Published online in advance by J-STAGE

ORIGINAL ARTICLE

Serum Neurogranin Measurement as a Biomarker of Central Nervous System Infections: A Preliminary Study

Isil Bektas Canturk,[1] Asim Kalkan,[2] Acelya Kirat Es,[3] Oner Bozan,[2] Sevilay Sema Unver,[2] Mucahit Senturk,[2] Mehmet Esat Ferhatlar[2] and Bilge Deniz Tayfun[2]

[1] Department of Emergency Medicine, 28 Aralık Gaziantep State Hospital, Gaziantep, Turkey
[2] Department of Emergency Medicine, University of Health Science, Prof. Dr. Cemil Tascioglu City Hospital, Istanbul, Turkey
[3] Department of Emergency Medicine, Van Training and Education Hospital, Van, Turkey

(Received for publication on November 18, 2021)
(Revised for publication on February 14, 2022)
(Accepted for publication on March 11, 2022)
(Published online in advance on June 17, 2022)

The early diagnosis of central nervous system infections is of great importance to minimize morbidity and mortality. Neurogranin is a postsynaptic neural protein, and when the blood–brain barrier is damaged, neurogranin levels increase in both the cerebrospinal fluid and serum. The aim of this study was to evaluate the level of serum neurogranin and to investigate its utility in the diagnosis of central nervous system infections. This study was conducted as a prospective case–control study of patients diagnosed with meningitis. The study initially included 55 patients, and 15 patients with proven central nervous system infection were ultimately included in the patient group. The results in the patient group were compared with those of the control group of 15 healthy subjects. The 15 patients comprised 4 women and 11 men with a mean cerebrospinal fluid neurogranin level of 432.4 ± 123.5 ng/ml. Correlation analysis revealed a moderate positive correlation between cerebrospinal fluid neurogranin levels and serum neurogranin levels. The mean serum neurogranin level was 198.6 ± 51.7 ng/ml in the control group but was significantly higher at 429.2 ± 104.3 ng/ml in the patient group. In conclusion, it may be useful to measure blood neurogranin levels in patients suspected of having central nervous system infections, especially in those for whom computed tomography, magnetic resonance imaging, or lumbar puncture cannot be performed. (DOI: 10.2302/kjm.2021-0019-OA)

Keywords: **neurogranin, biomarker, central nervous system infections**

Introduction

Infections of the nervous system are caused by pathogens such as bacteria, viruses, and fungi and are potentially life-threatening. Prompt recognition and treatment of a central nervous system (CNS) infection is crucial for patient survival, because such infections have high morbidity and mortality rates. It is of great importance that the preliminary diagnosis of patients with CNS infections be confirmed as promptly as possible.[1] Therefore, early diagnosis is important in the emergency setting, and a suitable biomarker is needed to facilitate the timely diagnosis of these infections.

Neurogranin is a postsynaptic protein expressed in the neocortex, amygdala, caudate nucleus, putamen, and hippocampus and is localized in the cortical areas and cerebral cortex layers II–IV of the human brain. Neurogranin is involved in signal transduction in postsynaptic neurons and in the apical and basal localized dendrites of pyramidal neurons.[2] Neurogranin, a small protein that can easily pass through a damaged blood–brain barrier, is used as a biomarker in chronic neurodegenerative diseases and has

Reprint requests to: Asım Kalkan, Assoc Prof, Department of Emergency Medicine, University of Heath Science, Prof Dr Cemil Tascioglu City Hospital, 34100 Istanbul, Turkey, E-mail: drasimkalkan@hotmail.com

recently also been used in acute brain injury. Neurogranin levels have been found to increase in traumatic brain injury as a result of damage to the blood–brain barrier.[3] Against this background, we hypothesized that, in CNS infections, neurogranin levels would increase in cerebrospinal fluid (CSF) and in serum because such infections cause brain damage and disrupt the blood–brain barrier. The aim of this study was to evaluate serum neurogranin levels and to investigate its utility in the diagnosis of CNS infections.

Methods

Study design and setting

This study was conducted between May 2018 and May 2019 in the Emergency Medicine Clinic of Okmeydani Training and Research Hospital, subsequent to the approval of the Local Ethics Committee (LEC Number: 904). Informed consent forms were obtained from the patients or their relatives. An average of 250,000 patients per year are admitted to the Emergency Department (ED) of our hospital. There are three different areas in the ED, color coded green, yellow, and red, that indicate the clinical status of the patients. Approximately 70% of ED patients are allocated to the green zone. Green zone patients are evaluated for minor trauma such as low back pain, upper respiratory tract infection, or ankle sprain. An average of 15 to 25 meningitis cases are diagnosed annually in our clinic. The patients enrolled in the current study were those with CNS infections who were admitted to the ED in the defined 1-year period and who met the inclusion criteria with none of the exclusion criteria. Of the patients with CNS infection, only those diagnosed with bacterial meningitis were included in this study. Patients with viral or fungal meningitis were excluded.

Participants

A total of 15 patients with CNS infection confirmed by lumbar puncture findings were ultimately included in the study, along with 15 healthy volunteers as the control group. The control group subjects were selected from patients who were admitted to the ED green zone with simple complaints such as fatigue and who met the following criteria: underwent blood tests for any reason, the results of which were normal; no pathology was determined; and who voluntarily agreed to participate in the study. Patients and control group subjects were excluded from the study if they had any neuropsychiatric disease such as schizophrenia, Parkinson's disease, any chronic cognitive disease such as dementia or Alzheimer's disease, intracranial lesions secondary to trauma, head trauma, a history of ischemic cerebrovascular disease within the previous 3 months, lesions located in the CNS, or were taking antidepressant or antipsychotic medications that primarily affected the CNS. Anti-depressant and anti-psychotic drugs change the level of serum neurogranin. In a postmortem study of schizophrenia patients, neurogranin levels in the prefrontal cortex were determined to be low. Some studies have shown increased neurogranin gene expression in patients with depression. Therefore, it was thought that in patients using anti-depressants or anti-psychotics, the serum neurogranin levels could be affected by the disease.[4,5]

Data collection and neurogranin measurements

The demographic data of the patients, such as the file number, date, complaint and date of onset, age, sex, and known comorbid diseases, were recorded. Blood pressure, pulse, O_2 saturation, body temperature, Glasgow Coma Score, and consciousness level were recorded on the same form. Additionally, complete blood counts, biochemical analysis, CSF analysis (cell counts and glucose, lactate dehydrogenase, and protein levels), brain computed tomography findings, and treatment received in the emergency department were recorded. Serum and CSF neurogranin levels were also added to this form.

Routine blood samples were collected for complete blood count and biochemical analysis. CSF samples were obtained from lumbar puncture performed for the diagnosis of CNS infection. The centrifuged serum samples were transferred from blood tubes to another Eppendorf tube to avoid possible hemolysis and were then stored at −80°C until assay. For ethical reasons, CSF was not obtained from the control group, but blood samples were taken. The neurogranin levels in the blood samples of both groups were examined using enzyme-linked immunosorbent assay (ELISA) with a 96-well human neurogranin ELISA kit from BenchTop Lab Systems (Geno Technology, Saint Louis, MO, USA). The results were recorded on the data collection form.

Statistical analysis

All data were evaluated using SPSS version 22.0 software (SPSS, Chicago, IL, USA). Descriptive statistics were applied to all study data, and the results were stated as mean ± standard deviation values for continuous variables, and number and percentage for categorical variables. Initially, the data were tested for conformity to the normal distribution using the Shapiro–Wilk test. The serum and CSF neurogranin levels were compared for patients with CNS infection, and the serum neurogranin levels were compared between the patient and control groups. A value of $P < 0.05$ was considered statistically significant.

Table 1. Demographic and clinical data of the patient and control groups

Parameter	Study group	Control group	*P*
Age (years)	37 ± 28 (20–80)	29 ± 18 (18–49)	0.061[a]
Female	4 (26.7%)	8 (53.3%)	0.001[a]
Male	11 (73.3%)	7 (46.7%)	
Body temperature (°C)	38.2 ± 1 (36–39)	36.35 ± 1 (36–37)	0.002[a]
Systolic blood pressure (mm/Hg)	127 ± 23 (107–154)	115 ± 15 (98–124)	0.001[a]
Diastolic blood pressure (mm/Hg)	95 ± 13 (60–100)	78 ± 11 (70–90)	0.000[a]
Pulse (/min)	89.5 (68–164)	70 (76–129)	0.000[a]
CRP (mg/l)	82.4 ± 43.2	10.8 ± 5.6	0.000[b]
Leukocyte (×10^3 units/mm^3)	13.7 ± 6.0	8.9 ± 3.9	0.000[b]
Sodium (mmol/l)	138.6 ± 1.7	135.7 ± 1.4	0.987[b]
CSF glucose (mg/dl)	43.9 ± 9.8		
CSF leukocyte (n/mm^3)	2383.9 ± 459.8		
CSF protein (mg/l)	658.69 ± 27.8		

Data are mean ± SD (range) or n (%).
[a] Mann-Whitney U test.
[b] Independent sample t-test

Table 2. Laboratory data of the patient group

Case no.	Serum neurogranin (ng/ml)	CSF neurogranin (ng/ml)	Case no.	Serum neurogranin (ng/ml)	CSF neurogranin (ng/ml)
1	252.97	333.69	9	133.60	213.26
2	234.89	292.04	10	211.68	250.07
3	305.59	398.84	11	219.60	255.05
4	169.32	247.17	12	218.81	310.00
5	167.84	288.15	13	175.63	216.43
6	1289.42	1248.83	14	1102.09	1205.17
7	256.30	280.83	15	482.76	556.32
8	1217.63	1287.74			

Results

Initially, 55 patients aged >18 years with suspected CNS infection were included in the study. No CNS infection was detected in the lumbar puncture samples of 19 patients; a further 14 patients were excluded because of dementia or Alzheimer's disease, and 7 patients were excluded because they were using antidepressant drugs. Consequently, 15 patients with CNS infection confirmed by lumbar puncture findings and 15 healthy control group subjects were evaluated. The study group consisted of 4 (26.7%) women and 11 (73.3%) men with a mean age of 37 ± 28 (range, 20–80) years. The mean age of the control group was 29 ± 18 (18–49) years, and there was no significant difference between the mean ages of the two groups. The sex distribution of the control group was 8 (53.3%) women and 7 (46.7%) men. In the patient group, the mean systolic blood pressure was 127 ± 23 mmHg (107–154) and the mean diastolic blood pressure was 95 ± 13 mmHg (60–100). In the control group, the mean systolic blood pressure was 115 ± 15 mmHg (98–124) and the mean diastolic blood pressure was 78 ± 11 mmHg (70–90). There was a statistically significant difference between the groups in respect of both the systolic and diastolic blood pressures ($P < 0.001$). The mean body temperature was 38.2 ± 1°C (36–39) in the patient group, and 36.35 ± 1°C (36–37) in the control group, with a statistically significant difference between the two. The mean heart rate was 89.5/min in the patient group and 78/min in the control group, and the difference was statistically significant. The leukocyte levels and C-reactive protein (CRP) values of the patients were significantly higher than those of the control group, but there was no significant difference in serum sodium levels (**Table 1**).

Lumbar punctures were not performed in the control group because it is an invasive procedure. Therefore, the CSF findings of only the patient group could be evaluated. The mean CSF protein value of the study group was 658.69 ± 27.8 mg/l, the CSF leukocyte value was 2383.9 ± 459.8 cells/mm^3, and the CSF glucose level was 43.9 ±

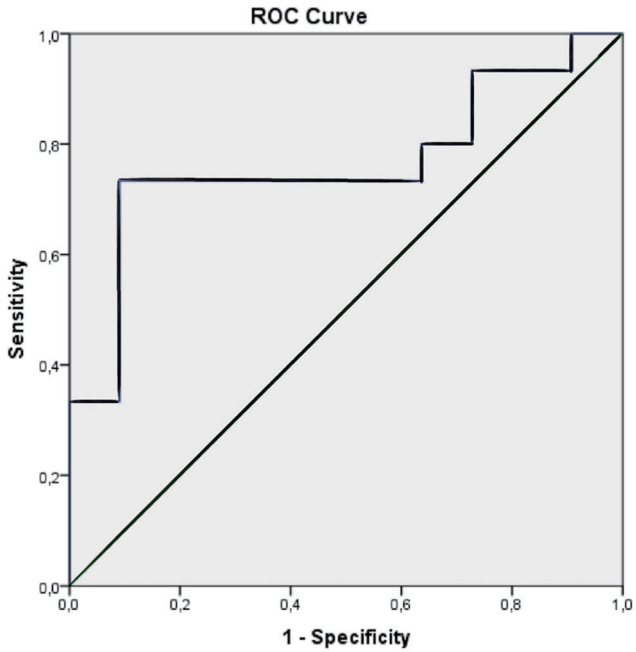

Fig. 1. Characteristic curve for predicting central nervous system infections based on neurogranin levels.

9.8 mg/dl.

The mean CSF neurogranin level in the patient group was 432.4 ± 123.5 ng/ml (**Table 2**). There was a positive correlation between the CSF neurogranin level and the serum neurogranin level (r = 0.615, $P < 0.05$). As the level of CSF neurogranin increased, so did the level of serum neurogranin. A cutoff value for serum neurogranin of 204.26 ng/m yielded 73% sensitivity and 82% specificity for the determination of central nervous system infection (**Fig. 1**).

When the serum neurogranin values were compared between the patient group and the control group, the mean of the control group was 198.6 ± 51.7 ng/ml and the mean of the study group was 429.2 ± 104.3 ng/ml. The mean serum neurogranin level in the current study was elevated in patients with CNS infection, and there was a statistically significant difference between the two groups ($P < 0.01$) (**Table 3**).

Table 3. Neurogranin values of the study and control groups

	Study group (mean ±SD)	Control group (mean ±SD)	*P*
Serum neurogranin (ng/ml)	429.2 ± 104.3	198.6 ± 51.7	0.001[a]
CSF neurogranin (ng/ml)	432.4 ± 123.5		

[a]Independent sample t-test.

Discussion

The results of this study demonstrated that serum neurogranin levels were significantly higher in patients with CNS infection than in the control group. The main reason for this may be disruption of the blood–brain barrier resulting from CNS infection. One of the most important structures constituting the blood–brain barrier are the so-called tight junctions between cells. These structures can be degraded due to the action of the chemical mediators of inflammation caused by infection, such as TNFα, IL-1b, histamine, serotonin, bradykinin, thrombin, and free oxygen radicals. It is known that many factors adversely affect the blood–brain barrier. Intracranial hemorrhage, ischemia and reperfusion, radiation, acute hypertension, encephalomyelitis, brain tumors, hypercapnia, hypoxia, and convulsions all cause an increase in the permeability of the blood–brain barrier.[6,7] With the deterioration of the blood–brain barrier, it is inevitable that the inflammatory mediators in the CNS will pass into the systemic circulation. The increases in neurogranin levels in both the CSF and serum determined in this study are thought to be caused by bacteria or viruses disrupting the blood–brain barrier.

The findings of this study are supported by those of De Vos et al.[8] In that study, serum and CSF neurogranin levels were high in patients with acute ischemic stroke, and a positive correlation was found between the size of the ischemic area and serum neurogranin levels. De Vos et al. attributed this increase to cell death and the deterioration of the blood–brain barrier.[8] Similarly, in a study by Yang et al., the serum neurogranin levels of patients with traumatic brain injury were compared with those of a healthy control group.[9] High levels of serum neurogranin were determined in patients with traumatic brain injury, which was attributed to the breakdown of the blood–brain barrier as a result of the trauma and the permeation of neurogranin into the serum.[9] Thorsell et al. found that levels of neurogranin in both serum and CSF were increased in Alzheimer's patients with CNS degeneration, and stated that neurogranin can be used as a biomarker in diseases leading to brain cell degeneration, such as Alzheimer's disease.[10] To avoid affecting the results of the current study, patients with CNS degeneration were excluded. From these results, it can be hypothesized that neurogranin might be of use as a biomarker not only to detect disease in patients with chronic brain injury, but also in acute brain injuries.

The three most common symptoms or signs of CNS infections are fever, neck stiffness, and changes in mental status. Unfortunately, these three clinical conditions do not coexist in the majority of patients. Headache, fever, nausea, vomiting, chills and shivering, which are found in many infectious diseases, are also found in 85% of CNS infections.[11] Body temperature changes are almost always present. Many patients have body temperatures above 38°C, but hypothermia may also be present.[12] The

clinical findings of most of the current study patients were consistent with those in the literature. The results of the current study suggest that the measurement of serum neurogranin levels could support the diagnosis of CNS infection, especially for undiagnosed patients. The evaluation of serum neurogranin levels may support the diagnosis of CNS infection in patients who do not always present with consistent clinical findings or who cannot undergo brain computed tomography or brain magnetic resonance imaging to evaluate CNS pathology. Serum neurogranin levels may be helpful, especially in patients with CNS infection, when lumbar puncture cannot be performed. However, there are currently no data in the literature on serum neurogranin levels in conditions such as nausea, vomiting, and fever. It would be useful to conduct studies to determine whether the serum levels are affected by these symptoms alone.

In the current study, the CRP levels were high in the patient group. The reason for evaluating this acute phase reactant was to be able to show more clearly that there was a systemic infection in the patients. Whenever there is synaptic damage, neurogranin can increase. Although no studies have evaluated the neurogranin level in infections affecting the brain, it can be considered that future studies will support this hypothesis. It is thought that neurogranin levels will increase in particular in infections affecting the brain together with systemic infection, such as sepsis.

In a study by Jia et al. that evaluated levels of exosomal synaptic proteins, it was stated that evaluation of these proteins in CSF and blood could reflect synaptic changes in the brain.[13] Exosomes are protein-carrying microparticles found in many types of cells. They have small structural dimensions, are similar to the cells in the region where they are found, and can easily cross the blood–brain barrier. Therefore, exosomes can easily remove pathological proteins from the CNS.

Jia et al. evaluated exosomal and CSF protein levels, one of which was neurogranin. In that study of patients with Alzheimer's disease, it was determined that, as the level of exosomal neurogranin decreased, so neurogranin in CSF increased. It was concluded that evaluation of neuronal-derived exosomal or free (serum) neurogranin was a promising biomarker reflecting pathologies in the brain.[13] The findings of the study by Jia et al. support the results of the current study. In the current study, it was found that the synaptic damage resulting from CNS infection increased the serum neurogranin level.

Study Limitations

The most important limitation of this study was the small number of patients. The small number of patients with CNS infections admitted to our hospital annually resulted in this study being conducted as a preliminary study. However, it is anticipated that future studies over a longer time frame and with more patients will support the current findings. Another limitation was that, although the CSF neurogranin levels of the patients were measured, it was not possible to measure the CSF neurogranin levels of the control group. It is unethical to perform lumbar puncture on completely healthy individuals; however, animal experiments could be performed to support these findings. The absence of a statistically significant difference between the blood neurogranin levels and CSF neurogranin levels in the patient group suggests that CSF neurogranin levels increase when the serum neurogranin level increases in cases where the blood–brain barrier is disrupted. Another limitation of this study was that only cases of bacterial meningitis were evaluated. However, it is thought that future studies could show an increase in neurogranin levels both in the serum and CSF in patients with viral or fungal meningitis or encephalitis. The blood–brain barrier is disrupted in both viral and bacterial meningitis and in encephalitis, and new studies on this subject could support this hypothesis. CRP was selected in the current study as a marker of systemic infection. If a correlation had been made with biomarkers more specific to brain tissue, such as SB100 protein or neuron-specific enolase, which increase after disruptions to the blood–brain barrier, the study results would have been strengthened. Future studies incorporating these biomarkers together with neurogranin will increase the reliability of this study.

Conclusion

In conclusion, in accordance with the findings of the current study, the measurement of serum neurogranin levels may be a useful procedure for the diagnosis of CNS infections, especially in patients for whom computed tomography, magnetic resonance imaging, or lumbar puncture cannot be performed.

Conflicts of Interest

No conflicts of interest were declared by the authors.

References

1. Giovane RA, Lavender PD: Central nervous system infections. Prim Care 2018; **45**: 505–518. PMID:30115337, DOI:10.1016/j.pop.2018.05.007
2. Gerendasy DD, Gregor Sutcliffe J: RC3/neurogranin, a postsynaptic calpacitin for setting the response threshold to calcium influxes. Mol Neurobiol 1997; **15**: 131–163. PMID:9396008, DOI:10.1007/BF02740632
3. Li L, Li Y, Ji X, Zhang B, Wei H, Luo Y: The effects of retinoic acid on the expression of neurogranin after experimental cerebral ischemia. Brain Res 2008; **1226**: 234–240. PMID:18602376, DOI:10.1016/j.brainres.2008.06.037
4. Xiang Y, Xin J, Le W, Yang Y: Neurogranin: a potential biomarker of neurological and mental diseases. Front Aging Neurosci 2020; **12**: 584743. PMID:33132903, DOI:10.3389/fnagi.2020.584743

5. Broadbelt K, Ramprasaud A, Jones LB: Evidence of altered neurogranin immunoreactivity in areas 9 and 32 of schizophrenic prefrontal cortex. Schizophr Res 2006; **87**: 6–14. PMID:16797925, DOI:10.1016/j.schres.2006.04.028
6. Abbott NJ: Astrocyte-endothelial interactions and blood-brain barrier permeability. J Anat 2002; **200**: 629–638. PMID:12162730, DOI:10.1046/j.1469-7580.2002.00064.x
7. Obermeier B, Daneman R, Ransohoff RM: Development, maintenance and disruption of the blood-brain barrier. Nat Med 2013; **19**: 1584–1596. PMID:24309662, DOI:10.1038/nm.3407
8. De Vos A, Bjerke M, Brouns R, De Roeck N, Jacobs D, Van den Abbeele L, Guldolf K, Zetterberg H, Blennow K, Engelborghs S, Vanmechelen E: Neurogranin and tau in cerebrospinal fluid and plasma of patients with acute ischemic stroke. BMC Neurol 2017; **17**:170. PMID:28854881, DOI:10.1186/s12883-017-0945-8
9. Yang J, Korley FK, Dai M, Everett AD: Serum neurogranin measurement as a biomarker of acute traumatic brain injury. Clin Biochem 2015; **48**: 843–848. PMID:26025774, DOI:10.1016/j.clinbiochem.2015.05.015
10. Thorsell A, Bjerke M, Gobom J, Brunhage E, Vanmechelen E, Andreasen N, Hansson O, Minthon L, Zetterberg H, Blennow K: Neurogranin in cerebrospinal fluid as a marker of synaptic degeneration in Alzheimer's disease. Brain Res 2010; **1362**: 13–22. PMID:20875798, DOI:10.1016/j.brainres.2010.09.073
11. Putz K, Hayani K, Zar FA: Meningitis. Prim Care 2013; **40**: 707–726. PMID:23958365, DOI:10.1016/j.pop.2013.06.001
12. Tunkel AR, Glaser CA, Bloch KC, Sejvar JJ, Marra CM, Roos KL, Hartman BJ, Kaplan SL, Scheld WM, Whitley RJ, Infectious Diseases Society of America: The management of encephalitis: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis 2008; **47**: 303–327. PMID:18582201, DOI:10.1086/589747
13. Jia L, Zhu M, Kong C, Pang Y, Zhang H, Qiu Q, Wei C, Tang Y, Wang Q, Li Y, Li T, Li F, Wang Q, Li Y, Wei Y, Jia J: Blood neuro-exosomal synaptic proteins predict Alzheimer's disease at the asymptomatic stage. Alzheimers Dement 2021; **17**: 49–60. PMID:32776690, DOI:10.1002/alz.12166