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Diagnostic Value of Serum Levels of GFAP, pNF-H, and NSE Compared With Clinical Findings in Severity Assessment of Human Traumatic Spinal Cord Injury

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Study Design. An analytical cohort study.

Objective. This study aimed to evaluate severity of traumatic spinal cord injury (SCI) based on the serum levels of phosphorylated form of heavy subunit of neurofilament (pNF-H), neuron-specific enolase (NSE), and glial fibrillary acidic protein (GFAP), which are axonal, neural cell body, and glial cell injury markers, respectively.

Summary of Background Data. Prior studies have reported elevated serum levels of pNF-H, NSE, and GFAP as biomarkers for the detection of traumatic SCI in animals. However, in this study, these biomarkers were studied in humans and with an extended level of timing.

Methods. The study included 35 patients with SCI with a mean age of 36.5 years. All patients were evaluated using the American Spinal Injury Association Impairment Scale, followed by examinations including radiography and spinal computed tomography for determining the injury level. Serum levels of NSE, pNF-H, and GFAP were determined using enzyme-linked immunosorbent assay.

Results. The mean serum level of GFAP was significantly higher in patients with SCI than in the control group. Mean serum levels of pNF-H and NSE were significantly higher during 24 and 48 hours after injury in patients with SCI than in the control group. The serum level of GFAP was appropriate for estimating the severity of SCI in the first 24 hours after injury.

Conclusion. Our findings suggest that increased serum levels of GFAP, NSE, and pNF-H can be used for the diagnosis and degree of SCI severity in trauma patients. During 48 hours after injury, estimation of serum levels of pNF-H, NSE, and GFAP, combined with neurological testing, could predict the presence of SCI and severity prior to spinal computed tomography and surgical or conservative interventions.

Key words: traumatic, spinal cord injury, severity, biomarker, pNF-H, GFAP, NSE, computed tomographic scan, MRI, human.

Level of Evidence: 2

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Despite the difficulty associated with traumatic spinal cord injury (SCI), there is no method for rapid assessment of its severity. Clinical examinations, followed by medical imaging, are critical in detecting spinal fractures, but they do not have optimal sensitivity and specificity for assessing spinal cord tissue injury and severity and predicting patient outcome, especially in patients with SCI. Although multidetector computed tomography (CT) with multiplanar and 3-dimensional reconstructions can demonstrate subtle abnormalities of the spinal column, it is very helpful in detecting unstable injuries of the bones of spine and paraspinal soft tissues. Neurochemical responses to SCI are complex and characterized by changing levels of mediators and biomarkers of primary and secondary spinal cord damage. Many studies conducted on animal and human traumatic SCI have focused on the detection of specific biomarkers in serum or cerebrospinal fluid (CSF) to predict the presence and severity of SCI in trauma patients.[1] Detection and validation of the so-called SCI biomarkers allow the prediction of severity and monitoring of spinal cord damage and disease states in animal models, and evaluating the effectiveness of some neuroprotective drug treatments in the animal SCI model may provide quick and simple clinical diagnosis and prognosis of traumatic SCI in human.[2,3]

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Evaluation of the specific biomarkers for neural tissue and non-neural cells including neural cell body, axonal protein components, and glial cells may help diagnose the severity of SCIs. Neurofilament subunits (NF-L, NF-M, and NF-H) are important protein components of axonal structure, and an increased level of neurofilaments has been shown in CSF or serum of different SCI models in laboratory animals.[4,5] Hyperphosphorylated form of NF-H has been confirmed as a serum biomarker of axonal injury in rats.[6–8]

Neuron-specific enolase (NSE) is a glycolytic enzyme (2-phospho-D-glycerate hydrolase) that catalyzes the conversion of 2-phosphoglycerate to phosphorenol pyruvate. It consists of homo- or heterodimers of 3 different monomer isoforms (α, β, and γ). NSE is a 78-kD γ-homodimer and represents the dominant enolase isoenzyme found in neuronal and neuroendocrine tissues. Its levels in other tissues, except erythrocytes, are negligible. Its biological half-life in body fluids is approximately 24 hours.[9] Loy *et al*[10] reported that serum levels of NSE increased in 6 hours after SCI in the weight-drop SCI model and this enzyme can be used as a rapidly elevated biomarker for acute SCI.

Glial fibrillary acidic protein (GFAP), which was first isolated from the astrocyte skeleton by Eng *et al* in 1971, is a class III intermediate protein found in the structural network of glial cells.[11,12] Elevated serum levels of GFAP have been reported as a biomarker for the detection of traumatic SCI, traumatic brain injury, and brain hemorrhage in the acute phase of stroke.[13] Guéz *et al*[14] and Brisby *et al*[15] have shown that levels of GFAP and NFL increase in CSF of patients with SCI.

This study was aimed to evaluate the diagnostic value of serum levels of NF-H, NSE, and GFAP for the prediction of SCI severity in trauma patients.

MATERIALS AND METHODS

This study was conducted at the Department of Emergency Medicine, Shohadye Haftom Tir Hospital and Neuroscience Research Center. The study was conducted from January to April 2013. After approval of the study design by the university ethical committee, informed written consent from patients and their nearest relatives was obtained. Patients who had spinal fracture and SCI were enrolled in the study. Sixteen patients had severe SCI and underwent immediate spinal traction or surgical interference for decompression. Patient exclusion criteria included brain injury, death, and referral to other centers after admission. All patients with suspected SCI were clinically evaluated using the American Spinal Injury Association (ASIA) Impairment Scale and spinal CT using the Toshiba 16 Aquilion system. In this study, 9 case patients with spinal fractures but without neurological symptoms were considered as the control group. Patients were divided into 3 age-groups, 16 to 25, 26 to 45, and 46 to 65 years, and serum samples were obtained from all patients.

Imaging Assessment

Spinal CT scans were obtained with the Toshiba 16 Aquilion multidetector CT scanner, working at 50 to 150 mA, pitch factor 1.2 to 1.5, and 120 kV. Scans of the thoracolumbar spine were obtained at 100 to 150 mA, whereas those of the upper cervical spine were obtained at 50 to 100 mA. For all patients, multiplanar reconstruction (MPR) images in both coronal and sagittal planes were obtained whereas 3-dimensional images for some case patients were reconstructed. Diameter of the spinal canal at the level of injury in axial and sagittal MPR images was measured. The sagittal diameter of the spinal canal at midline on the level of injury and the adjacent normal spine at above and below the injury was measured.

Laboratory Assays

After confirmation of SCI by neurological examinations and CT, venous blood samples were obtained from patients and clot formation was allowed and then serum was separated by centrifugation at 2000 rpm for 5 to 8 minutes. Serum was removed and placed in Eppendorf microtubes and stored at −20°C and then at −80°C until enzyme-linked immunosorbent assay (ELISA) was performed. The following ELISA kits were used: Abnova (Taipei, Taiwan), for NF-H protein; Millipore (Billerica, MA), for GFAP; and Eagle Biosciences (Nashua, NH), for NSE.

RESULTS

In total, 35 patients including 30 males and 5 females with a mean age of 36.5 (range, 16–64) years were evaluated. Nine case patients with spinal fracture and no evident trauma were considered as the control group. Of 26 patients with confirmed traumatic SCI, 8 patients had cervical injury, 8 case patients had thoracic injury, and 10 patients had lumbar injury. According to the neurological test before CT scan, 10 patients had severe (grade A), 7 patients had moderate (grade B), and 9 patients had mild (grades C and D) spinal injury. On the basis of the Hashimoto formula, the percentage of spinal canal narrowing on CT scans was calculated. The mean percentage of canal narrowing in patients was 57.5%, with a range from 18% to 100% (Table 1).

During the study period, the mean serum level of GFAP was significantly higher in patients with SCI than in the control group (Figure 1). Serum levels of GFAP in patients with severe SCI (grade A) were higher than those in patients with moderate (grade B) ($P < 0.05$) or mild SCI ($P < 0.01$). The level of GFAP was also different between patients with moderate SCI and those with mild SCI ($P < 0.05$; Figure 2).

TABLE 1. Distribution of the Patients With Spinal Cord Injury According to Injury Segments and Canal Compromise

Injury Segment	%	n	Mean Canal Compromise, %
Cervical	30.7	8	70
Thoracic	30.7	8	50
Lumbar	38.4	10	63
Total	100	26	64.3

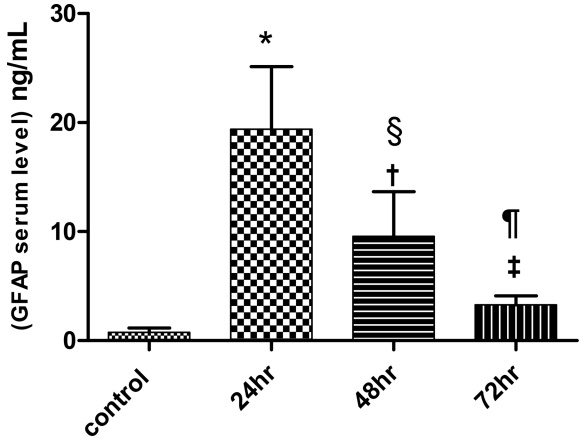

Figure 1. Serum level of GFAP (mean ± SEM) in patients with SCI compared with that in the control group during 72 hours after injury: 24, 48, and 72 hours after injury, serum level of GFAP in patients with SCI is significantly higher than that in the control group (*$P < 0.001$, †$P < 0.01$, and ‡$P < 0.05$), respectively. Decrease in the level of GFAP is observed 24 hours after injury, which is significantly lower at 48 and 72 hours after injury (§$P < 0.001$, ¶$P < 0.05$). GFAP indicates glial fibrillary acidic protein; SCI, spinal cord injury; SEM, standard error of mean.

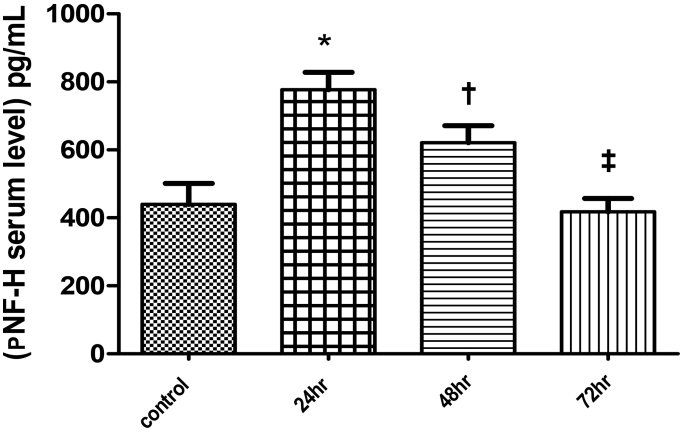

Figure 3. Serum level of pNF-H (mean ± SEM) in patients compared with control during 3 time courses: in the first and second 24 hours, serum level of pNF-H is significantly higher than that in the control group (*$P < 0.01$, †$P < 0.05$). After 24 hours, pNF-H decreased. In the first 24 hours, the level of pNF-H is significantly higher than that in the third 24 hours (‡$P < 0.001$). PNF-H indicates phosphorylated form of heavy subunit of neurofilament; SEM, standard error of mean.

Generally, mean serum levels of NSE and phosphorylated form of heavy subunit of neurofilament (pNF-H) were significantly higher in patients with SCI than in the control group. Mean serum levels of pNF-H were significantly higher during the first and second 24 hours in patients with SCI than in the control group ($P < 0.001$), but there was no significant difference compared with the control group during the third 24 hours after injury. Because serum level of NF-H in the first 24 hours was significantly higher than in the second 24 hours ($P < 0.05$), it seems that the best time for NF-H sampling should be considered in the first 24 hours. Moreover, serum level of NF-H in the first 24 hours was higher than in the third 24 hours ($P < 0.001$) and that in the second 24 hours is significantly higher ($P < 0.05$) than in the third 24 hours (Figure 3). Serum level of pNF-H in patients with severe SCI compared with patients with moderate or mild SCI was not significantly different. However, serum level of pNFH in patients with severe (grade A) and moderate SCI (grade B) compared with those who had mild (grade D) injury was also significantly higher ($P < 0.05$; Figure 4). Serum levels of NSE in the first and second 24 hours in patients with SCI were

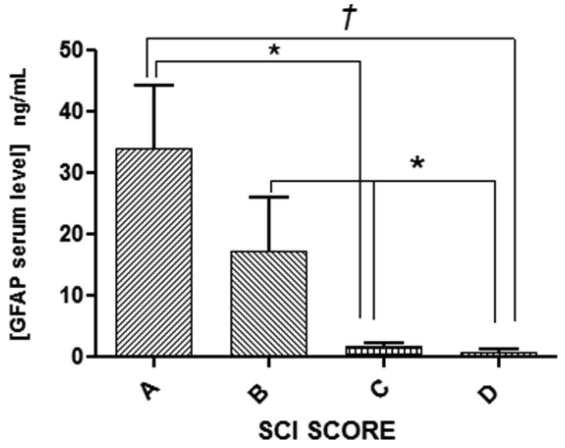

Figure 2. Serum level of GFAP (mean ± SEM) 24 hours after injury in patients with SCI according to the ASIA score. There is no significant difference in the level of GFAP between groups A (severe lesion) and B (moderate), but the level of GFAP is significantly lower in the C and D (mild) groups than in group A (*$P < 0.01$). Serum level of pNF-H in the second 24 hour is significantly higher than the control group (†$P < 0.01$). GFAP indicates glial fibrillary acidic protein; SCI, spinal cord injury; ASIA, American Spinal Injury Association; SEM, standard error of mean.

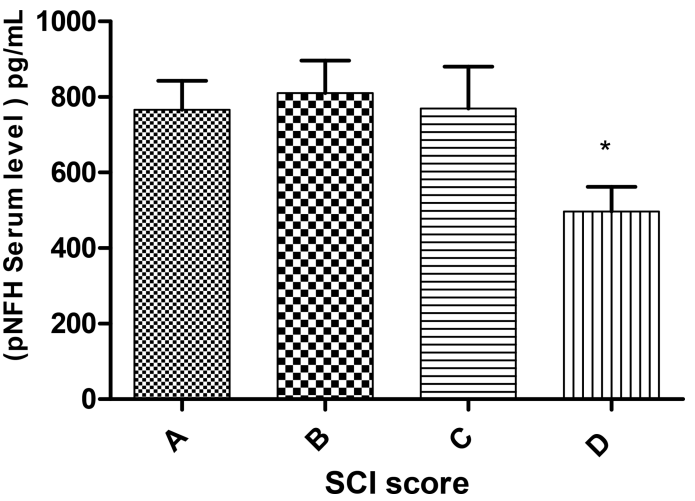

Figure 4. Serum levels of pNF-H (mean ± SEM) in the first 24 hours in patients with SCI according to the ASIA score. There is no difference in groups A (severe lesion) and B (moderate) and C. But serum level of NSE in the A, B, and C groups is significantly higher than that in group D (*$P < 0.05$). NSE indicates neuron-specific enolase; SCI, spinal cord injury ASIA, American Spinal Injury Association; SEM, standard error of mean; pNF-H, phosphorylated form of heavy subunit of neurofilament.

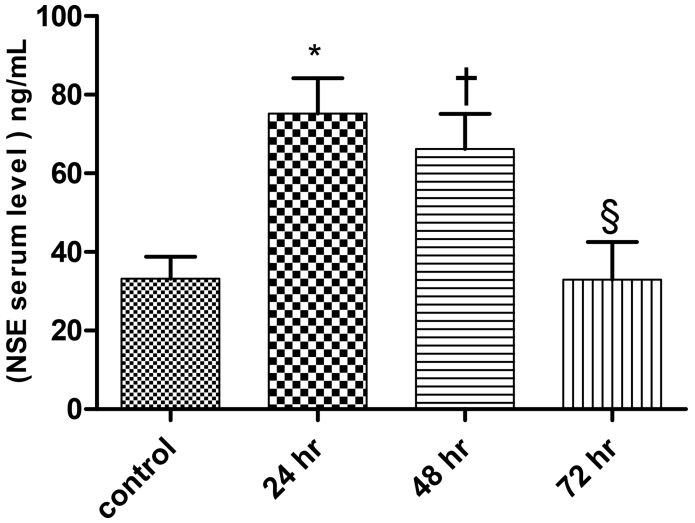

Figure 5. Serum level of NSE (mean ± SEM) during 72 hours after injury in patients with SCI (n = 23). The level of NSE is significantly higher in the control group at 24 and 48 hours after injury (*$P < 0.01$ and †$P < 0.05$, respectively). As shown, the level of NSE decreased 24 hours after injury, which is significantly lowered during 72 hours (‡$P < 0.001$, §$P < 0.05$). NSE indicates neuron-specific enolase; SCI, spinal cord injury; SEM, standard error of mean.

significantly higher than those in the control group ($P < 0.01$ and $P < 0.05$, respectively). After 24 hours, the level of NSE had a decreasing pattern; somehow in the second and third 24 hours, it was significantly lower ($P < 0.001$ and $P < 0.05$, respectively; Figure 5). Serum level of NSE in patients with SCI was not appropriate according to the ASIA Impairment Scale score, but it was significant between those with grade C and grade D injury ($P < 0.05$; Figure 6).

Serum level of GFAP in patients with SCI tended to decrease with increasing age, but the difference was not significant between groups (Figure 7). There was no significant difference in serum levels of pNF-H based on age in patients

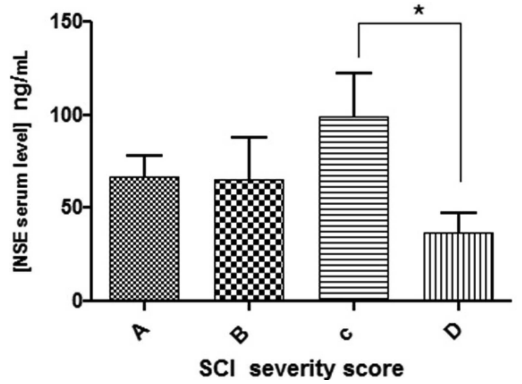

Figure 6. Serum level of NSE (mean ± SEM) in the first 24 hours in patients with SCI according to the ASIA score. There is no difference between groups A (severe lesion) and B (moderate) and C. But serum level of NSE in group C is significantly higher than that in group D (*$P < 0.05$). NSE indicates neuron-specific enolase; SCI, spinal cord injury; ASIA, American Spinal Injury Association; SEM, standard error of mean.

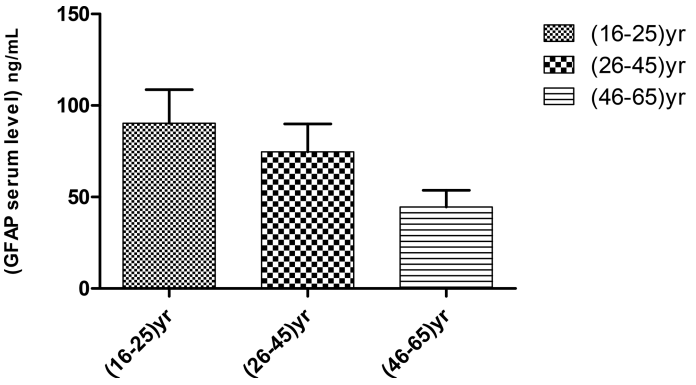

Figure 7. Serum levels of GFAP (mean ± SEM) in patients with SCI show decreasing pattern with increase in age. But the differences among the 3 age groups were not significant. GFAP indicates glial fibrillary acidic protein; SCI, spinal cord injury; SEM, standard error of mean.

with SCI (Figure 8). Serum level of NSE in patients with SCI showed an increasing pattern with increasing age because it was significantly higher in older case patients (46–65 yr) than in younger ones (16–25 yr) (Figure 9).

Unfortunately, 8 patients with SCI died postoperatively; 7 case patients had severe SCI at C5–C6 level and 1 case at T8 level. As an important point, nonsurvivors had significantly higher serum levels of GFAP than survivors in the first 24 hours (Figure 10). Serum levels of pNF-H in nonsurvivors compared with survivors were not significant in the first 24 hours. On the contrary, the level of pNF-H did not decrease in the second 24 hours (Figure 11). Serum level of NSE increased in the second 24 hours in nonsurvivors compared with survivors (Figure 12).

DISCUSSION

This study aimed to evaluate the diagnostic value of serum levels of NF-H, NSE, and GFAP in comparison with neurological tests and spinal CT findings to assess the severity of damage in patients with SCI. Cervical spine injuries often

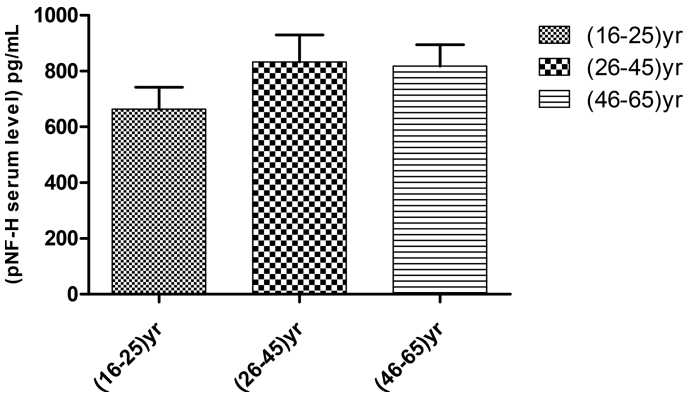

Figure 8. Serum levels of pNF-H (mean ± SEM) in patients with SCI show increasing pattern with increase in age. But the differences among the 3 age groups were not significant. PNF-H indicates phosphorylated form of heavy subunit of neurofilament; SCI, spinal cord injury; SEM, standard error of mean.

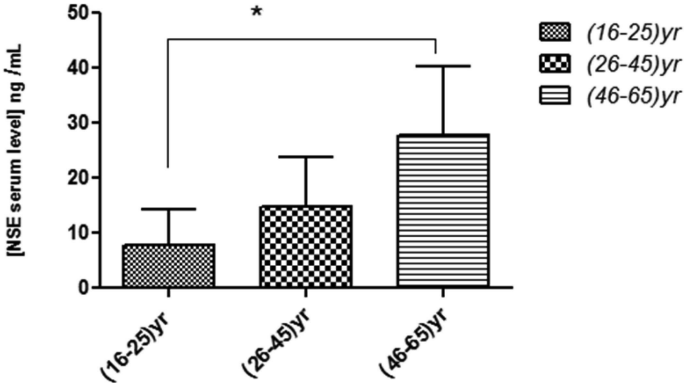

Figure 9. Serum levels of NSE (mean ± SEM) in patients with SCI show increasing pattern with increase in age. The level of NSE in older case patients (46–65 yr) was significantly higher than that in younger case patients (16–25 yr). But the differences between patients aged 16 and 25 years and those aged 26 and 45 years and the differences between patients aged 26 and 45 years and those aged 56 and 65 years were not significant. NSE indicates neuron-specific enolase; SCI, spinal cord injury; SEM, standard error of mean.

occur at 2 spinal levels: C2 in the upper part and C5, C6, or C7 in the lower part, and T12–L1 in the thoracolumbar spine.[16,17]

Lesions in patients who were included in this study were often located in the lower cervical, lower thoracic, and lumbar sections (Table 1). Although multidetector CT is sufficient to rule out traumatic spinal injuries, the use of spinal CT, especially in the cervical region, in high-risk traumatic patients is a cost-effective imaging modality and prevents complications. Magnetic resonance imaging (MRI) is the only modality that shows the spinal cord and soft-tissue injuries of the spines.[18,19]

MRI is less sensitive than CT for bone injuries and should be used in addition to CT in some patients; however, it has disadvantages in unconscious patients, due to difficulty monitoring vital signs in severely injured patients during transport

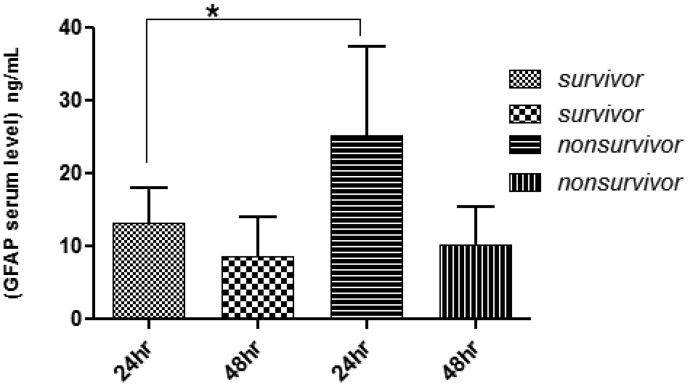

Figure 10. Serum level of GFAP (mean ± SEM) in survivors and nonsurvivors. Serum level of GFAP in nonsurvivors (n = 8) is significantly higher than that in survivors during 24 hours after injury (n = 15) (*$P < 0.05$), but there was no significant difference between nonsurvivors and survivors after 48 hours. GFAP indicates glial fibrillary acidic protein; SEM, standard error of mean.

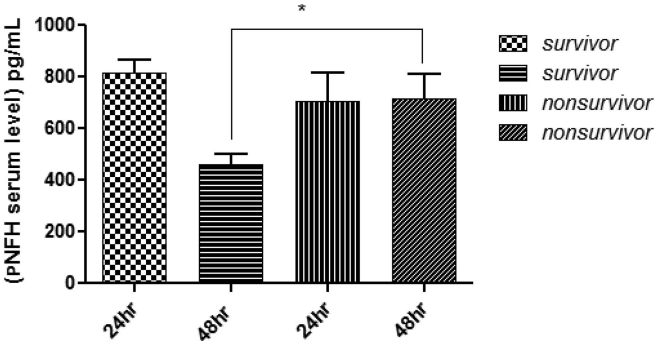

Figure 11. Serum level of pNF-H (mean ± SEM) in survivors and nonsurvivors. Serum level of NF-H in nonsurvivors (n = 8) is significantly higher than that in survivors in the second 24 hours (n = 15) (*$P <$ 0.05). PNF-H indicates phosphorylated form of heavy subunit of neurofilament; SEM, standard error of mean.

and the imaging procedure.[20] The presence of metallic equipment or non–MRI-compatible prosthesis and bone fixatives can decrease image quality and sometimes make it impossible,[21] but MRI can detect new cord damages such as contusions, especially in SCIWORA (spinal cord injury without radiological abnormality) cases.[22,23]

Several biomarkers have been introduced as candidate for the assessment of nervous system injury in trauma, inflammation, neurodegeneration, neurotoxicity, and tumors.[24,25]

Our findings indicated that the first 24 hours is the best time period for sampling and assessment of biomarkers in human SCI. Serum levels of GFAP, NSE, and pNF-H decreased in the second and third 24 hours. Shaw *et al*,[7] in an animal model of SCI, reported that the level of pNF-H rises rapidly and reaches its peak value in about 16 hours after injury. The levels then decline to baseline slowly. We observed that the biomarker serum levels were not similar during the first, second, and third 24 hours after injury. This could be due to several reasons. One of these reasons is transportation of patients between the emergency department, imaging room, and operation room that causes more movement of the spinal bone fragment at the lesion site or may be due to surgical interventions in the first 24 hours. The decrease in the next time course could be due to spinal cord decompression,

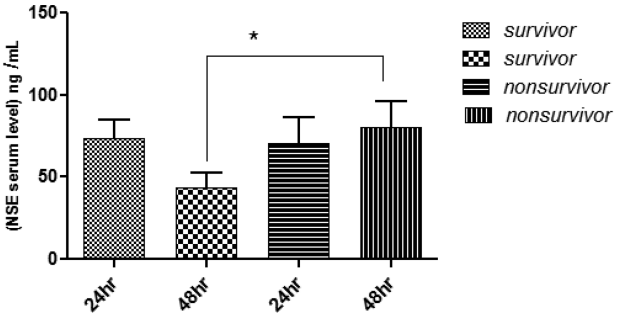

Figure 12. Serum level of NSE (mean ± SEM) in survivors and nonsurvivors. Serum level of NSE in nonsurvivors (n = 8) is significantly higher than that in survivors in the second 24 hours (n = 15) (*$P < 0.05$). NSE indicates neuron-specific enolase; SEM, standard error of mean.

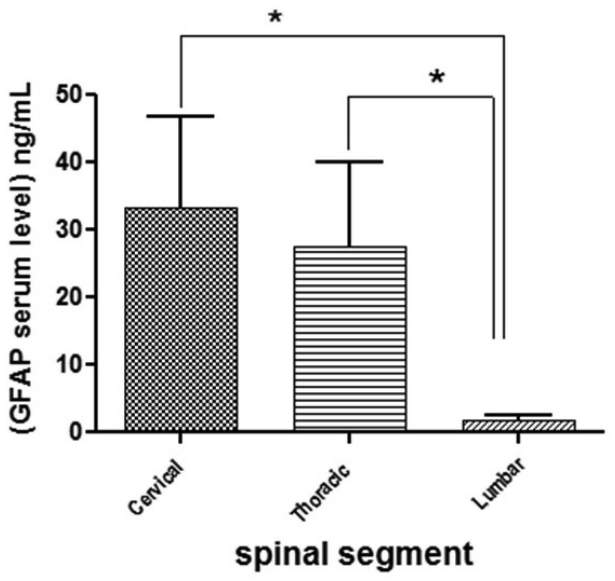

Figure 13. Serum level of GFAP (mean ± SEM) in patients categorized according to spinal segments. Levels of GFAP in patients with cervical and thoracic lesions are significantly higher than in those with lumbar lesions (*$P < 0.05$), but no significant difference was observed between serum level of GFAP in patients with cervical and thoracic lesions. GFAP indicates glial fibrillary acidic protein; SEM, standard error of mean.

drug administrations (*e.g.*, methyl prednisolone and dexamethasone), and other treatments including patient stabilization and bed rest after the first 24 hours. Treatment with methyl prednisolone decreases the expression of GFAP and prevents neurofilament degradation and therefore decreases serum levels of neurofilament in rats after SCI.[26] Moreover, it might be due to half-life of the biomarker, which returns to the basal level after reaching its peak concentration. Second, patients with SCI had significantly elevated mean serum levels of GFAP compared with the control group and significantly higher levels in those with severe and moderate SCI than in the controls who had just spinal fractures. This finding is consistent with that of Kwon *et al*,[27] who reported injury severity–dependent pattern of GFAP expression at 24 hours after injury in patients with SCI. Our findings indicate that SCI is associated with the release of these biomarkers in accordance with severity of spinal cord trauma and canal compression related to the injury site. Higher levels of GFAP in patients with injury at the cervical and thoracic segments rather than at the lumbar segment may be due to the lower density of glial cells in the lumbar segment than in the upper spinal segment as shown in Figure 13. Therefore, it can be concluded that GFAP is an optimal biomarker for upper SCI and brain injury. This is in agreement with the findings of Missler *et al*[28] and Pelinka *et al*,[29] who reported that the level of GFAP is increased after brain injury and its level is proportional to the severity of central nervous system injury. Foerch *et al*[30] also showed that in hemorrhagic stroke, serum levels of GFAP can be used as a biomarker for the detection of brain hemorrhage in the acute phase of stroke. On the basis of our results, among 3 biomarkers (GFAP, NF-H, and NSE) measured by ELISA, only serum levels of GFAP showed changes in a time-dependent manner in the first 24 hours after injury. These findings indicate that the level of GFAP slowly increases in serum and reaches its maximum level slower than those of NSE and pNF-H. For the first time, Petzold and Rejdak[31] showed pNF-H in blood in patients with acute optic neuritis. Petzold *et al*[32] also presented an ELISA kit for NF-H, which was sensitive to phosphorylated forms of NF-H, the kit that was very similar in specificity to the kit that we used in the present study. In the other study, Hu *et al*[33] reported an increase in CSF level of pNF-H in Alzheimer disease. NSE is expressed in platelets and blood cells. This biomarker is increased in serum of patients with active systemic sclerosis and also expressed in some kind of tumors such as small cell lung carcinoma, carcinoids, islet cell tumors, and neuroblastoma.[34,35]

Although these reports decrease the specificity of NSE in traumatic SCI, there is enough evidence that NSE can be used as an SCI biomarker. Brisby *et al*[15] reported an increased level of NSE in patients with disk herniation compared with control cases.

Zhang *et al*[36] reported that serum and CSF levels of NSE were increased in the animal model of SCI. Loy *et al*[37] reported that increases in serum levels of NSE were observed at 6 hours after injury. Our findings are in accordance with other research findings that revealed that NSE can be used as a rapid biomarker of SCI.

We did not observe significant difference in serum levels of pNF-H and NSE between patients categorized according to the ASIA Impairment Scale score. This may be due to the timing of blood sampling because in approximately all cases, their peak reached after some hours.

Shaw *et al*[7] confirmed pNF-H as a serum biomarker of axonal injury in rats and concluded that about 1% of the urea-soluble protein in rat spinal cord is pNF-H. They observed 2 peaks for pNF-H expression; a sharper one at about 16 hours, which is similar to our finding in human SCI.

Together, our results showed certain findings; first, these biomarkers could not replace neurological examination and imaging modality for emergency diagnosis, but they could be used only as an adjuvant diagnostic factor in patients with SCI. Second, serum levels of GFAP and NF-H are more specific biomarkers for SCI than NSE.

Evaluation of serum levels of NSE, GFAP, and pNF-H in survivors and nonsurvivors showed that nonsurvivors had sever SCI and had significantly higher serum levels of GFAP in the first 24 hours than survivors. In nonsurvivors, not only there is significant decrease in the 48-hour serum levels of GFAP, NSE, and pNF-H compared with survivors but also there are increased serum levels of NSE and pNF-H at 48 hours after injury (Figures 10, 11). This increase in serum levels of biomarkers can be a predictor for future outcome. Therefore, serum levels of GFAP, NSE, and NF-H in death case patients were significantly higher than those in survivors. In agreement, Honda *et al*[38] reported an increased level of GFAP as a biomarker for morbidity and mortality in traumatic brain injury.

All survivors showed decreased levels of NSE, GFAP, and pNF-H within the first 48 hours after trauma, whereas in nonsurvivors, levels of biomarkers were decreased nonsignificantly.

This study is more focused on SCI. However, future studies can be conducted on brain injuries and might have different results in comparison with SCIs. Moreover, in future studies, more evidence can be helpful for the outcome and recovery status of SCI.

CONCLUSION

Serum levels of GFAP, NF-H, and NSE, combined with clinical examinations and subsequently CT scans, could predict spinal cord damage in trauma patients irrespective of the extent of anatomical trauma. Serum biomarker research in patients with SCI is a new field, and our report about biomarkers of SCI may provide a better insight of potentials for biomarker research. More studies are required to determine which biomarkers are prominent in different features of SCI such as severity, outcome, recovery, and therapeutic interventions. Nowadays, the validation of SCI biomarkers is related to other examinations such as CT, MRI, and ASIA standards; however, in the future, biomarkers will provide accurate information in patients with SCI.

➢ Key Points

- ❑ We proposed to evaluate severity of traumatic SCI based on serum levels of pNF-H, NSE, and GFAP.
- ❑ Serum levels of NSE, pNF-H, and GFAP were determined using ELISA.
- ❑ Increased serum levels of GFAP, NSE, and pNF-H can be used for the diagnosis and degree of SCI severity in trauma patients.

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