

The correlation between CT findings of diffuse axonal injury and the expression of neuronal aquaporin in patients with craniocerebral injury

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Abstract. – OBJECTIVE: The paper aimed at exploring the correlation between CT findings of diffuse axonal injury and the expression of neuronal aquaporin in patients with craniocerebral injury.

PATIENTS AND METHODS: 150 patients with diffuse axonal injury diagnosed by CT and 50 healthy physical examiners were selected as the study objects. According to the craniocerebral CT and GCS scale scores, the patients were divided into DAI light group, medium group, and heavy group. The general conditions of patients were observed and recorded, and the brain pathological morphology, craniocerebral edema and CT imaging results of the patients in each group were compared. Changes in serum and brain AQP-4 levels were detected by RT-PCR and Western blot, and the correlation between CT manifestations of DAI and the expression of neuronal aquaporin was investigated.

RESULTS: The results of DAI's pathological morphology, cerebral edema and CT imaging showed that the brain tissue of each group of DAI had a certain degree of injury. With the increase of the injury degree, the degree of edema and the number of axonal injuries sharply increased, and the difference was significant (p -value < 0.05). Therefore, CT could be used as an effective basis for the rapid and efficient diagnosis of DAI. RT-PCR, Western blot and Spearman correlation analysis showed that the levels of AQP-4 in the serum and brain tissue of DAI patients were significantly increased. With the increase of the degree of diffuse axonal injury, the expression level of AQP-4 was further increased, and the difference was significant (p -value < 0.05). The CT manifestations of patients in each group were positively correlated with the expression level of AQP-4 protein.

CONCLUSIONS: AQP-4 can be used as an important molecular index to judge the condition and prognosis of DAI, providing a new non-invasive detection method for the clinical diagnosis and treatment of DAI, which has high clinical application value.

Key Words:

Diffuse axonal injury, Computed tomography (CT), Aquaporin.

Introduction

Diffuse axonal injury (DAI) refers to a primary brain parenchyma injury distributed diffusely with axonal injury as the main change caused by head trauma^{1,2}. DAI, as a common and frequently-occurring disease in neurosurgery, poses a serious threat to the health and life safety of patients^{3,4}. Computed tomography (CT) has long been the primary examination method for the diagnosis of DAI due to its fast imaging speed and good fracture signal display, which plays a positive role in the early diagnosis and treatment of DAI, as well as in improving the curative effect and prognosis of patients^{5,6}.

Aquaporin (AQP) is widely distributed on cell membrane and plays an important regulatory role in cell water metabolism. It is a group of cell membrane transporters closely related to water permeability⁷⁻¹¹. Studies¹⁰ have shown that AQP-1, AQP-4 and AQP-9 are abundantly present in the brain tissue and play an important role in the generation of cerebrospinal fluid. In recent years, literature has confirmed that AQP-4 is highly correlated with craniocerebral injury¹¹, but the correlation between the CT findings of DAI and the expression of neuronal aquaporin in patients has been little investigated. On this basis, we studied the CT manifestations and neuronal aquaporin expression levels in patients with DAI after craniocerebral injury and analyzed the correlation between the two.

Patients and Methods

General Information

A total of 150 patients who visited the Department of Neurosurgery of our hospital from January 2019 to December 2019 for traumatic brain injury were selected as the research subjects. After CT examination and clinical diagnosis, all the

patients were diagnosed with diffuse axonal injury. Clinical manifestations included nausea, vomiting, headache and coma to different degrees.

Patients with DAI were graded according to the craniocerebral CT and GCS (Glasgow Coma) scale to determine the degree of head injury, and were divided into three groups: mild, medium and severe. In the mild group, GCS score was 13-15, and there was no significant abnormality in cerebral CT. In the medium-sized group, GCS score was 9-12, and cerebral CT showed partial hematoma and hemorrhage, with the midline structure showing a shift of < 3 mm. In the severe group, GCS score was 3-8, and cerebral CT showed intracranial disseminated hemorrhage and subarachnoid hemorrhage, blocked or narrowed infarction of the cerebral pool, and shift of the midline structure more than 3 mm. Fifty healthy people who were admitted to hospital at the same time were selected as the control group. General indicators, such as age, gender, and the first CT time of the research subjects were recorded, and the differences between groups were compared and excluded.

Inclusion Criteria

Inclusion criteria for patients with DAI: 1) diagnosis was confirmed through head CT and clinical diagnosis; 2) Patients with GCS score > 3 on admission, showing different degrees of language and cognitive impairment, and the cause of cerebral craniocerebral injury was clear; 3) Surgical treatment with craniotomy; 4) those accompanied with clinical symptoms such as nausea, vomiting, headache and coma to different degrees.

Exclusion Criteria

Exclusion criteria for patients with DAI: 1) patients who deteriorated rapidly and died within a short time; 2) patients with open craniocerebral injury; 3) patients with missing or incomplete clinical information; 4) Patients requiring immediate surgery for the first CT examination; 5) Patients with mental system diseases such as depression, mania, and schizophrenia; 6) Heart disease, diabetes, liver and renal insufficiency, etc.; 7) Patients with malignant tumor; 8) Patients with autoimmune diseases; 9) Pet-name ruby with past history of brain injury, epilepsy and other mental diseases.

Ct Examination Methods

All patients underwent CT scans using GE Highspeed dual-slice spiral CT machine in the United States within 24 h of head injury. The pa-

tient was in the supine position with the neck in the middle of flexion and extension (the plane of eyes and ears was perpendicular to the ground). The family members cooperated with the patient to keep the head in a static state as far as possible to reduce the generation of motion artifacts. The transverse continuous scanning was performed under the following scanning parameters: voltage of 120 kv, current of 150 mA, field of view of 25 cm, matrix of 512×512 , layer thickness of 10 mm and layer spacing of 10 mm. The baseline of the scan was parallel to OML (the line connecting external eye and the ipsilateral external auditory meatus). The continuous gapless scanning was performed from the skull base to the vertex, and a thin-layer scan with a layer thickness of 5 mm was performed at the position of suspected lesion.

Area of Craniocerebral Edema

The cerebral edema was assessed based on the edema area in the CT image of the patient's brain, where the edema area was calculated as the largest edema slice in the CT image. The formulae were as follows: area of encephaledema zone = (long diameter of edema zone * long diameter of edema zone) - (long diameter in edema zone * short diameter in edema zone).

Glass Nerve Fiber and Axonal Staining

In the process of craniotomy, brain tissues 1-2 cm around the edema or contusion foci in three groups were taken, and relatively normal brain tissues at the remote cerebral hematoma and contusion parts were taken as the control group. The specimens were fixed with 20% paraformaldehyde for 24 h, immersed in gradient ethanol, embedded in paraffin, and planar brain tissue sectioned, and then baked at 60°C for 25 min. After dewaxing, hydration and soaking in distilled water, the samples were immersed in 20% of freshly prepared silver nitrate for 30 min and reduced in 10% of formalin for three times. Subsequently, the samples were immersed in freshly prepared Glass amine silver solution for 30 s, reduced with 10% of formalin for 3 times, washed repeatedly with distilled water for 2 times, fixed with sodium thiosulfate (5%) for 2 min, and washed with water. The sections were immersed in gradient alcohol for dehydration, transparentized in xylene solution, and finally sealed with neutral gum to dry. The sealing piece was placed under a microscope to observe the pathological changes of brain tissue and record the number of axonal injuries in the field of view.

Effect of Detection of Aqp-4 Gene Expression in Serum of Patients With RT-PCR

Fasting venous blood (within 24 h, 3 d and 7 d after craniocerebral injury) was collected from the control group and the three DAI patient groups, and serum AQP-4 levels were detected by polymerase chain reaction (RT-PCR). The PCR amplification conditions were as follows: pre-denaturation at 94°C for 2.5 min, denaturation at 93°C for 30 s, annealing at 60°C for 25 s, extension at 70°C for 30 s, 35 cycles, and finally extension at 71°C for 60 s. Then, 10 µL of amplification products and 6 µL of Maker were simultaneously loaded, separated by 3.0% agarose gel electrophoresis (120 V, 40 min), stained with silver, and analyzed by UV gel imaging system and photographed.

Change of AQP-4 Protein Expression Level Detected by Western Blot

A certain concentration of SDS-PAGE electrophoresis gel was prepared and placed on the electrophoresis tank. The proteins (CREB and pCREB) prepared by the cells under each group of conditions were loaded and electrophoresis was conducted. After electrophoresis, the samples were transferred to PVDF membrane, blocked, and washed with TBST. Diluted protein antibodies (primary antibodies) were added respectively, and the samples were washed at 4°C overnight with TBST four times (5 min each time). HRP-labeled secondary antibody was then added, incubated at 37°C for 2 h, and washed with TBST for 4 times *5 min. The negative control group was established with GAPDH monoclonal antibody as primary antibody and HRP-labeled IgG as secondary antibody.

Statistical Analysis

SPSS 20.0 statistical analysis software (IBM Corp., Armonk, NY, USA) was used for data processing. Statistical results were expressed as

“mean standard deviation”; One-way analysis of variance was used for mean comparison among multiple samples, and the least significant difference LSD-*t* test was used for pairwise comparison of mean between groups. *p*-value <0.05 indicated that the difference had statistical significance

Results

General Conditions of Patients

The basic conditions of the DAI patients in the control group and the three groups are shown in Table I. As shown in the table, there were no significant differences in age, the ratio of men to women and the first CT time after craniocerebral injury among all groups (*p*-value > 0.05). The interference of the above factors on the test results could be ruled out, and the data were comparable (Table I).

Pathological Changes of Brain Tissue in Each Group

The pathomorphological changes of diffuse axonal injury in patients of each group were shown in Table II. Microscopic observation showed that the brain tissue structure of the control group was complete, and the nerve cells were arranged neatly, with abundant cytoplasm. The intercellular space was normal, and there was no axonal injury. In the DAI mild group, the brain tissue was loose, and the clear space appeared around the neuron cells, which was slightly edematous. The number of axonal injuries was significantly different from that in the control group (*p*-value < 0.05). In the DAI medium-sized group, there were edema in neurons, nuclear concentration, edema and aggravation of axonal injury (*p*-value < 0.05). In the DAI severe group, the lesions were more obvious, the blood vessels and the pericellular space were further enlarged, and the vacuoles were evident. The edema was more serious, and the number of axonal injuries was the largest (Table II).

Table I. General information of patients.

Group	Number of cases	Age (years)	Male/female kg/m ²	First CT time after craniocerebral injury (h)
Control group	50	45.27±7.46	38/12	16.44±1.27
DAI light group	50	46.42±8.25	39/11	17.01±2.24
DAI medium group	50	46.57±7.06	39/11	16.25±1.85
DAI heavy group	50	45.91±7.34	41/9	16.22±2.21
Variance ratio	-	0.43	0.28	0.25
<i>p</i> -value	-	>0.05	>0.05	>0.05

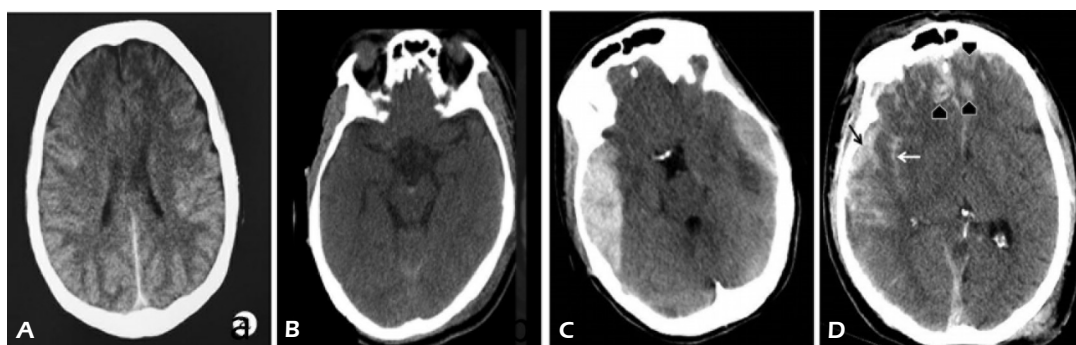


Figure 1. Craniocerebral edema of patients in each group (A) healthy control group; (B) DAI light group; (C) DAI medium group; (D) DAI heavy section.

Comparison of Intracranial Edema in Patients

The encephaledema of patients with DAI in the control group and each group are compared in Figure 1. As shown in Figure 1, T brain CT scan of healthy control group showed no edema. After craniocerebral injury, craniocerebral edema appeared in every DAI group. With the increase of the degree of diffuse axonal injury, the edema area was significantly larger than that in the mild group, and the difference between groups was significant (p -value < 0.05). The results showed that with the increase of the degree of diffuse axonal injury, the craniocerebral edema in our patient became more serious.

CT Manifestations of Diffuse Axonal Injury After Craniocerebral Injury

Typical CT findings of diffuse axonal injury after craniocerebral injury are shown in Figure 2 and 3. The lesion was nodular, oval or speckled

in shape, with high density on CT images, clear boundary and no obvious space-occupying effect. Some patients with intracranial injury presented with narrowing of brain fission, smaller brain pool, subarachnoid hemorrhage, subdural hematoma, etc.

Changes of Serum AQP-4 Gene Expression Level in Each Group

The primer designs of AQP-4 and GAPDH are shown in Table III. The serum AQP-4 levels in the control group and DAI patients in each group are shown in Table IV. As shown in Table IV, AQP-4 levels in the control group were stable at each time point. Serum AQP-4 levels in all DAI patient groups reached the peak on day 3 after operation and decreased on day 7 after operation. Serum AQP-4 levels of patients with craniocerebral injury in all groups were significantly different within 24 h, on days 3 and 7 (p -value < 0.05). Compared with the healthy control group, the serum AQP-4

Table II. Pathological changes of diffuse axonal injury in each group.

Group	Number of cases	Axle damage number
Control group	50	0
DAI light group	50	8±2
DAI medium group	50	28±7
DAI heavy group	50	51±12
Variance ratio	-	5.49
p -value	-	< 0.05

Table III. Primer sequence design during PCR.

Primer name	Leading	Back lead	Length (bp)
AQP-4	5'-GGAAGCGATCAACTAACATG-3'	5'-GAGCCTTGATCTCGTTACAG-3'	124
GAPDH	5'-CAGCATTGGAAGTGCTATGG-3'	5'-TAGAGCGAGTATGCATGACA-3'	128

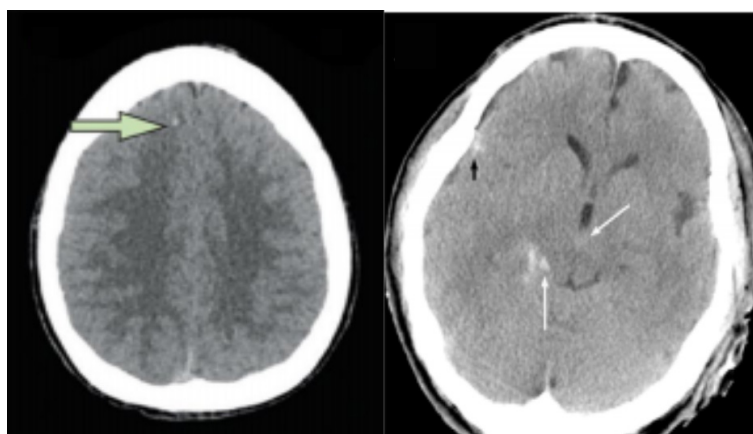


Figure 2. Typical CT image of diffuse axonal injury: oval or speckled lesion with no evident mass.

levels in the DAI patient group were significantly increased at each time point, and the differences were significant (p -value < 0.05). Moreover, with the increase of the diffuse axonal injury, the increase of AQP-4 was more significant. The results showed that cerebral diffuse axonal injury had the function of promoting the release of neuronal aquaporin AQP-4, which further improved the permeability of affected brain tissue and aggravated the edema and damage degree of brain tissue.

Changes of AQP-4 Protein Expression in Brain Tissue of Patients in Each Group

The expression levels of AQP-4 protein in the brain tissues of the control group and DAI patients in each group are shown in Table V. Compared with the control group, the AQP-4 level in the brain tissue of each DAI patient group was significantly increased, and the difference was statistically significant (p -value < 0.05). With the

increase of the degree of diffuse axonal injury, the expression level of AQP-4 protein was further increased, and the difference was significant (p -value < 0.05).

Correlation Between Ct Findings of Patients In Each Group and AQP-4 Protein Expression

Spearman correlation analysis showed that there was a significant positive correlation between CT findings of patients in each group and AQP-4 protein expression level ($r=0.78$, p -value < 0.05). These results suggested that AQP-4 protein could be used as a molecular marker for judging diffuse axonal injury in brain.

Discussion

Diffuse axonal injury (DAI) is a primary diffuse brain injury characterized by axonal fracture

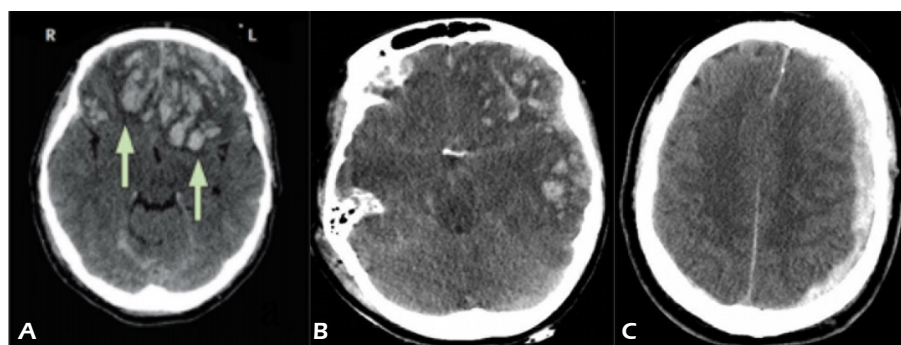


Figure 3. Typical CT image of diffuse axonal injury. **A**, contusion brain injury in frontal and temporal lobe region; **B**, Substantive brain contusion, high-density lesion, external model hematoma; **C**, Polar subdural hematoma, crescentic high-density lesion between dura mater and arachnoid.

Table IV. Detection of AQP-4 gene expression by RT-PCR.

Group	Within 24h (gray value)	3d after surgery (gray value)	7d after surgery (gray value)
Control group	0.13±0.02	0.12±0.03	0.13±0.01
DAI light group	0.42±0.03	0.55±0.50	0.19±0.02
DAI medium group	0.58±0.03	0.61±0.32	0.41±0.02
DAI heavy group	0.64±0.04	0.89±0.32	0.528±0.03
Variance ratio	6.37	8.76	7.76
<i>p</i> -value	<0.05	<0.05	<0.05

after external force applied to the head^{12,13}. At present, the assessment of the condition and prognosis of DAI mainly depends on medical effects, clinical symptoms and vital signs. CT can clearly display the cerebral edema and hemorrhagic focus in DAI, but it is not sensitive to non-hemorrhagic small lesions with a diameter shorter than 2 cm, which cannot accurately reflect the degree of the lesion^{14,15}. Neuronal aquaporin AQP-4 is mainly distributed around the blood vessels of the blood-brain barrier and plays an important regulatory role in the transport of liquid in the central nervous system. At the same time, it is also involved in the regulation of plasma colloid osmotic pressure¹⁶⁻¹⁸. Under normal physiological conditions, the level of AQP-4 in brain cells is low¹⁹. When nerve cells are damaged, AQP-4 can enter cerebrospinal fluid through the blood-brain barrier and plays an important role in the occurrence and development of diffuse axonal head injury²⁰.

At present, on the correlation between the CT findings of DAI and the expression of neuronal aquaporin in patients^{21,22}. On account of this, we selected 150 patients diagnosed with diffuse axonal injury by CT examination and clinical diagnosis as the research subjects. According to the craniocerebral CT and GCS scale scores, the patients were divided into three groups of DAI (light, medium and heavy), and 50 healthy subjects who were admitted for physical examination

at the same time as the control group. There were no significant differences in age, gender or the first CT time after craniocerebral injury among the four groups, and the data were comparable. The pathomorphological and craniocerebral edema results of diffuse axonal injury showed that the brain tissues of DAI groups were loose and edematous, and the axons were damaged to a certain extent. Along with the increase of the injury degree, the edema degree and the number of axonal injuries increased sharply with significant differences (*p*-value < 0.05). The results of cerebral CT images showed that typical DAI lesions were nodular, oval or speckled, with high density and no obvious space-occupying effect. Some patients with concomitant intracranial injury presented with narrowing of brain fission, smaller brain pool, subdural hematoma, which was consistent with the clinical diagnosis of patients and could be used as an effective basis for the rapid and efficient diagnosis of DAI. To further explore the correlation between the CT findings of diffuse axonal injury and the expression of neuronal aquaporin in our patient, we detected the changes of AQP-4 levels in serum and brain tissue of our patient using RT-PCR and Western blot. The results of RT-PCR and Western blot analysis showed that, compared with the healthy control group, the AQP-4 levels in serum and brain tissues of patients with DAI had significantly increased and the differences were

Table V. DAQP-4 protein expression.

Group	Number of cases	AQP-4 (gray value)
Control group	50	0.15±0.02
DAI light group	50	0.46±0.03
DAI medium group	50	0.62±0.03
DAI heavy group	50	0.74±0.03
Variance ratio	-	8.31
<i>p</i> -value	-	<0.05

statistically significant (p -value < 0.05). With the increase of the degree of diffuse axonal injury, the expression of AQP-4 had further increased, and the difference was significant (p -value < 0.05). The results showed that cerebral diffuse axonal injury had the function of promoting the release of neuronal aquaporin AQP-4, which further improved the permeability of affected brain tissue and aggravated the edema and damage degree of brain tissue. Spearman correlation analysis showed that there was a significant positive correlation between CT findings of patients in each group and AQP-4 protein expression, suggesting that AQP-4 protein could be used as a molecular marker for judging diffuse axonal injury in brain.

Conclusions

In summary, brain CT images could be used as an effective basis for the rapid and efficient diagnosis of DAI. The expression level of neuronal aquaporin AQP-4 in patients has a positive correlation with the CT findings of DAI, indicating that AQP-4 can be used as an important indicator to determine the condition and prognosis of DAI, which provides a new non-invasive test for the clinical diagnosis and treatment of DAI, with high clinical application value.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Informed Consent

Subjects followed the principle of voluntary participation and signed a study consent form.

Ethics Approval

The study was approved by the Ethics Committee of our Hengshui People's Hospital. The privacy and safety of subjects were adequately protected in accordance with clinical study guidelines.

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