High fibrosis indices in cerebrospinal fluid of patients with shunt-dependent post-traumatic chronic hydrocephalus

Abstract

Objective: A possible relationship between fibrosis along the route of cerebrospinal fluid (CSF) flow and the subsequent development of hydrocephalus has been indicated in previous studies. These changes in the fibrosis index may reflect the severity of hydrocephalus and could potentially become a diagnostic tool. The object of this study was to analyze the levels of procollagen type I C-terminal propeptide (PICP), procollagen type III N-terminal propeptide (PIIINP), hyaluronic acid (HA), and laminin (LN) in the CSF of patients with post-traumatic hydrocephalus and determine the significance of their presence. Subjects and methods: Forty-four patients were included in the study: 24 patients with shunt-dependent post-traumatic hydrocephalus (group A - hydrocephalus group); ten brain trauma patients without any sign of hydrocephalus (group B - trauma group); ten patients without brain trauma and hydrocephalus (group C - normal control group). CSF levels of PICP, PIIINP, HA, LN and transforming growth factor-β1 (TGF-β1) were detected using enzyme-linked immunosorbent assay (ELISA). Results: Levels of PICP, PIIINP, HA, and LN in the group of hydrocephalus patients were significantly higher than those in the post-trauma patients without hydrocephalus (p < 0.05) and normal control patients (p < 0.05). Moreover, the increased levels of PICP, PIIINP, HA, and LN were positively correlated with the level of TGF-β1 (p < 0.05). Conclusion: We demonstrated an increase of fibrosis factors including PICP, PIIINP, HA, and LN, that was positively correlated with TGF-β1 levels. This indicates an important role for the process of fibrosis in the development of post-traumatic chronic hydrocephalus and shows the potential utility of PICP, PIIINP, HA, and LN as a diagnostic index in shunt-dependent post-traumatic chronic hydrocephalus.

Keywords

• Fibrosis • Hyaluronic acid • Hydrocephalus • Laminin • Procollagen • TGF-β1

Introduction

Chronic hydrocephalus is a common complication after craniocerebral trauma. It is characterized by abnormalities in the circulation and absorption of cerebrospinal fluid (CSF) that result in ventricular dilatation. Most patients with malabsorption of CSF will become shunt-dependent. Previous research has implicated the malabsorption of CSF, caused by fibrosis along the route of CSF flow, in the pathogenesis of hydrocephalus [8, 9, 10, 18, 20, 34-36]. PICP, PIIINP, HA, and LN are widely recognized as biomarkers of fibrotic disease and elevated levels of these substances have been detected in the CSF of hydrocephalus animal models [11,12, 39, 42]. However, the role that fibrosis of the CSF circulation pathway plays in patients with post-traumatic hydrocephalus is still unclear. Our goal in the present study was to determine the fibrosis index in the CSF of patients with shunt-dependent post-traumatic hydrocephalus in order to further understanding of the pathophysiological mechanisms involved in hydrocephalus, and to promote the development of better therapies and diagnoses.

Methods

Patient selection

The patients were divided into 3 groups: group A (hydrocephalus group) consisted of 24 patients with shunt-dependent post-traumatic chronic hydrocephalus; group B (trauma group) consisted of 10 brain trauma patients without any sign of hydrocephalus determined by clinical exam and imaging; group C (normal control group) consisted of 10 patients without any trauma or hydrocephalus. Exclusion criteria included the following: congenital hydrocephalus, post intracranial infection hydrocephalus, tumor obstructive hydrocephalus and other causes of hydrocephalus. Ethics approval and informed consent were obtained for this study from Anhui Medical University and from either patients or their relatives. All procedures performed were in accordance with the ethical standards of national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The diagnosis of hydrocephalus was made by neurosurgeons and neuroradiologists.

Sample collection and detection

CSF samples from the hydrocephalus group were collected during shunt surgery; trauma group samples were collected during brain trauma surgery; normal control groups samples were collected in epilepsy and trigeminal neuralgia surgeries. All samples were stored

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at -70°C for the ELISA test. For the quantitative detection of PICP, PIIINP, HA, LN and TGF-β1 in serum and CSF, a commercial ELISA was used (Wuhan Boster Biological Technology Ltd., Wuhan, P.R. China) according to the manufacturer’s manual. In short, microtiter plates pre-coated with PICP, PIIINP, HA, LN, or TGF-β1 antibodies were filled with standard and undiluted samples and incubated for 2 hours at 37°C. After removal of both the standard and undiluted samples, we added a biotin-conjugated polyclonal antibody preparation specific to PICP, PIIINP, HA, LN and TGF-β1, and incubated the plates for 1 hour. Following the washing cycles, the plate was incubated with avidin conjugated to horseradish peroxidase for another hour at 37°C. After additional washing cycles, 3,3',5,5'-tetramethylbenzidine was added and the plates were incubated at 37°C for 30 minutes. The enzyme-substrate reaction was stopped by H2SO4. The optical density was measured immediately at 450 nm. According to the manufacturer’s specifications, no significant cross-reactivity or interference was observed.

**Statistical analysis**

Data were generally expressed as mean ± standard deviation (SD) values. Groups of data were compared by standard t-test. Comparisons were made between the hydrocephalus, trauma and normal control groups. The relationships between PICP, PIIINP, HA, LN and TGF-β1 levels were determined by Pearson correlation analysis. A difference between means was considered significant at p < 0.05.

**Results**

**Clinical states**

General information about the patients in all 3 groups is shown in Table 1. The clinical profiles of patients in the hydrocephalus group is shown in Table 2. The etiologies of primary brain trauma included SAH, intracerebral hematoma and slight ventricular hemorrhage. All patients were followed up for a period of at least 3 months. The Glasgow Coma Scale (GCS) of patients in the presence of brain trauma was 7.9 ± 2.8. Hydrocephalus occurred between the first and the tenth month post-trauma with the majority of cases developing in the second or third month. Once the symptom of hydrocephalus occurred, it progressed rapidly and become surgery dependent shortly thereafter. All patients’ symptoms were ameliorated following shunt surgery.

**Discussion**

According to the classical hypothesis of CSF hydrodynamics, CSF is produced within the brain ventricles, circulates toward the cortical subarachnoid space, and is finally absorbed.

| Group   | N  | Age (years) | Gender (M/F) |
|---------|----|-------------|---------------|
| Group A | 24 | 48.8 ± 11.4 | 75%           |
| Group B | 10 | 43.5 ± 17.1 | 70%           |
| Group C | 10 | 52.6 ± 9.9  | 60%           |

| Patient number | Gender (M/F) | Age (years) | Duration of shunting (weeks) | GCS |
|----------------|--------------|-------------|-----------------------------|-----|
| 1              | F            | 23          | 8                           | 7   |
| 2              | M            | 52          | 12                          | 14  |
| 3              | M            | 47          | 12                          | 6   |
| 4              | M            | 59          | 12                          | 5   |
| 5              | M            | 39          | 12                          | 6   |
| 6              | M            | 47          | 8                           | 8   |
| 7              | F            | 43          | 16                          | 4   |
| 8              | F            | 57          | 4                           | 10  |
| 9              | M            | 56          | 8                           | 6   |
| 10             | M            | 40          | 4                           | 6   |
| 11             | M            | 66          | 16                          | 15  |
| 12             | F            | 60          | 32                          | 8   |
| 13             | M            | 44          | 32                          | 8   |
| 14             | M            | 47          | 8                           | 8   |
| 15             | F            | 60          | 8                           | 10  |
| 16             | M            | 43          | 12                          | 8   |
| 17             | F            | 25          | 8                           | 4   |
| 18             | M            | 54          | 16                          | 8   |
| 19             | M            | 67          | 4                           | 8   |
| 20             | F            | 61          | 28                          | 10  |
| 21             | M            | 44          | 20                          | 8   |
| 22             | F            | 55          | 4                           | 4   |
| 23             | M            | 45          | 4                           | 9   |
| 24             | M            | 39          | 8                           | 11  |

CSF, cerebrospinal fluid; GCS, Glasgow Coma Scale.
into the venous sinuses. But a more recent, well-accepted hypothesis suggests that CSF is permanently produced and absorbed in the whole CSF system as a consequence of filtration and reabsorption of water volume through capillary walls into the surrounding brain tissue [6, 15, 24, 25]. Therefore, we believe that the fibrosis may affect the whole CSF pathway and lead to malabsorption of CSF.

One of the main features of fibrotic disease is excessive extracellular matrix repair. It is generally accepted that when primary damage surpasses the repair capabilities of parenchymal cells, interstitial connective tissue (extracellular matrix) hyperplasia will repair the defect. Studies have shown that the pathological changes of the CSF circulation pathways found in hydrocephalus involve an increase in the quantity of extracellular matrix.
and a decreased number of parenchymal cells [17, 28]. The hyperplasia of the extracellular matrix along the CSF circulation pathway may affect CSF absorption [7, 22, 26].

The extracellular matrix is predominantly composed of collagen, 85% of which is type I collagen, 11% type III, and 4% types IV and V. Therefore, PICP and PIIINP were selected as indicators of the formation of collagen fibrils. Hyaluronic acid is a glycosaminoglycan that is non-covalently bound by proteins in the extracellular space. It is the main component of proteoglycan. Laminin, one of the main glycoproteins of the basement membrane, is the index that reflects the level of glycoprotein. In liver fibrosis [19, 24, 29], idiopathic pulmonary arterial hypertension, cystic fibrosis [25], renal failure [38] and cardiac fibrosis [16, 30], serum concentrations of PICP, PIIINP, HA, and LN are believed to reflect the level of fibrosis. In experimental models of subarachnoid hemorrhage, increased concentrations of PICP and PIIINP in the CSF, as well as leptomeningeal deposition of collagen Types I and III, have been found [23, 30, 32, 41]. The fibril-forming collagens (types I–III) are synthesized by mesenchymal cells as procollagens. In searching for the origin of hyaluronan in CSF, studies have found that its concentration in the choroid plexus and leptomeninges is low and that it accumulates in the superficial layer of the cerebral cortex. Serial screening for hyaluronan in CSF may be valuable in cases of inflammatory diseases, tumors, and obstruction of CSF flow [14]. According to our results, increased levels of PICP, PIIINP, HA, and LN may indicate that hydrocephalus is a type of fibrotic disease.

Many factors have been demonstrated in the CSF of patients with hydrocephalus: enasin-C, GFAP, Aβ, VEGF and TGF-β [1, 19, 21, 33]. Concentrations of TGF-β1 have been found elevated in the CSF of adults with ventricular dilation due to subarachnoid and intraventricular hemorrhage [39], and both TGF-β1 and -β2 have been shown to be elevated in infants with post hemorrhage ventricular dilation [37]. Ventricular dilation has been produced in mice by injecting TGF-β1 into the cerebral subarachnoid space [27].

The TGF-β1 superfamily of cytokines is comprised of several homologous polypeptides that transduce a range of signals involved in cell growth and differentiation, as well as in the response to inflammation and tissue damage. The family members TGF-β1, -β2 and -β3 are present in all mammalian tissues, including the central nervous system. They have potent desmoplastic activity, through increased synthesis of collagen and several other components of the extracellular matrix [40], and are involved in a number of serious diseases involving excessive deposition of collagen and extracellular matrix, including diabetic nephropathy [2] and cirrhosis [5, 8]. In patients with post-traumatic chronic hydrocephalus, TGF-β1 is released into the CSF, and evidence indicates that this cytokine stimulates the laying down of extracellular matrix proteins. This process may produce permanent obstruction of the CSF pathway [20]. According to our results, the increased concentrations of PICP, PIIINP, HA, and LN are positively correlated with TGF-β1 levels (a recognized fibrosis factor). These results further our understanding of the pathogenesis of hydrocephalus from the perspective of fibrosis.

The traditional theory of CSF absorption through arachnoid granulations may not be complete. There is evidence that CSF can cross the ependyma and endothelium to reach the surface of brain by accessory pathways including nasal lymphatics, nerve sheaths and the spinal subarachnoid space [4, 13, 27, 36, 39]. Previous research has shown that in HT-X hydrocephalus rat models, there is decreased CSF outflow to the nasal lymphatics compared with control animals [31]. Lymphatic CSF absorption is impaired in a kaolin-communicating hydrocephalus model and the degree of this impediment may contribute to the severity of the hydrocephalus [22]. Lymphatic circulation obstruction can also induce further fibrotic disease [42]. Therefore, continued investigation into the relationship between lymphatic circulation and CSF pathway fibrosis will be an attractive research direction.

At the same time, we note that regulating fibrosis may delay the appearance of hydrocephalus. A recent finding by Botfield demonstrates that decorin can regulate factor-β/ Smad2/3 and its anti-fibrosis effect can delay the development of hydrocephalus [3]. It may also provide evidence for the role of CSF flow pathway fibrosis in the pathogenesis of hydrocephalus.

This study has some limitations. To exclude the possibility of an increase in PICP, PIIINP, HA, and LN concentration caused solely by brain trauma, we used brain trauma patients without hydrocephalus in the trauma group. However, the timing of CSF sample collection could not be uniformly regulated between the hydrocephalus and trauma groups. Therefore, we cannot conclude that the absence of an increase in fibrosis indices indicates that brain trauma patients will not develop hydrocephalus in the future. At the least, our results indicate that the increase of PICP, PIIINP, HA, and LN is not caused by trauma but rather by hydrocephalus, and that before hydrocephalus occurs, the level of fibrotic factors remain normal in the CSF of traumatic brain injury patients.

Conclusion

In summary, we confirmed that the levels of PICP, PIIINP, HA, and LN are increased in post-traumatic chronic hydrocephalus, but not increased in brain trauma patients without hydrocephalus. The fibrosis indices are positively related to TGF-β1 levels in hydrocephalus and have potential for use as biomarkers of post-traumatic hydrocephalus. Our results also demonstrated that fibrosis along the route of CSF flow may play a causal role in the development of chronic post-traumatic hydrocephalus and thus provide a new therapeutic orientation.

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