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Changes in serum leptin levels as well as sICAM-1 and sVCAM-1 soluble adhesion molecules during carotid endarterectomy

S. MAKRIS[1], K. VENETSANOU[2], E. SPARTALIS[1], C. KONTOGIANNIS[3], G. GEORGIOPOULOS[3], M. SPARTALIS[1], D.I. TSILIMIGRAS[1], D. MORIS[4], I. KAKISIS[5], G. KARAOLANIS[6], N. PATELIS[6], V. ZYMVRAGOUDAKIS[5], T.I. PAPASILEKAS[7], K.M. THEMISTOKLIS[7], A. LAZARIS[5]

[1]Laboratory of Experimental Surgery and Surgical Research "N.S. Christeas", National and Kapodistrian University of Athens, School of Medicine, Athens, Greece
[2]Research Unit General Hospital of Athens KAT, Athens, Greece
[3]Department of Clinical Therapeutics, "Alexandra" Hospital, University of Athens, Athens, Greece
[4]Department of Surgery, Duke University Medical Center, Durham, NC, USA
[5]Department of Vascular Surgery, Athens General Hospital "Attikon", National and Kapodistrian University of Athens, School of Medicine, Athens, Greece
[6]First Department of Surgery, Vascular Unit, Laikon Hospital, National and Kapodistrian University of Athens, School of Medicine, Athens, Greece
[7]1st Department of Neurosurgery, National and Kapodistrian University of Athens, "Evaggelismos" General Hospital, Athens, Greece

Abstract. – OBJECTIVE: Leptin is an adipokine, known to be associated with oxidative stress, inflammation, and atherogenesis. Leptin plays an essential role in atheromatosis-associated inflammatory cascade through stimulation of inflammatory mediators such as soluble intracellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1). However, little is known about this association in patients with atherosclerosis and severe internal carotid artery (ICA) stenosis undergoing carotid endarterectomy (CEA). Our objective was to evaluate the variations of serum leptin levels, as well as sICAM-1 and sVCAM-1 levels in these patients during the process of CEA and 24 hours postoperatively.

PATIENTS AND METHODS: The study group enrolled 50 patients undergoing CEA for ICA stenosis (> 70%). Serum leptin, sICAM-1 and sVCAM-1 plasma concentration measurements were performed at 4 distinct time points: before clamping of the ICA, 30 minutes after clamping of the ICA, 60 minutes after declamping of ICA and 24 hours postoperatively.

RESULTS: Leptin was significantly decreased during CEA, but an overshooting in its levels was observed at 24 hours after the operation. Both sICAM-1 and sVCAM-1 initially followed the pattern of leptin changes but after completing CEA and up to 24 hours postoperatively a steep increase in their levels was not established. sVCAM-1 and sICAM-1 correlated with indices of oxidative stress at peak inflammatory burden.

CONCLUSIONS: Leptin is a circulating marker of carotid atherosclerosis. Oxidative stress and expression of sVCAM-1 and sICAM-1 on vascular endothelial cells are key features in the pathophysiological process of atherosclerosis.

Key Words:
Leptin, Adhesion molecules, Atherosclerosis, Oxidative stress, Carotid endarterectomy.

Abbreviations

CCA = common carotid artery; CEA = carotid endarterectomy; CTA = computed tomography angiography; ELISA = enzyme-linked immunosorbent assay; ICA = internal carotid artery; MDA = malondialdehyde; mRNA = messenger ribonucleic acid; ROS = reactive oxygen species; sICAM-1 = soluble intracellular adhesion molecule-1; sVCAM-1 = soluble vascular cell adhesion molecule-1; TAC = total antioxidant capacity; TNF = tumor necrosis factor.

Introduction

Carotid atherosclerosis is characterized by the presence of atheromatous plaques, most frequently located in the carotid bifurcation and the proximal internal carotid artery (ICA)[1]. Rupture

Corresponding Author: Michael Spartalis, MD; e-mail: msparta@med.uoa.gr

of unstable carotid plaques resulting in the formation of intraluminal clot and cerebral emboli is the leading cause of ischemic stroke and transient ischemic attack in these patients[2]. Several randomized clinical trials have established carotid endarterectomy (CEA) as safe and effective for decreasing the risk of cerebrovascular events in both symptomatic and asymptomatic patients with carotid stenosis[3,4]. Of note leptin, an adipose tissue-derived hormone seems to play a key role in the lesion progression and plaques instability through its deleterious effects on endothelial cells[2,5]. In particular, leptin stimulates inflammatory mediators and induces oxidative stress by enhancing the generation of reactive oxygen species (ROS)[6]. In this way, ROS increase the expression of soluble intracellular adhesion molecule-1(sICAM-1), soluble vascular cell adhesion molecule-1(sVCAM-1)[7] and monocyte chemoattractant protein-1[8], which play a substantial role in atheromatosis-associated inflammatory cascade[9,10]. Additionally, leptin enhances the activity of metalloproteinase -2 and -9 and promotes pathological angiogenesis though vascular endothelial growth factor- A, increasing plaques destabilization and friability[11,12]. In the present study, we sought to evaluate the variations of serum leptin levels, as well as sICAM-1 and sVCAM-1 levels in patients with atherosclerosis and severe ICA stenosis undergoing carotid endarterectomy, both during the process of CEA and 24 hours postoperatively.

Patients and Methods

Study Setting and Population

In this prospectively designed study, we analyzed data from 50 patients undergoing carotid endarterectomy for ipsilateral internal carotid artery (ICA) stenosis (> 70%). Patients were recruited between April 2013 and May 2015 from the Vascular Surgery Clinic of University Hospital of Athens "Attiko", Greece. The study was conducted in accordance with the principles underlined in the Declaration of Helsinki and the Ethics Committee of the National and Kapodistrian University of Athens. The severity of ICA stenosis was evaluated by carotid duplex imaging and the analysis of the peak systolic and end-diastolic velocities and was validated by computed tomography angiography (CTA). Exclusion criteria included: a) history of acute or chronic inflammatory disease b) current treatment with anti-inflammatory drugs c) current use of vitamins or antioxidant supplements and d) active cancer. All patients were designated as low-risk for CEA, provided written informed consent and presented no contraindications to the procedure.

Surgical Technique

Carotid endarterectomy was performed with a transverse incision in a skin crease at the level of the carotid bulb under general anesthesia. The subcutaneous tissues and underlying muscles were divided, the carotid sheath was exposed, and the ICA was identified. Afterwards, a careful dissection was performed between the common carotid artery (CCA) and a point distal to the ICA plaque, in order to allow for clamping of the normal soft artery. The internal, common and external arteries were clamped sequentially, and a longitudinal arteriotomy was performed below the level of bifurcation, extended distally. The carotid plaque was isolated and successfully removed through a dissection plane developed in the layers of the deep media. After removal of any residual plaque and artery repair, suture line was completed, and carotid clamps were sequentially released to restore the carotid flow.

Blood Collection and ELISA Measurements

Peripheral blood samples were obtained from a venous catheter inserted into the ipsilateral jugular bulb at 4 distinct time points: before clamping of the ICA (T_0), 30 minutes after clamping of the ICA (T_1), 60 minutes after declamping of ICA (T_2) and 24 hours postoperatively (T_3). The serum was separated by centrifugation at 3000 rpm for 10 min. Serum leptin, sICAM-1 and sVCAM-1 levels were determined by enzyme-linked immunosorbent assay (ELISA) at the aforementioned time points using commercially available kits from Cusabio Biotech., (College Park, MD, USA).

Statistical Analysis

Continuous variables are presented as mean ± standard deviation or median (interquartile range) for variables not following the normal distribution. Nominal variables are presented as counts and percentages. Normal distribution of continuous variables was assessed by the Shapiro-Wilk test and graphically inspected by percentile-percentile plots and histograms. Levels of leptin and sICAM-1 and VCAM-1 were compared among pre-specified time points (baseline, 30 min post carotid clamping, 1 hour after declamping and 24

hours after endarterectomy.) with the non-parametric Friedman test. A correction for multiple comparisons was performed (i.e. Dunn's Multiple Comparison Test). All statistical tests were two-tailed. A value of $p < 0.05$ was considered statistically significant. Statistical analysis was performed with SPSS 23.0, (SPSS Inc., Armonk, NY, USA).

Results

Baseline Characteristics of Patients

Fifty patients (27 men, 23 women) with a mean age of 68.6 ± 8.7 years were enrolled. All patients were diagnosed with high-grade ICA stenosis (at least 70%) by using carotid duplex imaging and CTA. Thirty-six patients (72%) were asymptomatic, whereas 10 of them presented clinical symptoms of a transient ischemic attack (n=6), amaurosis fugax (n = 1) and stroke (n = 3). Comorbidities associated with ICA stenosis included coronary artery disease (n = 23), chronic renal disease (n = 4), systemic arterial hypertension (n = 46), diabetes mellitus (n = 16) and dyslipidemia (n = 32).

Serum Leptin Levels

Leptin levels during CEA and 24 hours postoperatively are shown in Figure 1. In particular, leptin levels significantly decreased 30 min after clamping of ICA (between T_0-T_1 interval, $p < 0.05$) as well as 60 min after the release of the clamping (between T_1-T_2 interval, $p < 0.05$) compared to baseline. A significant increase was recorded 24 hours postoperatively compared to all the predefined time points ($p < 0.01$ for T_0, $p < 0.001$ for T_1 and T_2).

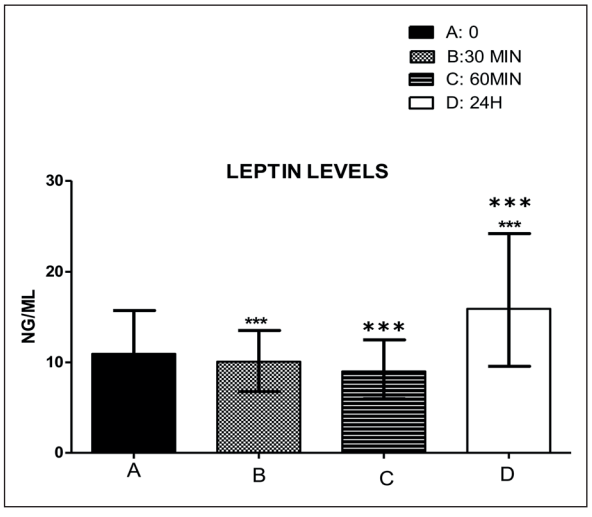

Figure 1. Leptin levels during and after carotid endarterectomy. A time 0 min, B after 30 min, C after 1 hour, D 24 hours after endarterectomy. ** $p < 0.05$ for pairwise differences between A and B, C. ***$p < 0.001$ for pairwise differences between D and B, C. ** $p < 0.01$ for pairwise difference between D and A.

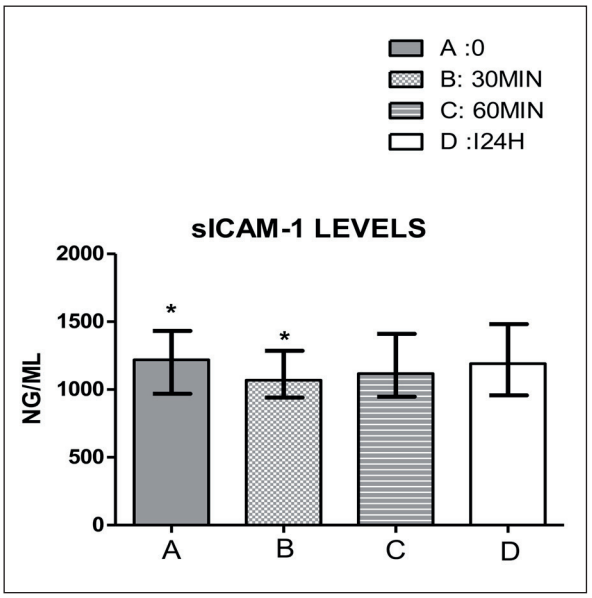

Figure 2. s-ICAM levels during carotid endarterectomy. A time 0 min, B after 30 min, C after 60 min, D 24 hours after endarterectomy.

Serum sICAM-1 and sVCAM-1 Levels

A significant decrease of sICAM-1 was found between (A) baseline and (B) clamping ($p = 0.005$) (Figure 2), whereas sVICAM significantly increased between (B) clamping and (C) declamping ($p = 0.05$) (Figure 3). Soluble ICAM-1 and sVCAM-1 were significantly correlated at all examined endpoints ($p < 0.01$ for all) (Table I). At declamping (C), s-VCAM significantly correlated with malondialdehyde (MDA) (Spearman = 0.324, $p < 0.05$) and was inversely associated with total antioxidant capacity (TAC) (Spearman's rho = 0.408, $p = 0.003$).

Discussion

In this study, we have demonstrated temporal changes in circulating levels of leptin, and soluble adhesion molecules during and post carotid endarterectomy. Leptin was significantly decreased during CEA, but an overshooting

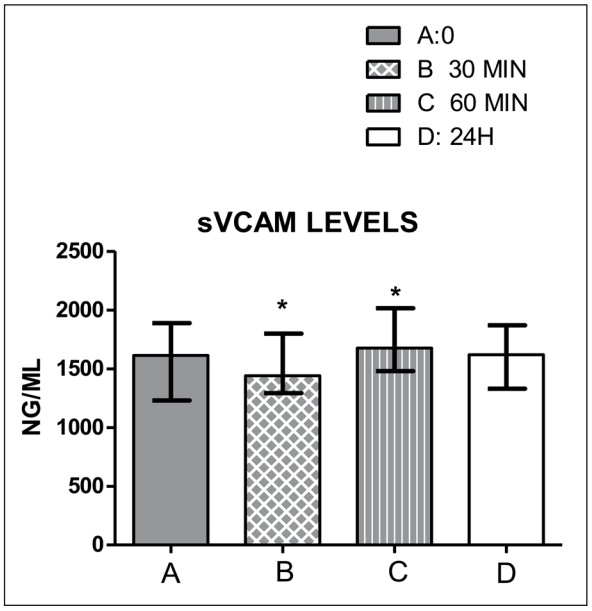

Figure 3. s-VCAM levels during carotid endarterectomy, A time 0 min, B after 30 min, C after 60 min, D 24 hours after endarterectomy.

in its levels was observed at 24 hours after the operation. Both sICAM-1 and sVCAM-1 initially followed the pattern of leptin changes but after completing CEA and up to 24 hours postoperatively a steep increase in their levels was not established. Notably, sVCAM-1 and sICAM-1 correlated with indices of oxidative stress at peak inflammatory burden (i.e. at CEA completion). To date, no study has examined acute changes in leptin levels during CEA. In general, circulating levels of leptin are found to be elevated in obese control patients and those with atherosclerosis irrespective of their body mass index[13]. In addition, leptin is found to be positively correlated to serum triglycerides, insulin, and TNF-a[14]. Regarding pro-inflammatory molecules, circulating levels of s–VCAM-1 have been shown to be increased during the endarterectomy especially in patients with diabetes mellitus[15]. Nevertheless, the finding that in carotid stenosis, sVCAM-1 measurement improves the stratification of prognosis provided by a well-established risk factor, encourages further efforts in this field[15]. Furthermore, the observation that increased levels of s–VCAM-1 are associated with restenosis after endarterectomy, suggests that these markers may be of some value in the selection of patients for endarterectomy and could constitute a new therapeutic target[16]. In contrast, our study demonstrated that s–VCAM-1 levels do not present significant changes 24 hours after CEA, despite the fact that a notable decrease was recorded 30 min post carotid clamping. Circulating levels of s-VCAM-1 s-ICAM-1 all have been shown to be increased in peripheral artery disease and patients with atherosclerosis[13], although the findings of different studies are not consistent. Elevated levels of adhesion molecules have been detected in patients with diabetes mellitus, but conflicting results were reported in non-diabetics[17-19]. Increased levels of the adhesion molecules are due to a variety of molecular and cellular factors, including the increased transcription of genes, alteration of mRNA stability, changes in translation and enhanced proteolytic cleavage from the cell surface[20,21]. Oxidative stress and expression of the VCAM-1 on vascular endothelial cells are early features in the pathogenesis of atherosclerosis and the inflammatory diseases[6]. Regulation

Table I. Correlation among sICAM-1, TAC, MDA, sVCAM-1 at pre-specified time points (A time 0 min, B after 30 min, C after 1 hour, D 24 hours after endarterectomy).

	sVCAM-1							
	A		B		C		D	
	p	Spearman	*p*	Spearman	*p*	Spearman	*p*	Spearman
sICAM-1	0.002	0.473	< 0.001	0.656	0.003	0.458	0.002	0.479
TAC A	NS	–	NS	–	0.009	-0.408	NS	–
MDA A	NS	–	NS	–	0.044	0.324	NS	–

Abbreviations: sICAM-1, soluble intracellular adhesion molecule-1; TAC, total antioxidant capacity; MDA, malondialdehyde sVCAM-1, soluble vascular cell adhesion molecule-1, NS, no significant.

of VCAM-1 gene expression may be coupled to oxidative stress through specific reduction-oxidation (redox) sensitive transcriptional or posttranscriptional regulatory factors[22,23]. In cultured human vein endothelial cells activated VCAM-1 gene expression through mechanism that was repressed approximately 90% by antioxidants and N-acetylocysteine[24]. Selectively antioxidants inhibited the induction of VCAM-1. These suggest a molecular linkage between an antioxidant sensitive transcriptional regulatory mechanism and VCAM-1 gene expression that expands on the notion of oxidative stress as an important regulatory signal in the pathogenesis of atherosclerosis. Our findings are in accordance with these mechanisms since VCAM-1 and ICAM-1 correlated with indices of oxidative stress.

Certain limitations should be acknowledged in our study. Firstly, our results provide a "proof of principle" that leptin, sICAM-1 and sVCAM-1 levels are significantly reduced during CEA, while a notable increase in leptin levels is recorded 24 hours postoperatively. However, we examined only 4 distinct time points (baseline, 30 minutes post carotid clamping, 1 hour after declamping and 24 hours after endarterectomy) and no outcomes were assessed 24 hours after the CEA. Secondly, it was a single-center study, and the number of patients who were finally included was quite low.

Conclusions

We showed that leptin is a circulating marker of carotid atherosclerosis. Oxidative stress and expression of sVCAM-1 and sICAM-1 on vascular endothelial cells are key features in the pathophysiological process of atherosclerosis. Leptin is significantly decreased during CEA, but an overshooting in its levels was observed at 24 hours after the operation. Both sICAM-1 and sVCAM-1 initially followed the pattern of leptin changes but after completing CEA and up to 24 hours postoperatively a steep increase in their levels was not established. Long-term multi-center interventional studies are warranted in order to explore both acute and chronic changes in serum leptin levels and soluble adhesion molecules during and after carotid endarterectomy, as well as its clinical implications in the management of symptomatic and asymptomatic carotid stenosis.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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