VIA MEDICA

ORIGINAL ARTICLE

Acta Angiol
Vol. 29, No. 3, pp. 85–93
DOI: 10.5603/aa.96853

ISSN 1234–950X
e-ISSN 1644–3276

Visinin-like protein (VLP-1) as a potential marker of brain damage after carotid endarterectomy — preliminary study

Jędrzej Tkaczyk[1], Stanisław Przywara[2], Bożena Kiczorowska[3], Piotr Terlecki[4], Agata Stanek[5], Marek Iłżecki[6]

[1]Doctoral School, Department of Vascular Surgery and Angiology, Medical University of Lublin, Lublin, Poland
[2]Department of Vascular Surgery and Angiology, Medical University of Lublin, Lublin, Poland
[3]Institute of Animal Nutrition and Bromatology, University of Life Sciences in Lublin, Lublin, Poland
[4]Chair and Department of Vascular Surgery and Angiology, Medical University of Lublin, Lublin, Poland
[5]Department and Clinic of Internal Medicine, Angiology and Physical Medicine, Faculty of Medical Sciences in Zabrze, Medical University of Silesia, Zabrze, Poland
[6]Private Specialist Medical Practice Marek Iłżecki, Lublin, Poland

Abstract

Introduction: *Stroke is the second leading cause of disability and death worldwide. Carotid endarterectomy (CEA) reduces the incidence of ischemic stroke or death in patients with sympatomatic carotid artery stenosis more effectively than pharmacological therapy alone. Visinin-like protein 1 (VLP-1) is a potential marker of brain injury. An increased serum level of VLP-1 was observed in neurodegenerative diseases, ischemic stroke, and traumatic brain injury.*

Material and methods: *The objective of the study was to report the changes in serum level concentrations of VLP-1 in patients undergoing CEA. The study group consisted of 22 patients with severe carotid artery stenosis, qualified to CEA. Serum levels of VLP-1 were measured by an enzyme-linked immunosorbent assay (ELISA) test at 24 h before CEA, 12 and 48 h after the surgery.*

Results: *Serum VLP-1 levels were significantly reduced 48 h after CEA compared to the levels before and 12 h after surgery.*

Conclusions: *VLP-1 serum level decreases after an uncomplicated CEA in patients with high-grade carotid artery stenosis. Alterations in this curve may be a marker of neurological events after the procedure. Higher VLP-1 baseline levels before CEA may reflect brain damage caused by chronic ischemia.*

Key words: carotid endarterectomy; carotid artery stenosis; stroke; visinin-like protein

Acta Angiol 2023; 29, 3: 85–93

Introduction

Stroke is the second leading cause of disability and death worldwide, especially in low-and middle-income countries [1]. The most common etiology of ischemic stroke (IS) is atherothrombotic occlusion of the carotid artery, and the others include embolic stroke, cerebral hypoperfusion, and venous thrombosis [2].

Carotid endarterectomy (CEA) is a surgical procedure that reduces the incidence of IS or death in patients

Address for correspondence: Jędrzej Tkaczyk, MD, Doctoral School, Department of Vascular Surgery and Angiology, Medical University of Lublin, S. Staszica 11, 20–081 Lublin, Polska, e-mail: jedrzej.tkaczyk@gmail.com

Received: 06.08.2023 Accepted: 08.08.2023 Early publication date: 25.09.2023

with severe (≥ 70 to 99% of stenosis), symptomatic (IS, TIA, or retinal TIA) carotid artery stenosis more effective than pharmacological therapy alone [3–6]. However, this method is not completely free of complications in the perioperative period, and this may include micro and macro embolism resulting in brain ischemia or ischemia-reperfusion injury and brain edema, caused by clamping and declamping of the internal carotid artery (ICA) during the CEA [7–10].

Visinin-like protein 1 (VLP-1) is a neuron-specific molecule from the highly homologous family of neuronal calcium sensor (NCS) proteins [11], which are involved in several calcium-dependent signal transduction processes in neurons [12]. VLP-1 is responsible for multiple roles in normal neuron function, such as regulation of neuronal ion channels, membrane traffic, learning, neuronal growth, and survival, however, it is also a neurotoxic factor under the condition of the disruption of calcium homeostasis caused by stroke [13, 14]. In this case, calcium overload, an increased level of cytosolic Ca2+ concentration in neurons may lead to the involvement of NCS proteins in numerous necrotic and apoptotic pathways in the central nervous system (CNS) [15].

In 2006, Laterza et al. found VLP-1 in the cerebrospinal fluid (CSF) of a rat model of stroke and in the plasma from patients after stroke, suggesting it to be a potential marker of brain injury [16]. Subsequently, the potential to diagnose ischemic stroke using VLP-1 was confirmed in a pilot study conducted by Stejskal et al. [17]. Recently, Li et al. found that an increase in the level of VLP-1 in patients after stroke can serve as an independent predictor of cognitive dysfunction [18]. In 2020 Liu et al. concluded that serum levels of VLP-1 were associated with poor clinical outcomes in stroke patients [14].

Changes in the VLP-1 level can also be observed in neurodegenerative diseases such as Alzheimer's disease (AD) [19]. A recent meta-analysis conducted by Hao in 2021, which contained 51 studies, confirmed that the level of VLP-1 increased in AD, compared to healthy controls, which confirmed that it could be a valuable marker for the diagnosis of AD [20]. The serum level of VLP-1 also increased after an epileptic seizure, suggesting that it may be a useful biomarker for seizure-induced neuronal injury [21]. Down-regulation of VLP-1 has been reported in amyotrophic lateral sclerosis [ALS] [22]. Later, Liebl et al. suggested, that VLP-1 is a redox sensor that integrates Ca2+ homeostasis and the level of oxidative stress within a cell, and that the presence of VLP-1 dimers and aggregates correlates with the progression of motor neuron disease [23].

VLP-1 was also investigated as a possible biomarker of traumatic brain injury. Wu et al. found that VLP-1 may be a suitable biomarker for the identification of sports-related concussion (SRC) [24]. Similar results were obtained by Bradley-Whitman et al. [25], who found that the serum level of ubiquitinated-VLP-1 was significantly elevated after the brain injury in a well-characterized rat unilateral cortical contusion model. In contrast, Shahim et al. did not observe a significant difference in the plasma level of VLP-1 at 1 h after the concussion compared to the pooled baseline preseason levels, in a group of 288 professional ice hockey players [26].

In the light of the above findings, the aim of our study was to investigate changes in serum level of VLP-1 in patients undergoing CEA.

Material and methods

Patients

The study group consisted of patients with internal carotid artery stenosis, scheduled to undergo CEA in the Department of Vascular Surgery and Angiology of Medical University in Lublin, Poland. The study involved 22 patients aged from 57 to 82 with a mean age of 71.36 (standard deviation = 6.51) years. The degree of internal carotid artery stenosis ranged from 70 to 90%. The inclusion criteria were carotid artery stenosis > 50% in symptomatic patients (symptoms of stroke/TIA < 6 months before), or > 60% in asymptomatic patients with at least 1 feature suggesting higher stroke risk on BMT. The exclusion criteria were: inability to give informed consent, complete occlusion of the internal carotid artery, intracranial artery lesion more significant than the proximal carotid lesion, brain damage in the course of other nervous system diseases, prior ipsilateral CEA, history of disabling stroke (modified Rankin score ≥ 3), active inflammation and expected survival time < 5 years. A Neurological examination was performed by a neurologist prior to and after CEA. In this neurological examination, there were no deviations from normal state in all patients included in the study. Conventional CEA was performed under local anesthesia without the use of a shunt. CEA was performed through a longitudinal arteriotomy, running from the carotid bifurcation to the anterolateral surface of the internal carotid artery ICA. The carotid artery was clamped, and the arteriotomy was closed with primary sutures. No postsurgical complications were observed. Demographic information and pertinent medical history of the patients are summarized in Table 1.

The estimation of internal carotid artery stenosis degree

The degree of internal carotid artery stenosis was determined based on a high-resolution USG Doppler examination, performed with a Toshiba Aplio 500 de-

Table 1. Characteristics of patients

Patient ID	Sex	Age	Location	%	Stroke/TIA	Symptoms	Other diseases
1	M	74	R	90	No	Tinnitus, hypoacusis	None
2	F	68	L	90	No	Tinnitus, dizziness	Diabetes, arterial hypertension, ischemic heart disease
3	M	57	R	70	No	None	Ischemic heart disease
4	M	78	R	80	No	Visual disturbances	Diabetes, arterial hypertension
5	M	74	L	90	No	Tinnitus, dizziness	Diabetes, arterial hypertension, ischemic heart disease
6	F	67	R	90	Stroke	Dizziness	Diabetes, arterial hypertension
7	M	67	L	90	Stroke	Hemiparesis	Diabetes, arterial hypertension
8	F	79	L	80	No	None	Arterial hypertension
9	M	78	R	90	Stroke	Hemiparesis	Arterial hypertension
10	F	63	L	90	No	Tremor	Diabetes, arterial hypertension
11	M	63	L	80	No	None	Arterial hypertension, ischemic heart disease
12	M	74	R	90	Stroke	None	Arterial hypertension
13	M	63	L	90	Stroke	Hemiparesis	Arterial hypertension
14	F	82	L	80	No	Dizziness	Arterial hypertension
15	M	74	L	90	No	None	Diabetes, arterial hypertension, ischemic heart disease
16	M	76	L	80	Stroke	None	Arterial hypertension
17	M	76	L	85	TIA	None	Diabetes, arterial hypertension, ischemic heart disease
18	F	64	L	90	TIA	None	Diabetes, arterial hypertension
19	F	72	L	70	No	None	Arterial hypertension
20	M	77	L	90	Stroke	Hemiparesis	Arterial hypertension
21	M	67	R	90	Stroke	Hemiparesis	Diabetes, arterial hypertension
22	M	77	L	80	No	Dizziness, visual disturbances	Arterial hypertension

ID — identification; M — male; F — female; TIA — transient ischemic attack; L — left; R — right

vice with a high-frequency (11 MHz) linear probe. The sonographer was a vascular medicine specialist who was unaware of the subject's clinical state.

Based on Doppler studies, patients were qualified for the CEA procedure as determined by the guidelines established by the European Society of Vascular Surgery [27]. Patients with severe carotid artery stenosis were identified using criteria established by NASCET (North American Symptomatic Carotid Endarterectomy Trial) according to the following formula: % ICA stenosis = (1-[narrowest ICA diameter/diameter normal distal cervical ICA]) × 100 [6].

Biochemical analysis of visinin-like protein 1

Serum samples were taken from the antecubital vein of the patients at three different times: within 24 hours preoperatively to CEA, 12 hours postoperatively, and 48 hours postoperatively.

Serum for the analysis of specific proteins was obtained by centrifugation of whole blood at 3000 rpm (603 × g) for 15 min in a laboratory centrifuge at a temperature of 4°C and stored at –80°C prior to analyses. Plasma without signs of hemolysis was analyzed using a quantitative sandwich enzyme-linked immunosorbent assay (ELISA) technique. The protocols were adapted from a commercially developed assay manufactured by Bioassay Technology Laboratory (BT Lab, Zhejiang, China). The concentrations of visinin-like protein 1 (VLP-1); Cat. No: E4118Hu were quantified based on the optical density (OD) at 450 nm using the BioTek ELx808™ Absorbance Microplate Reader (BioTek, Winooski, VT, USA). The samples for each participant were diluted to fit the range of the standard curve and

Table 2. Serum levels of visinin-like protein 1 (VLP-1) and a comparative analysis

VLP-1 level (μg/l)						
	N	Mean	Median	SD	SE	p
Before	22	0.065	0.060	0.022	0.00477	p = 0.00041*
12 h after	22	0.054	0.053		0.024	0.00514
48 h after	22	0.0417	0.042		0.018	0.00392
Difference					**Significance**	
Before — 12h after					p = 0.03251	
Before — 48h after					p = 0.00009*	
12h after — 48h after					p = 0.00639*	

*statistically significant; SE — standard error; SD — standard deviation; N — number of patients

run in duplicate on the same plate. Briefly, the plates were precoated with a human antibody, specific for each analyzed protein. A specific biotinylated antibody was also added to each sample. Then, streptavidin-HRP was added to the sample and standard wells. After incubation, plates were washed with 5× washing buffer with an automatic plate washer. Substrate solutions were added and once again the plates were incubated. The reaction was terminated by the addition of a stop solution. The concentration of protein levels in samples was calculated based on the standard curves using the average of the duplicate values.

Statistical analysis

The distribution of the collected data was evaluated using the Shapiro-Wilk test showing normal distribution for VLP-1. Furthermore, the data on the VLP-1 levels were analyzed using the one-way repeated measures ANOVA test with post hoc student's t-test using Bonferroni correction. Correlation analysis was performed using the Spearman rank correlation. The VLP-1 values were expressed in μg/l. The values of $p < 0.05$ were considered significant.

Results

The repeated measures ANOVA test showed that a sampling time significantly affected ($p < 0.05$) serum VLP-1 levels; $F(2, 42) = 12.96$, $p = 0.00041$. Serum VLP-1 concentrations in patients and a comparative analysis are presented in Table 2.

The post-hoc paired student's t-test using a Bonferroni corrected $\alpha = 0.0166$ (0.05/3) indicated that the VLP-1 level was statistically significantly decreased 48 h after CEA as compared to the levels measured prior to surgery (Fig. 1), and 12 h after the surgery (Fig. 2) ($p < 0.0166$). However, the difference in serum

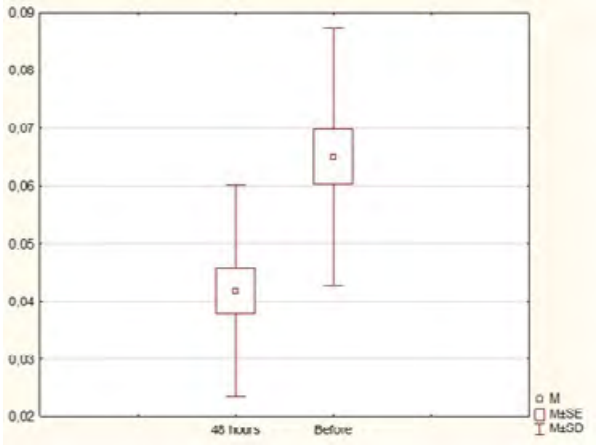

Figure 1. Serum visinin-like protein 1 (VLP-1) levels in patients before and 48 hours after the procedure, Bonferroni p = 0.00009** (M — mean; SE — standard error; SD — standard deviation); *statistically significant

VLP-1 levels prior to surgery and 12 h after CEA (Fig. 3) was not statistically significant ($p > 0.0166$).

There were no differences in serum VLP-1 concentrations in three measurements between males and females ($p > 0.05$). The difference in serum VLP-1 concentrations between younger (< 69 years) and older (> 69 years) patients in the three measurements was also not significant ($p > 0.05$). There was no correlation between serum creatinine level and the level of VLP-1 in all three measurements ($p > 0.05$).

Discussion

Our study revealed that the serum VLP-1 level decreased statistically significantly 48h after CEA compared to the levels measured before surgery and 12h after surgery.

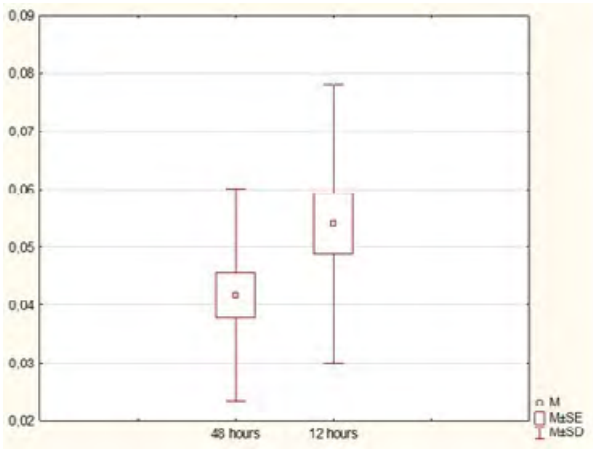

Figure 2. Serum visinin-like protein 1 (VLP-1) levels in patients 48 and 12 hours after the procedure, Bonferroni p = 0.00639** (M — mean; SE — standard error; SD — standard deviation); *statistically significant

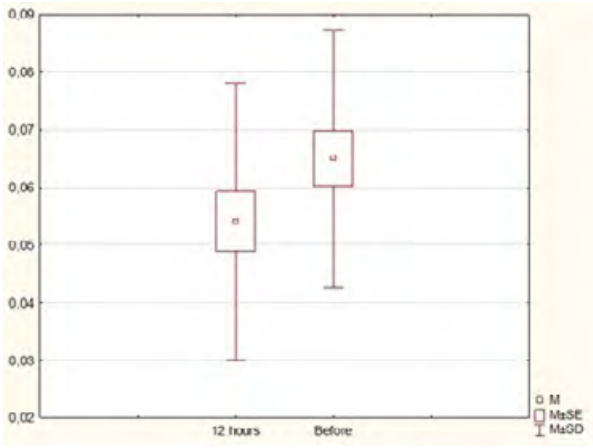

Figure 3. Serum visinin-like protein 1 (VLP-1) levels in patients before and 12 hours after the procedure, Bonferroni p = 0.03251. (M — mean; SE — standard error; SD — standard deviation)

To the best of our knowledge, this study is the first to evaluate the influence of the uncomplicated CEA on the level of VLP-1, and it presents data on a characteristic time curve of this molecule in the perioperative period of CEA.

At the beginning of 2006, Laterza et al. identified VLP-1 in cerebrospinal fluid from a rat model of a retrospectively collected stroke, and in plasma from 18 patients after stroke [16].

In 2011 Stejskal et al. developed the ELISA sandwich method for the determination of VLP-1. The study was held on a group of 33 patients: 17 healthy subjects and 16 suffering from ischemic stroke. The authors presented a statistically significant ($p < 0.01$) difference in the level of VLP-1 in both serum and cerebrospinal fluid (CSF), between these two groups, assuming that VLP-1 may be an independent marker of brain injury. The authors did not find significant differences in VLP-1 concentration between men and women in either serum or CSF in the group of healthy subjects, nor in the group of patients with ischemic stroke. The limitations of the study included a small number of patients and the selection of the control group, which did not allow one to estimate the precision for differentiating stroke from other neurological diseases [17].

A larger group of 110 patients with a stroke, was investigated by Li et al. in 2018. The authors found that in the stroke group, the level of VLP-1 was significantly ($p < 0.001$) higher than that in the control group, and in the patients with cognitive dysfunction, the level of VILIP-1 was higher than that in the normal cognition group. Authors therefore concluded that this molecule can serve as a predictor for cognitive dysfunction after stroke [18].

In 2020 Liu et al. investigated a group of 80 stroke patients and found that infarct volume and serum VLP-1 concentrations were highly and significantly correlated, and VLP-1 levels showed a strong association with poor stroke outcome [14].

In contrast, the study conducted by Park et al. in 2013 in a group of 175 patients found that the peripheral blood level of VLP-1 did not increase after stroke and was therefore not associated with stroke outcome, probably due to sampling of blood markers within 24 hours after stroke onset, when the release of proteins from the damaged brain to peripheral blood has not reached the peak yet [28].

Up to now, VLP-1 has not been investigated in the perioperative period of carotid endarterectomy; however, some other molecules, considered markers of brain damage, were previously studied before and after this procedure.

In 2001 Connolly ES Jr et al. conducted a study on a group of 25 patients who underwent CEA, measuring serum levels of S100B protein and neuron-specific enolase before (24h and pre-clamping), and after (24, 48, and 72 hours) the procedure. The study group was divided to: injured (those who exhibited significant declines in neuropsychometric test performance), and uninjured patients. As a result, injured patients presented significantly higher S100B levels, compared with uninjured patients, at 24, 48, and 72 hours after surgery ($P < 0.05$). However, S100B levels were elevated not only during the postoperative period but also during the preclamp period. Although the mechanism of cerebral injury among CEA patients demonstrating a decrease in neuropsychometric test performance is unknown, hypoperfusion or microemboli could be responsible. There were no significant differences in neuron-specific

enolase levels for injured and uninjured patients at any time point [29].

Our study showed that VLP-1 levels decreased after CEA. Rasmussen et al. presented similar results. The authors measured serum levels of neuron -specific enolase (NSE) and S100B, a different marker of brain damage, in a group of 22 patients undergoing carotid endarterectomy, preoperatively and postoperatively (12, 24, 36 and 48 h after CEA). The results showed that NSE level was significantly higher before carotid artery surgery and decreased postoperatively, while S100B level did not significantly change. The authors found no correlation between the change in cognitive function and the changes in blood levels of NSE or S100B protein [30].

The same molecules were investigated by Brightwell et al. [31], on a group of. 52 patients with carotid artery stenosis, of whom 28 underwent CEA and 24 carotid artery stenting (CAS). Both NSE and S100B were significantly higher at the baseline compared to the normal population. After the intervention, there was a non--significant trend of a transient rise of S100B levels in the CAS group, while the levels in the CEA group appeared unchanged. NSE appeared to increase at 48 hours postoperatively in the CEA group and decline in the CAS group, however, differences were not statistically significant. S100B alone increased significantly at 24 hours in those patients with postoperative neurological deficit ($p = 0.015$), and in those with emboli detected by the perioperative transcranial Doppler examination.

Mussack et al. conducted a study on a group of 46 patients with carotid artery stenosis, treated with CEA or CAS, and found that CEA but not CAS was associated with a transient increase in serum levels of S-100B, which later returned to baseline levels. The prolonged elevation of serum S-100B levels corresponded to the development of postoperative neurological deficits [32].

Earlier, a study group from our Department investigated a number of different molecules, that were potential brain damage markers, in the perioperative period of CEA.

In 2014 Terlecki et al. investigated the plasma level of kynurenic acid (KYNA) in a group of 40 patients, who underwent CEA or carotid artery stenting (CAS). Patients who underwent CEA were divided into groups with stable and unstable carotid artery plaque. KYNA level was measured before surgery and 1, 6, 24, and 48 hours after surgery. The authors found that the baseline value of plasma KYNA concentrations determined before surgery were higher in patients with unstable carotid plaque undergoing CEA than in patients with stable carotid plaque undergoing CEA and patients undergoing CAS. Independent of the baseline KYNA level, its concentration increased during the postoperative period in all studied groups. Higher plasma KYNA concentrations were noted in patients with postoperative neurological disorders. KYNA value was associated with the degree of inflammation measured by NLR (neutrophil-lymphocyte ratio) [33].

Later, in 2016 Ilzecki et al. investigated serum levels of several molecules in the perioperative period of patients undergoing uncomplicated CEA. Serum levels of carnosine dipeptidase 1 (CNDP1) and terminal hydrolase L1 (UCHL1) Ubiquitin C decreased significantly 12 h after CEA compared to the level before surgery and then normalized 48 h after CEA [34]. Microtubule--associated protein tau (MAPt) and myelin basic protein (MBP) presented a similar time curve, and significantly decreased 12 hours after CEA compared to the level before the surgery ($p < 0.05$), but then normalized 48 hours after CEA [35]. Three other molecules: glial fibrillary acidic protein (GFAP), neurofilament light polypeptide (NEFL), and brain lipid-binding protein (FABP7) levels were not statistically different between all the three measurements ($p > 0.05$) [36, 37].

In the other study, published by Ilzecki et al. in 2016, authors showed in the group of 25 patients undergoing uncomplicated CEA, that the serum level of NSE was statistically significantly increased 48 h after CEA as compared with the levels measured 12 h after surgery and prior to surgery ($p < 0.05$) [38].

Therefore, the results obtained in our study appear to be similar to some of the previous research, in which the serum level of brain damage markers (CNDP1, UCHL1, MAPt, MBP) significantly decreased in uninjured patients, after the uncomplicated CEA. The reason for that could be chronic brain ischemia caused by a high-grade internal carotid artery stenosis which could be responsible for the chronic damage of the brain, and therefore higher baseline level of brain damage markers such as VLP-1. Then, after the CEA, when the perfusion normalizes, the VLP-1 level drops with time. Similar results obtained by Rasmussem and Brightwell, who investigated NSE levels before and after uncomplicated CEA, and Ilzecki, who investigated levels of CNDP1, UCHL1, MAPt, and MBP seem to support this hypothesis. However, other levels of some other brain damage markers, such as S100B and KYNA appeared to increase after CEA.

The reason why different molecules established as brain damage markers act differently after CEA surgery remains unknown. It is possible, that some of them show higher sensitivity in detecting damage to the brain, and only these could detect small injuries of the brain such as silent ischemia after the CEA, caused by clamping and declamping the artery, microemboli, ischemia-reperfusion etc.

Higher VLP-1 levels before the procedure may represent brain damage caused by chronic ischemia, which is reduced by successful, uncomplicated CEA. Chronic cerebral hypoperfusion (CCH) has previously been investigated to activate a molecular and cellular injury cascade that leads to the breakdown of the blood-brain barrier (BBB) and neurodegeneration [39]. Therefore, brain damage markers, such as VLP 1, may be elevated, and found in the peripheral blood, due to a BBB impairment.

Up to now, there is no data on the possibility of release of the VLP-1, e.g. directly from the atherosclerotic plaque. However, there is some evidence of the presence of VLP-1 in extra-neuronal tissues. Buttgereit et al. [40] described VLP-1 expression in the human heart, rat cardiomyocytes, and H9c2 cells, and demonstrated that VLP-1 regulates the cell surface localization of natriuretic peptide receptor B. Dai et al. have shown that VLP-1 is expressed in pancreatic beta-cells and modulates insulin secretion [41]. Guerrico et al. showed that VLP-1 decreased cell adhesion and invasiveness of highly invasive squamous carcinoma cells in mice [42]. In addition, Akagi et al. demonstrated a prognostic role for VLP-1 at the mRNA level, suggesting its possible usefulness as a predictor of lymph node metastasis and a poor prognosis in colorectal cancer [43].

The authors are aware that one of the limitations of our study is the small study group (22 patients), without a control group of healthy subjects or patients with neurological complications after the CEA. However, the aim of the study was to investigate how the VLP-1 level acts in patients who underwent successful, uncomplicated CEA, and therefore it can be treated as a pilot study, while there is still a need for the next, more complex research taking into account these issues in the future.

The other limitation is only one surgical technique of CEA (primary suture), without the use of a shunt in all of the patients. However, in a group of 22 patients differentiating the surgical technique and dividing the study group into smaller subgroups could make the statistical analysis unreliable.

Conclusions

On the basis of the obtained results, it may be concluded that alternations in the curve of the serum level of VLP-1 may be a marker of neurological complications after the CEA procedures, however, further investigations are needed. Additionally, higher VLP-1 baseline levels before CEA may reflect brain damage caused by chronic ischemia.

Conflict of interest

None.

References

1. Saini V, Guada L, Yavagal DR. Global epidemiology of stroke and access to acute ischemic stroke interventions. neurology. 2021; 97(20 supl. 2): S6–SS16, doi: 10.1212/WNL.0000000000012781, indexed in Pubmed: 34785599.
2. Yip HK, Sung PH, Wu CJ, et al. Carotid stenting and endarterectomy. Int J Cardiol. 2016; 214: 166–174, doi: 10.1016/j.ijcard.2016.03.172, indexed in Pubmed: 27061654.
3. Randomised trial of endarterectomy for recently symptomatic carotid stenosis: final results of the MRC European Carotid Surgery Trial (ECST). The Lancet. 1998; 351(9113): 1379–1387, doi: 10.1016/s0140-6736(97)09292-1.
4. Mayberg MR. Carotid endarterectomy and prevention of cerebral ischemia in symptomatic carotid stenosis. Veterans Affairs Cooperative Studies Program 309 Trialist Group. JAMA: The Journal of the American Medical Association. 1991; 266(23): 3289–3294, doi: 10.1001/jama.266.23.3289.
5. Barnett HJ, Taylor DW, Eliasziw M, et al. Benefit of carotid endarterectomy in patients with symptomatic moderate or severe stenosis. North American Symptomatic Carotid Endarterectomy Trial Collaborators. N Engl J Med. 1998; 339(20): 1415–1425, doi: 10.1056/NEJM199811123392002, indexed in Pubmed: 9811916.
6. Ferguson GG, Eliasziw M, Barr HW, et al. The North American Symptomatic Carotid Endarterectomy Trial: surgical results in 1415 patients. Stroke. 1999; 30(9): 1751–1758, doi: 10.1161/01.str.30.9.1751, indexed in Pubmed: 10471419.
7. Gupta N, Corriere MA, Dodson TF, et al. The incidence of microemboli to the brain is less with endarterectomy than with percutaneous revascularization with distal filters or flow reversal. J Vasc Surg. 2011; 53(2): 316–322, doi: 10.1016/j.jvs.2010.08.063, indexed in Pubmed: 21129899.
8. Backhaus R, Boy S, Fuchs K, et al. Hyperperfusion syndrome after MCA embolectomy - a rare complication? Am J Case Rep. 2013; 14: 513–517, doi: 10.12659/AJCR.889672, indexed in Pubmed: 24340127.
9. Lieb M, Shah U, Hines GL. Cerebral hyperperfusion syndrome after carotid intervention: a review. Cardiol Rev. 2012; 20(2): 84–89, doi: 10.1097/CRD.0b013e318237eef8, indexed in Pubmed: 22183061.
10. Capoccia L, Sbarigia E, Rizzo AR, et al. Contralateral occlusion increases the risk of neurological complications associated with carotid endarterectomy. Int J Vasc Med. 2015; 2015: 942146, doi: 10.1155/2015/942146, indexed in Pubmed: 25705519.
11. Braunewell KH, Klein-Szanto AJ. Visinin-like proteins (VSNLs): interaction partners and emerging functions in signal transduction of a subfamily of neuronal Ca2+ -sensor proteins. Cell Tissue Res. 2009; 335(2): 301–316, doi: 10.1007/s00441-008-0716-3, indexed in Pubmed: 18989702.
12. Zhao C, Braunewell KH. Expression of the neuronal calcium sensor visinin-like protein-1 in the rat hippocampus. Neuroscience. 2008; 153(4): 1202–1212, doi: 10.1016/j.neuroscience.2007.10.067, indexed in Pubmed: 18440708.
13. Groblewska M, Muszyński P, Wojtulewska-Supron A, et al. The role of visinin-like protein-1 in the pathophysiology of

Alzheimer's disease. J Alzheimers Dis. 2015; 47(1): 17–32, doi: 10.3233/JAD-150060, indexed in Pubmed: 26402751.
14. Liu D, Dong X, Yang R, et al. Visinin-like protein-1 level is associated with short-term functional outcome of acute ischemic stroke: A prospective cohort study. Medicine (Baltimore). 2020; 99(9): e19252, doi: 10.1097/MD.0000000000019252, indexed in Pubmed: 32118731.
15. Blandini F, Braunewell KH, Manahan-Vaughan D, et al. Neurodegeneration and energy metabolism: from chemistry to clinics. Cell Death Differ. 2004; 11(4): 479–484, doi: 10.1038/sj.cdd.4401323, indexed in Pubmed: 14713955.
16. Laterza OF, Modur VR, Crimmins DL, et al. Identification of novel brain biomarkers. Clin Chem. 2006; 52(9): 1713–1721, doi: 10.1373/clinchem.2006.070912, indexed in Pubmed: 16858073.
17. Stejskal D, Sporova L, Svestak M, et al. Determination of serum visinin like protein-1 and its potential for the diagnosis of brain injury due to the stroke: a pilot study. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2011; 155(3): 263–268, doi: 10.5507/bp.2011.049, indexed in Pubmed: 22286812.
18. Li Y, Wu Xqa. Study on the correlation of cognitive dysfunction after stroke with the levels of VILIP-1 and HS-CRP in serum. Acta Medica Mediterranea. 2018 Oct 17. ; 6: 1895–9, doi: 10.19193/0393-6384_2018_6_294.
19. Tarawneh R, D'Angelo G, Macy E, et al. Visinin-like protein-1: diagnostic and prognostic biomarker in Alzheimer disease. Ann Neurol. 2011; 70(2): 274–285, doi: 10.1002/ana.22448, indexed in Pubmed: 21823155.
20. Hao Y, Liu Xu, Zhu R. Neurodegeneration and glial activation related CSF biomarker as the diagnosis of Alzheimer's disease: A Systematic Review and an Updated Meta- analysis. Curr Alzheimer Res. 2022; 19(1): 32–46, doi: 10.2174/1567205018666211208142702, indexed in Pubmed: 34879804.
21. Tan Z, Jiang J, Tian F, et al. Serum visinin-like protein 1 Is a better biomarker than neuron-specific enolase for seizure-induced neuronal injury: a prospective and observational study. Front Neurol. 2020; 11: 567587, doi: 10.3389/fneur.2020.567587, indexed in Pubmed: 33071949.
22. Lederer CW, Torrisi A, Pantelidou M, et al. Pathways and genes differentially expressed in the motor cortex of patients with sporadic amyotrophic lateral sclerosis. BMC Genomics. 2007; 8: 26, doi: 10.1186/1471-2164-8-26, indexed in Pubmed: 17244347.
23. Liebl MP, Kaya AM, Tenzer S, et al. Dimerization of visinin-like protein 1 is regulated by oxidative stress and calcium and is a pathological hallmark of amyotrophic lateral sclerosis. Free Radic Biol Med. 2014; 72: 41–54, doi: 10.1016/j.freeradbiomed.2014.04.008, indexed in Pubmed: 24742816.
24. Wu Y, Abner EL, Conley C, et al. Preliminary evaluation of a novel point of care diagnostic device for sports-related concussion. Clin J Sport Med. 2022; 32(6): 623–626, doi: 10.1097/JSM.0000000000001056, indexed in Pubmed: 36315821.
25. Shahim P, Mattsson N, Macy EM, et al. Serum visinin-like protein-1 in concussed professional ice hockey players. Brain Inj. 2015; 29(7-8): 872–876, doi: 10.3109/02699052.2015.1018324, indexed in Pubmed: 25955117.
26. Bradley-Whitman MA, Roberts KN, Abner EL, et al. A novel method for the rapid detection of post-translationally modified visinin-like protein 1 in rat models of brain injury. Brain Inj. 2018; 32(3): 363–380, doi: 10.1080/02699052.2017.1418907, indexed in Pubmed: 29283288.
27. Naylor AR, Ricco JB, de Borst GJ, et al. Editor's Choice - management of atherosclerotic carotid and vertebral artery disease: 2017 Clinical Practice Guidelines of the European Society for Vascular Surgery (ESVS). Eur J Vasc Endovasc Surg. 2018; 55(1): 3–81, doi: 10.1016/j.ejvs.2017.06.021, indexed in Pubmed: 28851594.
28. Park SY, Kim J, Kim OJ, et al. Predictive value of circulating interleukin-6 and heart-type fatty acid binding protein for three months clinical outcome in acute cerebral infarction: multiple blood markers profiling study. Crit Care. 2013; 17(2): R45, doi: 10.1186/cc12564, indexed in Pubmed: 23497639.
29. Connolly ES, Winfree CJ, Rampersad A, et al. Serum S100B protein levels are correlated with subclinical neurocognitive declines after carotid endarterectomy. Neurosurgery. 2001; 49(5): 1076–82; discussion 1082, doi: 10.1097/00006123-200111000-00010, indexed in Pubmed: 11846900.
30. Rasmussen LS, Christiansen M, Johnsen J, et al. Subtle brain damage cannot be detected by measuring neuron-specific enolase and S-100beta protein after carotid endarterectomy. J Cardiothorac Vasc Anesth. 2000; 14(2): 166–170, doi: 10.1016/s1053-0770(00)90012-0, indexed in Pubmed: 10794336.
31. Brightwell RE, Sherwood RA, Athanasiou T, et al. The neurological morbidity of carotid revascularisation: using markers of cellular brain injury to compare CEA and CAS. Eur J Vasc Endovasc Surg. 2007; 34(5): 552–560, doi: 10.1016/j.ejvs.2007.06.016, indexed in Pubmed: 17719806.
32. Mussack T, Hauser C, Klauss V, et al. Serum S-100B protein levels during and after successful carotid artery stenting or carotid endarterectomy. J Endovasc Ther. 2006; 13(1): 39–46, doi: 10.1583/05-1656.1, indexed in Pubmed: 16445322.
33. Terlecki P, Pawlik P, Iwaniuk A, et al. Carotid surgery affects plasma kynurenic acid concentration: a pilot study. Med Sci Monit. 2014; 20: 303–310, doi: 10.12659/MSM.890212, indexed in Pubmed: 24561546.
34. Ilzecki M, Ilzecka J, Przywara S, et al. Serum carnosine dipeptidase 1 and ubiquitin C - terminal hydrolase L1 as markers of brain damage in patients after carotid endarterectomy. Ulutas Med J. 2016; 2(3): 132, doi: 10.5455/umj.20160713014033.
35. Ilzecki M, Przywara S, Ilzecka J, et al. Serum microtubule associated protein tau and myelin basic protein as the potential markers of brain ischaemia-reperfusion injury in patients undergoing carotid endarterectomy. Acta Angiologica. 2016; 22(2): 37–43, doi: 10.5603/aa.2016.0008.
36. Iłżecki M, Iłżecka J, Przywara S, et al. Effect of carotid endarterectomy on brain damage markers. Acta Neurol Scand. 2017; 135(3): 352–359, doi: 10.1111/ane.12607, indexed in Pubmed: 27126899.
37. Ilzecki M, Przywara S, Ilzecka J, et al. Serum glial fibrillary acidic protein as a marker of brain damage in patients after carotid endarterectomy. Acta Angiologica. 2016; 22(1): 1–4, doi: 10.5603/aa.2016.0001.
38. Iłżecki M, Iłżecka J, Przywara S, et al. Serum neuron-specific enolase as a marker of brain ischemia-reperfusion injury in patients undergoing carotid endarterectomy. Acta Clin Croat. 2016; 55(4): 579–584, doi: 10.20471/acc.2016.55.04.07, indexed in Pubmed: 29117648.

39. Rajeev V, Fann DY, Dinh QN, et al. Pathophysiology of blood brain barrier dysfunction during chronic cerebral hypoperfusion in vascular cognitive impairment. Theranostics. 2022; 12(4): 1639–1658, doi: 10.7150/thno.68304, indexed in Pubmed: 35198062.
40. Buttgereit J, Qadri F, Monti J, et al. Visinin-like protein 1 regulates natriuretic peptide receptor B in the heart. Regul Pept. 2010; 161(1-3): 51–57, doi: 10.1016/j.regpep.2009.12.019, indexed in Pubmed: 20079378.
41. Dai FF, Zhang Yi, Kang Y, et al. The neuronal Ca2+ sensor protein visinin-like protein-1 is expressed in pancreatic islets and regulates insulin secretion. J Biol Chem. 2006; 281(31): 21942–21953, doi: 10.1074/jbc.M512924200, indexed in Pubmed: 16731532.
42. Gonzalez Guerrico AM, Jaffer ZM, Page RE, et al. Visinin-like protein-1 is a potent inhibitor of cell adhesion and migration in squamous carcinoma cells. Oncogene. 2005; 24(14): 2307–2316, doi: 10.1038/sj.onc.1208476, indexed in Pubmed: 15735716.
43. Akagi T, Hijiya N, Inomata M, et al. Visinin-like protein-1 overexpression is an indicator of lymph node metastasis and poor prognosis in colorectal cancer patients. Int J Cancer. 2012; 131(6): 1307–1317, doi: 10.1002/ijc.27341, indexed in Pubmed: 22052372.