



# Clinical usefulness of brain-derived neurotrophic factor and visinin-like protein-1 in early diagnostic tests for acute stroke <sup>☆</sup>

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## ARTICLE INFO

### Article history:

Received 22 December 2018

Received in revised form 12 February 2019

Accepted 24 February 2019

### Keywords:

VILIP-1

BDNF

Acute stroke

Emergency department

## ABSTRACT

**Objectives:** Lack of a rapid biochemical test for acute stroke is a limitation in the diagnosis and management of acute stroke. The aim of this study is to evaluate the efficacy of BDNF and VILIP-1 as diagnostic markers in acute ischemic stroke and as predictors of mortality.

**Methods:** The study included 75 patients with acute ischemic stroke older than 18 years. During the same period, 28 normal controls were recruited from the hospital ED. Blood samples were collected from all patients at admission to determine the levels of VILIP-1 and BDNF.

**Results:** The mean VILIP-1 levels in the study and control groups were  $0.547 \pm 0.081$  and  $0.515 \pm 0.035$  ng/mL, respectively, and the difference was not significant ( $p = 0.071$ ). The mean BDNF levels in the study and control groups were  $3.89 \pm 2.05$  ng/mL and  $14.9 \pm 4.7$  ng/mL, respectively, and the level was significantly ( $p < 0.0001$ ) lower in the stroke patients.

**Conclusion:** The BDNF level showed a significant ability to discriminate stroke and control patients but did not predict mortality. The VILIP-1 level showed insignificant ability to discriminate stroke patients and again did not predict mortality.

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## 1. Introduction

Stroke is a leading cause of mortality and morbidity worldwide. The rapid identification of stroke patients in the emergency department (ED) is necessary to administer therapies such as tissue plasminogen activator [1]. The diagnosis of ischemic stroke in the ED depends on physical examination, non-contrast computed tomography (CT), and diffusion-weighted magnetic resonance imaging (MRI). Non-contrast CT has poor sensitivity in ischemic stroke, and MRI is not available in every ED. Furthermore, intoxication, hypoglycemia, metabolic disorders, and cerebral mass lesions may mimic the presentation of acute stroke [2,3]. These limitations decrease the definitive diagnosis of stroke, and only a small portion of patients receive thrombolytic therapy. Diagnostic uncertainty has a major role in the underutilization of thrombolytic therapy. Therefore, the development of rapid diagnostic serum biochemical

markers of brain injury may improve the identification of ED patients with stroke [4].

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that facilitates neuronal survival and growth [5,6]. Neurotrophic factors are produced mainly by neurons, but also by cells of the immune system. In the healthy brain, neurons are the major source of neurotrophic factors. Pathological conditions stimulate additional BDNF synthesis from peripheral blood cells to compensate for a lack of BDNF [5,7,8]. The greatest concentrations of BDNF are seen in the memory and learning areas, mainly in the hippocampus and associative cortex. Recent studies showed that the BDNF concentration is correlated with the degree of vasogenic damage to the white matter of the brain. The BDNF genotype may also play a role in the development of cerebral ischemia [5,9].

Visinin-like proteins constitute a highly homologous subfamily of neuronal calcium-sensor proteins, including VILIP-1, VILIP-2, VILIP-3, hippocalcin, and neurocalcin delta [2]. VILIP-1 is found throughout the central nervous system, and it enters the cerebrospinal fluid (CSF) after the destruction of brain cells [10]. Only a few papers have reported the concentrations of VILIP-1 protein in blood or CSF in brain injuries [2].

<sup>☆</sup> The English in this document has been checked by at least two professional editors, both native speakers of English. For a certificate, please see: <http://www.textcheck.com/certificate/UZrooy>

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Therefore, this study evaluated the efficacy of BDNF and VILIP-1 as diagnostic markers in acute ischemic stroke and as predictors of mortality to identify biochemical parameters serving as components of a new biochemistry-based stroke diagnostic system.

## 2. Materials and methods

### 2.1. Study design

The study enrolled acute stroke patients admitted to the Department of Emergency Medicine of Adiyaman University Training and Research Hospital from April 2017 to July 2017 within 4 h of a new neurological event. The clinical diagnosis of stroke was validated by MRI performed on a 1.5 T Achieva scanner (Philips, Best, The Netherlands), as evaluated by radiologists. Stroke volume was measured using the Philips Extended MR Workspace R2.6.3.1.

Ultimately, the study included 75 patients with acute ischemic stroke older than 18 years. During the same period, 28 normal controls were recruited from the hospital ED. The control group consisted of voluntary patients who were admitted to the ED due to non-neurological complaints, who did not have a history of neurological disease or stroke and did not diagnosed with any neurologic condition after clinical evaluation. This prospective study was approved by the Ethics Committee of Adiyaman University (2017/2-7). All patients or their relatives provided written informed consent.

### 2.2. Data collection

Age, sex, vital signs, date, time of admission, stroke localization, infarct volume, Glasgow Coma Scale, National Institutes of Health Stroke Scale (NIHSS), and CHA<sub>2</sub>DS<sub>2</sub>-VASc<sub>2</sub> scores, mortality, body mass index (BMI), white blood cell count, biochemical parameters, and VILIP-1 and BDNF levels were recorded on standardized forms. None of the controls had any comorbidity or chronic disease.

Patients with malignant tumors, transient ischemic attacks, intracerebral hemorrhage, a history of surgery or trauma, severe edema, chronic renal insufficiency, autoimmune diseases, or febrile disorders and those under 18 years old were excluded from the study.

Blood samples were collected from all patients at admission to determine the levels of VILIP-1 and BDNF. All assays were completed within 60 min in the ED laboratory. Residual serum was stored at  $-80^{\circ}\text{C}$ . Blood cell counts were obtained using the CELL-DYN 3700 platform (Abbott Diagnostics, Santa Clara, CA, USA). Serum VILIP-1 and BDNF levels were measured using Cloud-Clone ELISA kits (Katy, TX, USA) and Sunred ELISA kits (Shanghai, China) [respectively]. The serum VILIP-1 and BDNF levels were measured using a quantitative sandwich enzyme immunoassay technique, following the manufacturer's instructions. Briefly, standards, samples, and solutions were added to plates, and then Stop solution was added to all wells. Subsequently, the plates were subjected to spectrophotometric analysis at 450 nm in the EZ Read 400 microplate reader (Biochrom, Cambridge, UK). A standard curve was drawn using the concentrations of the standards, and the OD values were calculated accordingly.

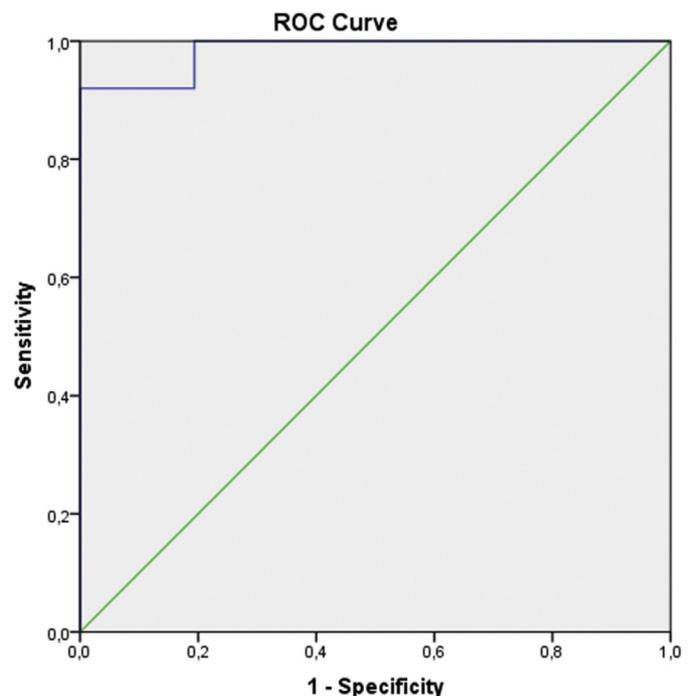
### 2.3. Statistical analysis

The categorical data was expressed as number and percentage, and the numerical data was expressed as mean  $\pm$  standard deviation. The independent samples *t*-test was used to examine normally distributed data. The Mann-Whitney *U* test and chi-square test were used to compare two groups. Spearman's rho was calculated for correlation analysis among infarct volume, NIHSS, VILIP-1,

**Table 1**  
Characteristics of acute stroke patients.

N = 75	(Mean $\pm$ standard deviation)
Age	73.22 $\pm$ 11.57
Infarct volume (cm <sup>3</sup> )	32.74 $\pm$ 63.95
GCS	12.76 $\pm$ 3.15
NIHSS	10.88 $\pm$ 7.72
CHA <sub>2</sub> DS <sub>2</sub> -VASc <sub>2</sub> score	4.11 $\pm$ 1.75
Body mass index	26.79 $\pm$ 4.74
VILIP-1	0.547 $\pm$ 0.081
BDNF	3.89 $\pm$ 2.05
HbA1-c (mmol/mol)	7.04 $\pm$ 2.37
D-dimer (mg/L)	4266.49 $\pm$ 9486.33
Ionize Ca <sup>+</sup> level (mmol/L)	1.17 $\pm$ 0.078
Albumin (g/dL)	3.48 $\pm$ 0.5
Corrected Ca <sup>+</sup> level (mg/dL)	9.41 $\pm$ 0.59
Hs-CRP (mg/dL)	2.49 $\pm$ 3.85
Sedimentation (mm/h)	22.51 $\pm$ 16.54
Lactate (mEq/L)	2.07 $\pm$ 0.88
Cholesterol (mg/dL)	181.25 $\pm$ 44.3
Triglycerides (mg/dL)	163.75 $\pm$ 93.23
HDL cholesterol (mg/dL)	36.17 $\pm$ 10.58
LDL cholesterol (mg/dL)	111.27 $\pm$ 32.91
B12 vitamin (pg/mL)	219.61 $\pm$ 211.66
Folic acid (ng/mL)	7.64 $\pm$ 3.24

and BDNF. The influence of the BDNF and VILIP-1 levels on mortality was examined using binary logistic regression analysis, which allows adjustment for confounding factors (age, sex, BMI, NIHSS score, infarct volume, and vascular risk factors). Results were expressed as adjusted odds ratios (ORs) with the corresponding 95% confidence interval (CI). Receiver operating characteristic (ROC) curves were used to assess the accuracy of the BDNF and VILIP-1 markers to predict mortality, and the results were reported as the area under the curve (AUC). All statistical analyses were performed using SPSS for Windows, ver. 20.0 (SPSS, Chicago, IL, USA). Statistical significance was defined at  $p < 0.05$ .



**Fig. 1.** Receiver operator characteristic curve for BDNF, demonstrating sensitivity as a function of 1-specificity for stroke group from control group based on the logistic model. This logistic model had an area under the receiver operator characteristic curve of 0.983.

**Table 2**

Multivariate analysis of predictors of mortality. Multivariable model included all of the following variables: infarct volume, NIHSS score, serum levels of HsCRP, sedimentation, lactate, B12, Folic acid, VILIP-1 and BDNF.

	OR	95% CI	P
Infarct volume (cm <sup>3</sup> )	1.02	1–1.04	0.055
NIHSS	1.27	0.94–1.7	0.119
GCS	1.06	0.59–1.89	0.845
CHA <sub>2</sub> DS <sub>2</sub> -VASc <sub>2</sub> score	1.42	0.78–2.6	0.257
VILIP-1	0.001	0–268,549,181	0.560
Hs-CRP	1.4	0.96–2.02	0.077
Sedimentation	1.09	1–1.18	0.050
Lactate	0.55	0.16–1.88	0.339
B12	0.98	0.96–1.01	0.127
Folic acid	0.99	0.61–1.62	0.978
BDNF	2.07	0.96–4.48	0.063

Abbreviations: OR, odds ratio; CI, confidence interval; NIHSS, National Institutes of Health Stroke Scale; Hs-CRP, high sensitive C-reactive protein; VILIP-1, Visinin like protein-1; BDNF, brain-derived neurotrophic factor; GCS, Glasgow Coma Scale.

### 3. Results

The 75 stroke patients included 34 (45.33%) men and 41 (54.67%) women. Ten (13.3%) stroke patients died. The mean ages of the survivor and non-survivor groups were 72.3 ± 11.76 and 78.1 ± 10.7 years, respectively, and did not differ significantly ( $p = 0.14$ ). Table 1 shows the characteristics of the study group.

The control group was consisting of 8 males (28.6%) and 20 females (71.4%). The mean age of the control group was 69.14 ± 9.68 years. The mean age of study and control did not differ significantly ( $p = 0.101$ ). The mean VILIP-1 levels in the study and control groups were 0.547 ± 0.081 and 0.515 ± 0.035 ng/mL, respectively, and the difference between groups was not significant ( $p = 0.071$ ) (Fig. 1). The mean VILIP-1 levels in the survivor and non-survivor groups were 0.55 ± 0.086 and 0.55 ± 0.036 ng/mL, respectively, and did not differ ( $p = 0.296$ ).

The mean BDNF levels in the study and control groups were 3.89 ± 2.05 ng/mL and 14.9 ± 4.7 ng/mL, respectively, and the level was significantly ( $p < 0.0001$ ) lower in the stroke patients. Based on the ROC curve, the optimum cutoff serum BDNF level as an indicator of stroke was 7.5 ng/mL, which yielded a sensitivity of 100% and a specificity of 92%. At an AUC of 0.983 (95% CI, 0.966–1), BDNF showed a significant ability to discriminate stroke and control patients (Fig. 1). The mean BDNF levels in the survivor and non-survivor groups did not differ (3.80 ± 1.99 vs. 4.50 ± 2.39 ng/mL, respectively;  $p = 0.296$ ).

NIHSS showed no statistically significant correlation with VILIP-1 and BDNF ( $r = 0.121$  and  $-0.5$ ;  $p = 0.303$  and  $0.971$ , respectively). However, NIHSS and infarct volume showed moderate significant positive correlation ( $r = 0.561$  and  $p < 0.0001$ ). Infarct volume showed not significant correlation with VILIP-1 and BDNF ( $r = 0.61$  and  $-0.064$ ;  $p = 0.601$  and  $0.584$ , respectively). VILIP-1 showed weak and significant positive correlation with age ( $r = 0.269$  and  $p = 0.012$ ). BDNF showed significant and moderate negative correlation with age ( $r = -0.465$  and  $p < 0.0001$ ). Table 2 shows the effects of the predictors of mortality on the study population.

### 4. Discussion

The diagnostic uncertainty associated with ischemia has a major role in the underutilization of thrombolytic therapy. In medical emergencies such as acute coronary syndrome with a compressed timeframe for initiating effective intervention, biomarker-based diagnostic approaches have proven extremely useful. This strategy may also help in diagnosing and managing acute stroke early [11]. A rapid biomarker-based test may facilitate

the diagnosis of stroke and increase the number of patients who have the chance to receive thrombolytic therapy [11].

The BDNF level was significantly lower in our study group than in the controls. BDNF showed a significant ability to discriminate stroke and control patients. Among acute stroke patients, the BDNF levels did not differ between the survivor and non-survivor groups and did not predict mortality. BDNF level was also negatively correlated with age.

This may be due to a decrease in the amount of BDNF released from neurons that decrease in the number and function in stroke patients or elderly population. As a matter of fact, a negative relation with age was found in other studies about BDNF [5]. There are studies showing that in patients with acute ischemic stroke, BDNF can be used as an indicator of poor prognosis in motor dysfunction. The reason for this is the role of BDNF protein in neuronal plasticity and repair mechanisms [12]. In neurodegenerative diseases such as Alzheimer's, Huntington's, Parkinson's diseases, BDNF showed statistically significant results as well. [5]. In the study of Wang et al., it has been shown that blood plasma BDNF can be used effectively both for the comparison of stroke with healthy individuals and for predicting mortality [12]. In our study, although BDNF showed a statistically significant decrease in stroke, it did not show significant results in mortality. Higher levels of BDNF were associated with the absence of stroke.

In our study, the VILIP-1 level showed no significant ability to discriminate stroke and control patients. Outside the brain, VILIP-1 is expressed in the pancreas and heart [13]. These alternative expression sites may decrease the specificity of VILIP-1. Among acute stroke patients, the VILIP-1 levels did not differ in the survivor and non-survivor groups and did not predict the mortality. It is known that increased VILIP-1 protein in trauma in CSF is effective in demonstrating neuronal damage. In addition, there are studies showing that VILIP-1 is effective in demonstrating neuronal damage in Alzheimer's patients [14,15]. Blood plasma VILIP-1 protein level has also been shown to be associated with neuronal damage. In the study of Stejskal et al., it was found that VILIP-1 levels in CSF were highly effective with 100% sensitivity and specificity in comparing acute stroke cases with healthy individuals [2]. However, in our study, VILIP-1 value in plasma did not show significant results in acute ischemic stroke. Therefore, more comprehensive studies on this subject is essential. Since CSF analysis was not performed in our study, the change of VILIP-1 levels in CSF in stroke cases is not known. However, in acute stroke cases, diffusion MRI is a fast and sensitive method and it is obvious that CSF sampling will not be the primary method in the diagnostic process.

There were significant limitations to this study. The small study population, patient status before admission to the ED, complications during hospitalization, and previous unknown chronic diseases may have affected the VILIP-1 and BDNF levels. Also, we didn't classify ischemic stroke patients as in TOAST classification, which could give significant results among subgroups. Due to insufficient data of time from stroke onset, we didn't assess its correlation with VILIP-1 and BDNF. Finally, our control group was consisting of healthy population with no neurologic symptoms. However, the real challenge is to differentiate stroke from other neurological conditions such as epilepsy, brain tumor, migraine, etc. So, further studies are needed to analyze these unexplained points.

### 5. Conclusions

Stroke mortality can be reduced by rapid identification of stroke patients and early administration of therapies such as tissue plasminogen activator. Lack of a rapid diagnostic test for acute stroke is

an important limitation in the diagnosis and management of acute stroke. In this study, the BDNF level showed a significant ability to discriminate stroke and control patients, but did not predict mortality. The VILIP-1 level also showed a significant ability to discriminate stroke patients but again did not predict mortality.

### Competing interests

The authors declare that they have no competing interests.

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