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Serum Carnosine Dipeptidase 1 and Ubiquitin C - Terminal Hydrolase L1 as Markers of Brain Damage in Patients after Carotid Endarterectomy

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Background: Carotid endarterectomy (CEA) is the recommended surgical procedure in the prevention of ischemic stroke. However, this procedure may lead to vascular neurological complications. The aim of the study was to measure serum carnosine dipeptidase 1 (CNDP1) and ubiquitin C - terminal hydrolase L1 (UCHL1)as markers of brain damagein patients that underwent CEA due to high-grade internal carotid artery stenosis.

Method: This study included in 25 patients. Blood samples were taken from the antecubital vein at three different intervals (within a 24 h period prior to CEA, 12 h following surgery, and 48 h after surgery). Serum CNDP1 and UCHL1 levels were measured by a commercially available enzyme-linked immunosorbent assay (ELISA).

Results: The study showed that serum CNDP1 and UCHL1 levels were significantly decreased 12 h after CEA when compared to level before the surgery. Furthermore, these enzymes levels were normalized 48 h after CEA.

Conclusion: Data from our study showed that CEA significantly affects serum CNDP1 and UCHL1 levels. Moreover, these enzyme levels seems to reflect a brain ischemia resulting from severe internal carotid artery stenosis in patients undergoing CEA.

Keywords: Carnosine dipeptidase 1, carotid endarterectomy, ischemic stroke, ubiquitin C, terminal hydrolase L1

Introduction

Stroke hassignificant mortality, morbidity, and socioeconomic consequences for patients, their partners, and society (1). Carotid endarterectomy (CEA) is the recommended surgical procedure in prevention of ischemic stroke (2). However, surgical treatment of atherosclerotic stenosis may lead to neurological vascular complications during the perioperative period. These complications include, among others, micro and macro embolism resulting in brain injury caused by ischemia. Moreover, acute ischemia and reperfusion by clamping and declamping of the internal carotid artery during CEA may cause cerebrovascular auto regulation damage, hyperperfusion, and brain edema (3-6).

Carnosinases are dipeptidases play diverse functions in all areas of life. Under appropriate conditions, human isoforms of carnosinase

catalyze the hydrolysis of the dipeptide carnosine (β-alanyl-L-histidine) and homocarnosine (γ-aminobutyryl-L-histidine). Alterations of serum carnosine dipeptidase 1 (CNDP1) activity have been associated with several pathological conditions, including neurological disorders, such as Parkinson's disease, Alzheimer's disease, multiple sclerosis, glioblastoma, and cerebrovascular disease (7-9).

Ubiquitin C-terminal hydrolase L1 (UCHL1) is a highly specific neuronal protein that is concentrated in the perikarya in grey matter. Its approximate molecular weight is 24 kDa. UCHL1 is expressed in a specific range of tissues, including the brain and numerous types of cancer. The protein is highly conserved and localized in neurons and neuroendocrine cells in vertebrates. It forms an estimated 5-10% of cytoplasmic protein in these cells. Its enzymic function is related to the removal of misfolded or oxidized proteins in the central nervous system (10-12). Data from the literature showed that UCHL1 may be a biomarker of brain damage after brain ischemia, traumatic brain injury, and subarachnoid hemorrhage (13, 14). This enzyme also participates in the pathogenesis of neurodegenerative diseases such as Parkinson's and Alzheimer's diseases (15).

Therefore, CNDP1 and UCHL1 could also be markers of brain damage in patients undergoing CEA. The aim of the study was to investigate CNDP1 and UCHL1 levels in the serum of these patients.

Study Design

Subjects

The study included patients hospitalized in the Department of Vascular Surgery and Angiology from September 2015 to March 2016. These candidates were approved to undergo CEA due to severe internal carotid artery stenosis. Based on Doppler studies, candidates were qualified for the CEA procedure as determined by the guidelines set forth by the European Society of Vascular Surgery. Using criteria established by NASCET (North American Symptomatic Carotid End arterectomy Trial), patients with severe stenotic carotid were identified. The study included 25 participants (15 male, 10 female); with an average age of 69 years (54-88 years). According to medical histories, the patients were divided into symptomatic patients (15 persons; 8 persons after ischemic stroke and 7 persons after transient ischemic attack) and asymptomatic patients (10 persons).

In the group of symptomatic patients, cerebrovascular events were observed from 2 months to 3 years prior to surgery. Additionally, patients with complete occlusion of the internal carotid artery, as well as those with brain damage in the course of other nervous system diseases were not qualified for the study. The measured degree of stenosis of the internal carotidartery in participants of this study was 60-90%. Conventional CEA was performed under local anesthesia without the use of a shunt. No postsurgical complications were observed.

Collecting Samples

Blood samples were taken from the antecubital vein at three different intervals (within a 24 hour period prior to CEA, 12 hours following surgery, and 48 hours after surgery). The samples were collected into the plastic tubes and centrifuged rapidly. Serum was stored at -80°C until assayed. CNDP1 and UCHL1 levels in serum samples were measured using a commercially available enzyme-linked immunosorbent assay (Enzyme linked Immunosorbent Assay Kit for CNDP1and ELISA for UCHL1;Cloud Clone Corp./USCN, Houston, TX, USA).

Approval of the study was granted by the Medical University Ethics Committee (KE-0254/218/2014). Informed consent was obtained from all participants included in the study.

Statistical analysis

The nonparametric Wilcoxon, Mann-Whitney, and Friedman tests (examination of differences) were used to determine the statistical significance of the recorded results. Spearman Rank Correlation was performed to analyze correlation of data. The CNDP1 and UCHL1 values are expressed in ng/ml; as median and range. The level of statistical significance was $p < 0.05$.

Results

The study showed that the serum CNDP1 level was significantly decreased 12 hours after CEA when compared to level before surgery ($p < 0.05$), and CNDP1 level was normalized 48 hours after CEA. The serum CNDP1 levels and a comparative analysis are presented in Table-1 and Figure-1.

Table-1. Serum CNDP1 & UCHL1 levels; comparative analysis

| Measurement	CNDP1 level [ng/ml]	UCHL1 level [ng/ml]
Before surgery - 1	1208.06 (660.9-1908.2)	0.36 (0.29-0.91)
12 hours after surgery - 2	949.1 (526.4-2523.7)	0.35 (0.25-0.85)
48 hours after surgery - 3	1062.89 (669.5-2262.5)	0.38 (0.27-1.06)
Comparison		
1-2 2-3 1-3	p=0.028* p=0.034* p=0.58	p=0.045* p=0.03* p=0.27

Data is expressed as median and range; Wilcoxon test; p*-statistically significant

The analysis of variance also showed that there were statistically significant differences in serum CNDP1 levels between all three recorded measurements (p:0.04).There was no statistical significant difference in serum CNDP1 levels between symptomatic and asymptomatic patients (p:0.69, p:0.26, and p:0.26; respectively).

Figure 1. Serum carnosine dipeptidase 1 level in patients
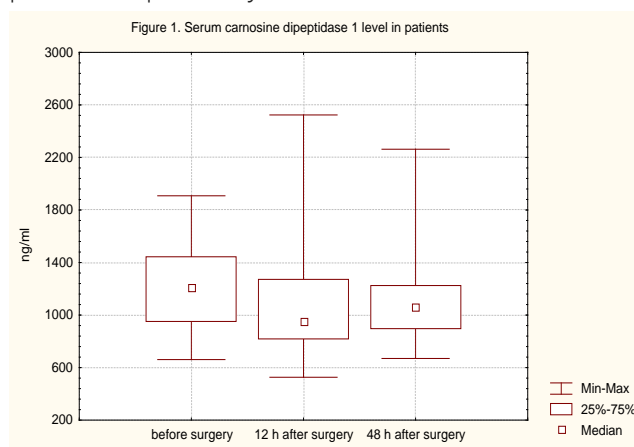
Additionally, there was no statistically significant correlation between serum CNDP1 level and age of the patients (r:-0.14, p:0.50). The study showed that the serum UCHL1 level was also significantly decreased 12 hours after CEA when compared to level before surgery ($p < 0.05$), and UCHL1 level was normalized 48 hours after CEA. The serum UCHL1 levels and a comparative analysis are presented in Table-1 and Figure-2.However, analysis of variance showed that there were no statistically significant differences in serum UCHL1 levels between all 3 recorded measurements (p:0.08).

Figure 2. Serum ubiquitin C - terminal hydrolase L1 in patients
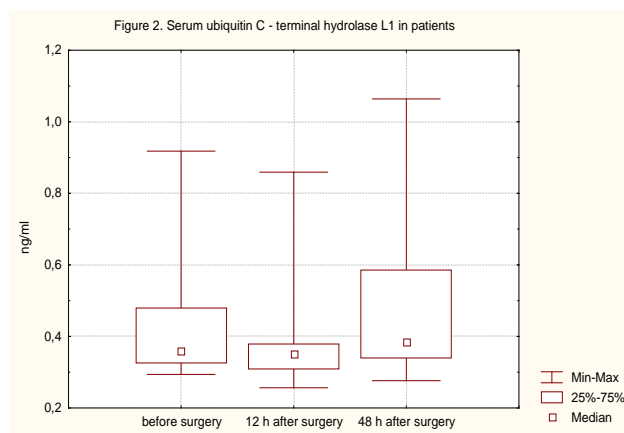
There was no statistical significant difference in serum UCHL1 levels between symptomatic and asymptomatic patients (p:0.26, p:0.29, and

p:0.06; respectively).Additionally, there was no statistically significant correlation bet-ween the serum UCHL1 level and the age of patients (r:-0.04, p:0.82).

Discussion

There are no studies in the literature concerning serum CNDP1 and UCHL1 levels after CEA. Our study showed that serum CNDP1 and UCHL1 levels were significantly decreased 12 hours after CEA compared to levels before surgery, and that these serum biomarkers normalized 48 hours after CEA. Rasmussen et al. (16) observed similar results in their study. The authors measured serum levels of another marker of brain injury, neuron-specific enolase (NSE), in patients before and after CEA. This study revealed that the serum NSE level decreased significantly after uncomplicated CEA. According to Brightwell et al. (17) a high-grade internal carotid artery stenosis may cause an increase in the background level of NSE. Additionally, it was found that upon overcoming such haemodynamically significant brain lesions after CEA, perfusion normalized and these levels of NSE subsequently fell with time.

It is difficult to say why CNDP1 and UCHL1 levels were higher before CEA in our study. It cannot be ruled out that this increase is a result of brain damage caused by chronic ischemia due to a significant stenosis of the internal carotid artery. It can, however, be hypothesized that a significant decrease in the serum CNDP1 and UCHL1 levels in the early period after CEA may be associated with improved blood supply to the brain while normalization of this enzyme may be the result of ischemic hyperperfusion brain damage at a later date after surgery.

Data from the literature showed that altered carnosine levels in neurological diseases may

be caused by disruption of the blood - brain barrier due to cerebral ischemia/reperfusion and/or damage to carnosinase, producing cells after ischemia (18,19). However, Butterworth et al. (20) observed that there was no correlation between size of the infarct and carnosinase activity.

It is known that carnosine may reduce ischemia and reperfusion damage in different animal models (21). An experimental investigation conducted on mice indicated that carnosine significantly decreased infarct size and neuronal damage when administered at points in time both before and after the induction of ischemia. Carnosine also decreased reactive oxygen species levels in the ischemic brain, preserved normal glutathione levels, and decreased matrix metalloproteinase protein levels and activity. Rajanikant et al. (22) concluded that carnosine is neuroprotective in focal cerebral ischemia and appears to influence deleterious pathological processes that are activated after the onset of ischemia.

Although a physiological role of carnosine has not been completely understood at this time, many beneficial actions have been attributed to carnosine. These benefits include its role as an antioxidant, antiglycating and ion-chelating agent, a wound healing promoter, and a free-radical scavenger.

Carnosine has been reevaluated as a molecular chaperon and inducer of antioxidant systems in oxidative stress conditions. Thus, beneficial effects on most of the common biochemical events that characterize neurological disorders make carnosine a very promising molecule among all endogenous compounds in the treatment and/or prevention of oxidative driven diseases (23).

The role of CNDP1 is to regulate the carnosine level. Moreover, this serum enzyme has been proposed as a novel specific bio-

marker for ischemic brain tissue damage (24). A number of animal studies have shown that circulating carnosine has health - protective biochemical properties, including reduction of oxidative stress (25). It was hypothesized that low carnosine activity promotes higher levels of circulating carnosine that may protect against oxidative stress. Moreover, Everaert et al. (26) showed that low plasma carnosinase activity promotes carnosinemia after carnosine ingestion in humans. If ischemia - hyper per-fusion syndrome after CEA has occurred, it cannot be ruled out that a decrease in serum CNDP1 level after surgery, as observed in our study, may be a neuroprotective mechanism, as indicated by carnosine's ability to reduce neurotoxicity through antioxidant capabilities.

According to Siman et al. (27), UCHL1 may be biomarker with clinical potential for the detection and management of ischemic central nervous system injury, including mild damage associated with surgically-induced circulation arrest. Data from the literature showed that UCHL1 may be a biomarker of hypoxic-ischemic encephalopathy (28, 29). Thus, UCHL1 could be also a biomarker of brain ischemic-hyperperfusion injury after CEA in our study.

It was showed that marrow isolated adult multilineage inducible (MIAMI) cells are cha-racterized by high level expression of UCHL1. Secretome analysis indicates that MIAMI cells secrete high levels of soluble mediators, which are known to play key roles in angiogenesis, arteriogenesis, atheroprotection, immunomo-dulation, neuroprotection, axonal growth, pro-genitor cell migration, and prevention of apoptosis (30).

Experimental investigation conducted on animals showed that UCHL1 contributes to neuroprotection after cerebral ischemia. The study conducted on rats after ischemic stroke showed upregulation of penumbra proteins, among others, involved in ubiquitin-mediated proteolysis. These changes in expression of some neuronal proteins, including UCHL1, were directed mainly for protection and tissue recovery in the penumbra. Demyanenko et al. (31) concluded that some upregulated proteins might serve as markers of protection proces-ses in a penumbra. Moreover, Peng et al. (32) observed that the downregulated miR-181b induces neuroprotection against ischemic injury by negatively regulating HSPA5 and UCHL1 protein levels, thus providing a poten-tial therapeutic target for ischemic stroke. As a result, the observed change in serum UCHL1 level after CEA in our study may serve also as a neuroprotective mechanism.

The study also showed that there were no differences in serum enzymes levels between symptomatic and asymptomatic patients divi-ded according to their medical histories. However, on the day of blood sampling, the patients did not have symptoms, which explains the result.

The study has some limitations because of the relatively small number of patients tested and absence of the control group. Addi-tionally, the patients did not demonstrate signs of stroke after CEA, therefore, computed tomography of the brain after surgery was not performed. Future studies related to this topic are planned. Data from our study showed that CEA significantly affects serum CNDP1 and UCHL1 levels. Moreover, these enzyme levels seems to reflect a brain ischemia resulting from severe internal carotid artery stenosis in pa-tients undergoing CEA. However, the observed change in serum CNDP1 and UCHL1 levels does not necessarily warrant a change in re-commendations concerning the use of CEA in patients with high-grade internal carotid artery stenosis.

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