Journal of Clinical Neuroscience 22 (2015) 1638–1642

Contents lists available at ScienceDirect

Journal of Clinical Neuroscience

journal homepage: www.elsevier.com/locate/jocn

Clinical Study

Phosphorylated neurofilament subunit levels in the serum of cervical compressive myelopathy patients

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ARTICLE INFO

Article history:
Received 12 January 2015
Accepted 14 March 2015

Keywords:
Biomarker
Cervical myelopathy
Complication
Neurofilament
Surgery

ABSTRACT

We investigated the serum levels of the phosphorylated form of the high molecular weight neurofilament subunit (pNF-H) in patients with cervical compressive myelopathy. pNF-H is becoming increasingly recognized as a biomarker for axonal injury, however, it remains unclear whether serum pNF-H is elevated in chronic spinal cord compression. We examined 26 patients who underwent surgery for cervical compressive myelopathy. Peripheral blood samples were obtained both preoperatively and 1 week after surgery to evaluate the serum pNF-H levels using an enzyme-linked immunosorbent assay. A history of recent aggravation of myelopathy was also investigated. Of the 26 myelopathy patients, the preoperative serum pNF-H level was negative in 20 patients and moderately elevated in six. Patients who were positive for pNF-H were more likely to have had a recent aggravation of myelopathy compared with the pNF-H negative patients (83 *versus* 25%; $p = 0.02$). All patients who were positive for pNF-H before surgery remained positive after surgery. Two patients who became positive after surgery demonstrated a neurologic deterioration associated with the surgery. In conclusion, the serum pNF-H level was negative in the majority of patients with cervical compressive myelopathy. Our results suggest that an elevated serum level of pNF-H is associated with an acute worsening of myelopathy and that a positive conversion of pNF-H after surgery is a marker of perioperative neural damage.

1. Introduction

Attempts have been made to establish a method for the quantitative evaluation of neural tissue damage using proteins derived from damaged neurons as biomarkers [1–3]. The phosphorylated form of the high molecular weight neurofilament subunit (pNF-H) is an axonal cytoskeleton protein, and it was first reported to be a potential biomarker in 2005 [4]. pNF-H, which is absent in the serum of healthy individuals [5], is known to appear in the serum of patients with various pathologies, including traumatic brain injury, subarachnoid hemorrhage, and febrile seizures [6–9]. More recently, the potential usefulness of pNF-H as a biomarker to evaluate the severity and prognosis of acute traumatic spinal cord injury (SCI) has been reported [10,11].

In pathological conditions associated with acute neural damage, such as traumatic SCI, direct destruction of neural tissue results in the leakage of pNF-H beyond the blood brain barrier and into the peripheral blood. But the association of pNF-H with chronic cord compression pathologies has not been investigated. As SCI sometimes occur due to conditions causing a narrow spinal canal and preexisting cord compression [12,13], understanding the baseline levels of pNF-H in patients with chronic spinal cord compromise is important to better estimate the incremental damage of neural tissue attributable to trauma. In this pilot study, we sought to investigate the role of serum pNF-H in patients with cervical compressive myelopathy.

2. Materials and methods

2.1. Patients

We prospectively and consecutively enrolled patients who underwent decompression surgery for cervical compressive myelopathy at our institution between April 2013 and March 2014. The surgical indication was determined by the senior surgeon according to the clinical symptoms and radiographical findings. Excluded patients included those with systemic diseases

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http://dx.doi.org/10.1016/j.jocn.2015.03.047
0967-5868/

that could potentially affect the cervical spine and compromise the neurological assessment, including autoimmune diseases, chronic renal failure with hemodialysis, and neurological disorders including Parkinson's disease, cerebral palsy, and myasthenia gravis. The preoperative and 3 month postoperative severity of myelopathy was evaluated using the modified Japanese Orthopaedic Association (mJOA) score [14]. The score ranges from 0 to 18. The recovery rate was calculated using the following formula (Hirabayashi method): recovery rate (%) = (postoperative mJOA − preoperative mJOA)/(18 − preoperative JOA) × 100 [15]. The medical charts were reviewed for the duration of the myelopathic symptoms and their acute aggravation (defined as those observed within 3 months). All patients underwent preoperative MRI, and signal intensity change in the spinal cord was also investigated. Although increased signal intensity on the T2-weighted image is indicative of myelopathy [16], it was not regarded as necessary to recommend surgery. Patients with idiopathic scoliosis without cord compromise, as confirmed by myelography and MRI, were used as a control group.

2.2. Samples and pNF-H assay

In the myelopathy group, blood samples were obtained preoperatively and 1 week after surgery. Some patients were discharged within 1 week and their postoperative blood samples were not available. In the control group, samples were obtained preoperatively. All blood samples were centrifuged to extract the serum, and then stored in a freezer until the measurements were performed. The pNF-H assay was carried out using a commercially available enzyme-linked immunosorbent assay kit (Human Phosphorylated Neurofilament H ELISA; BioVendor, Modrice, Czech Republic), as previously described [11]. The frozen plasma samples were allowed to thaw and were then diluted three-fold with a dilution buffer. The limit of detection was calculated from the mean absorbance of the blank (dilution buffer only) plus three standard deviations of the absorbance of the blank. The values of pNF-H below the limit of detection (70 pg/mL) were reported as negative for the statistical analyses.

2.3. Statistical analyses

All analyses were carried out using SPSS software (version 19; IBM Corporation, Armonk, NY, USA). For comparisons of the parameters between the groups, the Mann–Whitney U-test was used for continuous variables, and Fisher's exact test for proportions. p values less than 0.05 were considered significant for all statistical tests.

2.4. Ethics

The patients were educated about the study protocol on admission, and written consent was obtained from all enrolled patients or their parents if they were minors. The study was approved by the Institutional Review Board. We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during the course of this research.

3. Results

3.1. Patient characteristics

A total of 26 myelopathy patients and eight control patients were consecutively enrolled (Table 1). In the myelopathy group, there were 19 men and seven women with a mean age of 64.4 years (range: 38–86). The most common diagnosis was cervical spondylotic myelopathy (14 patients), followed by ossification of the posterior longitudinal ligament (nine patients) and cervical disc herniation (three patients). The control group consisted of one male and seven females with idiopathic scoliosis. The mean age was 17.9 years (range: 11–27), significantly younger than that of the myelopathy group ($p < 0.001$). All patients in the control group were neurologically intact.

3.2. Preoperative serum pNF-H levels

Of the 26 myelopathy patients, the preoperative serum pNF-H was negative in 20 patients (77%) and moderately elevated in six (range: 115–458 pg/mL). Control samples (n = 8) were all negative for pNF-H. Overall, the median duration of symptoms was 10 months (range: 1 month–13 years). The pNF-H positive group tended to have a shorter duration of symptoms compared with the pNF-H negative group (median 3.5 *versus* 12 months, respectively; $p = 0.07$), although this difference did not reach statistical significance. Patients with elevated pNF-H levels were more likely to have had a recent aggravation of myelopathy within the 3 months prior to the measurement (5/6 [83%] *versus* 5/20 [25%]; $p = 0.02$). In addition, patients with elevated pNF-H levels were more likely to have a lower preoperative mJOA score compared with pNF-H negative patients (median 10.5 *versus* 13.0; $p = 0.005$). The recovery rates of mJOA were not significantly different between the two groups (median 28% *versus* 20%; $p = 0.84$). Regarding the signal intensity change on T2-weighted MRI, there was no significant difference between the two groups (positive group: 5/6 [83%]; negative group: 13/20 [65%]; $p = 0.62$).

3.3. Postoperative serum pNF-H levels

Postoperative blood samples were available for 18 myelopathy patients. Among the 20 myelopathy patients with negative preoperative pNF-H levels, 14 were followed at 1 week postoperatively and their pNF-H levels remained negative in 12; two patients were positive. In one of the positive patients (Patient 5), the postoperative pNF-H level increased to 626 pg/mL. In addition, their intraoperative spinal cord monitoring showed an abnormally low amplitude during the fixation procedure, and the patient developed transient upper limb weakness after surgery. In the other (Patient 25), the patient underwent anterior decompression and fusion and the postoperative pNF-H level increased to 163 pg/mL. On the fourth postoperative day, the patient developed autograft dislodgement and pain in both the upper and lower extremities, requiring revision surgery. Among the six myelopathy patients with positive preoperative pNF-H levels, four were followed-up at 1 week after surgery. All four patients remained positive for pNF-H after surgery. The recovery rates (mJOA) were not different between those with and without elevated pNF-H levels (median 20% *versus* 20%; $p > 0.99$).

4. Discussion

There are two major findings in the present study. First, the serum pNF-H was negative in the majority of patients with cervical compressive myelopathy, as well as in the control patients. Patients who were positive for pNF-H were more likely to have had a recent aggravation of myelopathy compared with the pNF-H negative patients. Second, in most patients, the serum pNF-H level remained unchanged before and after surgery. Therefore, a postoperative elevation of serum pNF-H may be associated with the insult to the spinal cord during surgery.