen for 30-60 sec, and then the level was maintained between 1.0%-1.75% during the entire setup and imaging period to maintain a stable breathing rate. The patient took a prone position with a breathing pad placed under the abdomen. The breathing rate continuously monitored by adjusting the isoflurane level and kept it between 30-40 beats per minute. The core body temperature was maintained at 37°C using warm water recirculation system (SA Instruments, Inc., NY, USA).

Anatomical scans were collected using a 2D gradient echo sequence with the following parameters: 12 axial slices in the 256^2 plane, a field of view of 19.2 mm^2×0.75 mm, a flip angle of 20°, an average of 6, an echo time of 3.8ms, and repetition time of 100ms. 8 spin echo planar imaging (EPI) sequence was used to obtain diffusion weighted scan with repetition time of 2500 ms and echo time of 24ms. Localized whole brain voxel shimming (with full width at half maxima in the range from 30 Hz to 70 Hz) was completed, and the gradient delay before each acquisition was optimized. Additional reference data to correct the distortion in the EPI image reconstruction process was collected. Following acquisition parameters were used: 12 axial slices in the 256^2 plane, field of view of 19.2mm^2×0.75mm (planar resolution 150 mm^2), gradient amplitude of 29.7 gauss/cm, and RF pulse duration (δ) for 4 milliseconds (90° sinusoidal excitation and 180° refocusing pulse), gradient duration (Δ) for 10 milliseconds, maximum b value at 900 seconds/mm^2, 42-direction icosahedral sampling scheme (sampling on a hemisphere) and 6 B0 images. The total scan time of 3 measurements for each patient is 1 h and 40 min. A control study was conducted with an aqueous phantom containing 1M mannitol and 0.1M creatine to check the level of EPI ghosting and distortion, motion and eddy current artifacts. No directivity or high diffusion anisotropy was noticed in the phantom, and the sample phantom area of interest had a very low FA value (Zhao *et al*., 2018).

Tensor fitting and anisotropy index calculation

The first B0 image using affine registration was scanned and corrected. B0 image to manually generate each mask for removal of non-brain voxels was used. The weighted least squares fitting regression equation on the "dtifit" program on the FMRIB software library version 5.0 was used to perform tensor element reconstruction and the first, second and third eigenvector and eigenvalue estimation (λ1, λ2, λ3, Where λ1 is considered as the axial diffusivity D_{Ax}). In tensor element output of the FMRIB software library, Voxel-wise calculation of the additional diffusion anisotropy index and diffusion morphology measurement were performed. The specific indexes were D_{ave}, DR (λ2+λ3/2), FA index and A_{MO} (Shi *et al*., 2018).

Region of interest (ROI)

ROI-based analysis was done which was based on comparative analysis of the patient brain atlas on the manually plotted color-coded FA map (generated using the first feature vector), including: anterior commissure, corpus callosum, geniculate body (genu), splenium, striatum, frontal cortex, whole hippocampus, amygdala, internal capsule and substantia nigra. ROI values of FA, A_{MO}, D_{Ax}, D_{ave} and D_R are output in the form of a spreadsheet, and ANOVA is used for statistical analysis (Chen *et al*., 2018).

Detection of τ protein concentration in cerebrospinal fluid by double antibody sandwich ELISA

τ protein was prepareed by homogenizing the CSF in an extraction buffer containing 10 mM Tris-HCl (pH 7.5), 0.8 M NaCl, 10% sucrose, 1 mM EGTA, 2% SDS and incubated at 37°C for 30 min. After centrifugation at 20,000 g at 25°C for 10 min, the supernatant was taken, transferred to a 1.5 mL test tube, and centrifuged at 100,000 g at 25°C for 20 min. The sediment was washed by ultracentrifugation using 0.5 mL sterile saline and dissolved in the buffer. τ protein concentration was detected using double antibody sandwich ELISA test kit according to the instruction manual (Chen *et al*., 2018).

Statistical analysis

The data in this study were all processed by SPSS20.0 statistical analysis software (IBM, USA); Microsoft excel was used to generate graphs, tables, *etc*. Measurement data are expressed by mean ± standard deviation (mean±SD), one-way analysis of variance or repeated measurement analysis of variance was used for comparison between groups, LSD-t test was used for pairwise comparison between groups, and one-way analysis of variance was used for comparison among multiple groups. Logistic regression was used to calculate the regression equation and analyze the relationship between parameters like DIT quantitation and CSF τ protein expression in LA patients. χ^2 analysis was used for comparison between groups. $P<0.05$ indicates statistically significant difference.

RESULTS

Diffusion tensor imaging measurement

DT-MRI anisotropy index of the brains of the two groups was detected. The final FA result is shown in Figure 1. Compared with the control group, the experimental group has lower FA contrast and smaller overall brain size. Compared with the control group, the experimental group shows decreased FA contrast, a factor in relation to LA degree, which is observed in the anterior commissure (ANOVA analysis, P <0.05; tension: $F_{1,23} = 12.8$, $P= 0.002$; LA degree: $F_{2,23} = 4.6$, $P = 0.02$), CC (tension: $F_{1,23} = 24.6$, $P<0.0001$; LA degree: $F_{2,23} = 11.6$, $P= 0.0003$), IC (tension: $F_{1,23} = 9.4$, $P= 0.005$; LA degree: $F_{2,23} = 5.3$, $P= 0.01$), sCC (tension: $F_{1,23} = 4.2$, $P= 0.05$; LA degree: $F_{2,23} = 5.4$, $P= 0.01$) and Fmb (tension: $F_{1,23} = 10.9$, $P= 0.003$; LA degree: $F_{2,23}=1.8$, $P=0.2$). A_{MO} values of most WM and GM regions in the control and experimental groups range from 0.5 to close to 1. In fact, A_{MO} values are lower in the experimental group than in the control group (ANOVA analysis: $F_{1,23} = 7.9$, $P = 0.009$). The experimental group shows significant changes in A_{MO} values in 3 areas, with A_{MO} increased in one area (CTX) and decreased in the remaining 2 areas. By summarizing the DTI findings, our results show decreased FA, an indicator of LA degree. Where, D_R increases, A_{MO} decreases, and D_{ave} and D_{Ax} remain unchanged. Most FA reduction observed in white matter was observed in the corpus callosum (Okamura *et al*., 2018).

Cerebrospinal fluid τ protein expression level

CSF of the two groups was acquired by lumbar puncture, and CSF τ protein was detected by double antibody sandwich ELISA. There are statistical differences between the experimental group and the control group in the CSF τ protein concentration and CSF IgG index ($P<0.05$). In the experimental group, the correlation coefficient between CSF τ protein concentration and CSF IgG index is 0.32, showing no statistically significant difference (p>0.05) (Soumaya *et al*., 2018) (Table II).

Table II.- τ protein expression levels of the two groups.

Group	τ protein expression level	IgG index
Experimental group (n=58)	253.71±12.08	2.40±0.73
Control group (n=60)	224.22±5.37	2.15±0.43
t value	18.638	5.786
P value	0.001	0.032

DISCUSSION

The term LA (LA) was coined by Hachinski to describe the symmetrical patchy white matter hypodensity (CT scan) or hyperintensity (T2-weighted MRI) of the hemispheric white matter common in the elderly. White matter is more susceptible to ischemic damage than gray matter, firstly because the anastomotic substance protects the gray matter from infarction when penetrating blood vessel blocks abundant meninges. DTI is a relatively new technique that can provide quantitative information about white matter damage (Fernández *et al*., 2016). A further development of DWI is to measure diffusion in at least six non-collinear directions to provide a three-dimensional representation of water movement (Sullivan *et al*., 2014). In parallel fiber bundles, diffusion occurs preferentially along the fiber direction. Directionality of this diffusion can be quantified as FA, which varies from 0 (diffusion in all directions) to 1 (diffusion along a single axis). In normal white matter, highly oriented white matter bundles result in highly directional diffusion and therefore high FA. Since axon membrane and myelin hinder diffusion, damage to these structures results in increased average diffusivity. In LA, the loss of these ordered axon bundles and the concomitant non-directional gliosis results in a significant loss of FA (Müller *et al*., 2019; Hansen *et al*., 2020).

In acute ischemia, the apparent diffusion coefficient (ADC) value drops rapidly by 30-40%, which is an indicator of how easily water molecules diffuse in the tissue. In the ADC graph, tissues characterized by faster diffusion exhibit high signal, while the tissues with limited diffusion (usually in the case of acute cerebral ischemia) exhibit low signal (Boukrina *et al*., 2020). The signal is true in the diffusion weighted imaging (DWI). On the other hand, elevated CSF τ protein level is not unique to patients with Alzheimer's disease or senile dementia. In this study, ELISA method was used to detect CSF τ protein level of the two groups. Compared with normal patients, τ protein concentration value was significantly increased in the case of LA (Ammirati *et al*., 2019; Ryu *et al*., 2019). The research results found that LA patients might have higher CSF τ protein level than AD patients, indicating that the pathogenesis of LA is not simply caused by the release of τ protein into the CSF after the destruction of nerve cells. It is possible that there is a mutation of τ protein gene in patients with the disease, which leads to abnormally increased τ protein phosphorylation sites. It can be seen that for LA patients, determination of CSF τ protein concentration carries high clinical significance. There was no significant correlation between CSF τ protein concentration and CSF IgG index in the two groups. This result can be explained by at least two points: on the one hand, when neurons or axons are degenerated or damaged, τ protein in the cells is abnormally released into CSF, leading to increased protein concentration. The manifestation of nerve cell damage can vary depending on the location and degree. Intellectual impairment is only one manifestation. That is to say, even patients who do not show intellectual impairment may also have severe nerve cell damage, so τ protein level increases. On the other hand, as mentioned above, IgG sensitivity is not high. The intellectual impairment reflected by this scale is often serious end-stage damage, but due to activity of the pathogen, nerve cells may suffer from more serious damage in the early stage than in the terminal stage, thus releasing more τ protein compared to the terminal stage (Xie *et al*., 2018).

In summary, CSF τ protein expression can enable identification of the occurrence of LA symptoms to a certain extent, and DTI, as an important tool for non-invasive analysis of the internal brain structure, is playing an increasingly important role in medical clinical and basic research. DTI processes the diffusion tensor data and visually and clearly displays the distribution and metastasis of brain nerve fibers or white matter. The combination of the two carries important clinical significance (Shi *et al*., 2020).

CONCLUSION

τ Protein expression in CSF can cause the presence of LA symptoms to be detected to a certain degree. The integrated use of DTI, which is an effective instrument for non-invasive study of the internal anatomy of the brain, plays an increasingly important role in clinical and fundamental medical research.

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Statement of conflict of interest

The authors have declared no conflict of interests.

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