Endothelin-1 plasma concentration in patients with essential hypertension, atherogenic dyslipidemia and coronary artery disease

Concentrația plasmatică a endotelinei 1 la pacienți cu hipertensiune arterială, dislipidemie aterogenă şi boală coronariană

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Abstract

The aim of this study was to investigate endothelin-1 (ET-1) plasma concentration in patients with essential hypertension, atherogenic dyslipidemia, and coronary artery disease confirmed by coronarography and the relationship between ET-1 and subclinical atherosclerosis. The study comprised 32 patients with coronary artery disease, 12 hypertensive, 12 with atherogenic dyslipidemia and 12 healthy subjects. Antihypertensive medication was interrupted for two weeks before the study. The concentration of ET-1 was measured by ELISA. Endothelium-dependent, flow-mediated dilatation (FMD) of the brachial artery and carotid intima-media thickness (IMT) of the carotid artery were assessed by B-mode ultrasonography. ET-1 plasma concentration was significantly higher ($p < 0.001$) in coronary artery disease patients (25 ± 5.42 pg/ml) comparative with atherogenic dyslipidemia patients (19 ± 5.63 pg/ml), with hypertensive patients (16.8 ± 5.16 pg/ml) and with the control subjects (7.2 ± 2.53 pg/ml). A significant negative correlation was found between ET-1 and FMD of the brachial artery in coronary artery disease patients ($r = -0.81$, $p < 0.001$), in hypertensive patients ($r = -0.82$, $p < 0.001$), atherogenic dyslipidemia patients ($r = -0.85$, $p < 0.001$), and control subjects ($r= -0.84$, $p < 0.001$). A positive significant correlation between ET-1 and carotid IMT was found only in coronary artery disease patients ($r = 0.57$, $p<0.001$). We did not find significant correlations between endothelin-1 and carotid IMT in hypertensive ($r = 0.10$, $p=0.08$), dyslipidemic ($r = - 0.10$, $p=0.06$) patients, and control subjects ($r = - 0.14$, $p=0.03$).

Keywords: *endothelin-1, IMT, FMD*

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Rezumat

Scopul acestui studiu a fost investigarea concentraţiei plasmatice a endotelinei-1 (ET-1) la pacienţi cu hipertensiune arterială, dislipidemie aterogenă şi boală coronariană confirmată coronarografic şi relaţia dintre ET-1 şi ateroscleroza subclinică. Studiul a cuprins 32 de pacienţi cu boală coronariană, 12 hipertensivi, 12 cu dislipidemie aterogenă şi 12 subiecţi sănătoşi. Medicaţia antihipertensivă a fost întreruptă cu 2 săptămâni înainte de studiu. Concentraţia de ET-1 a fost măsurată prin Elisa. Vasodilataţia mediată de flux (FMD) la nivelul arterei brahiale şi grosimea intimei-media (IMT) la nivelul arterei carotide au fost evaluate prin ultrasonografie de tip B. Concentraţia plasmatică a ET-1 a fost semnificativ mai mare ($p < 0.001$) la pacienţii cu boală coronariană (25 ± 5.42 pg/ml) comparativ cu cei cu dislipidemie aterogenă ($19 \pm 5,63$ pg/ml), cu cei cu hipertensiune arterială (16.8 ± 5.16 pg/ml) şi cu subiecţii control ($7.2 \pm 2,53$ pg/ml). O corelaţie negativă, semnificativă a fost observată între ET-1 şi FMD la pacienţii cu boală coronariană ($r = -0.81$, $p < 0.001$), la cei cu hipertensiune arterială ($r = -0.82$, $p < 0.001$), la cei cu dislipidemie aterogenă ($r = -0.85$, $p < 0.001$) şi la subiecţii control ($r = -0.84$, $p < 0.001$). O corelaţie pozitivă, semnificativă între ET-1 şi IMT a fost observată doar la cei cu boală coronariană ($r = 0.57$, $p<0.001$). Nu am obţinut o corelaţie semnificativă între ET-1 şi IMT la cei cu hipertensiune ($r = 0.10$, $p=0.08$), cu dislipidemie aterogenă ($r = - 0.10$, $p=0.06$) şi la subiecţii control ($r = - 0.14$, $p=0.03$).

Cuvinte cheie: *endotelina-1, IMT, FMD*

Introduction

Endothelial dysfunction plays an important role in the pathogenesis of atherosclerosis and it had been demonstrated that endothelin-1 (ET-1) is elevated in atherosclerotic plaques. Endothelin (ET-1) is a polypeptide hormone secreted by the endothelial cells in some blood vessels, where it acts in a paracrine or autocrine manner on ETA and ETB receptors on adjacent endothelial or smooth muscle cells (1). The effects of endohelin-1 include cell proliferation, migration and contraction, and the induction of extracellular matrix components and growth factors (2). ET-1 appears to contribute to the maintenance of basal vascular tone and is activated in several diseases, including congestive heart failure, arterial hypertension, atherosclerosis, coronary artery diseases, renal failure, cerebrovascular disease, pulmonary arterial hypertension, and sepsis (3). Endothelial dysfunction is known to occur in patients with cardiovascular risk factors and usually it may precede structural changes. ET-1 may be an early marker and mediator of endothelial dysfunction, leading to enhanced vasoconstrictor responses and contributing to the development of atherosclerotic lesions (4).

Brachial flow-mediated dilatation (FMD) is a physiologic measure and carotid IMT is an anatomic structural measure of subclinical atherosclerosis (5).

The aim of our study was to determine the plasma concentration of ET-1 in patients with different cardiovascular pathologies (coronary artery disease, arterial hypertension, and atherogenic dyslipidemia) and to evaluate the correlations between ET-1 and carotid IMT and between ET-1 and brachial FMD in all groups of patients.

Material and methods

The study included 68 patients from the IVth Medical Clinic of the University of Medicine and Pharmacy "Victor Babeş" Timişoara, after informed consent was obtained: 32 patients with coronary artery disease (mean age 50±3 years, 71% males and 29% females), 12 hypertensive patients (mean age 55±4 years, 60% males and 40% females) and 12 patients with atherogenic dyslipidemia (mean age 53±5 years, 68% males and 32% females). The control group consisted of 12 healthy subjects (mean age 57 ± 4.25 years, 68% males and 32% females). Antihypertensive medication was interrupted for at least two weeks before the study.

The subjects in the control group had no history of heart or systemic disease; they had normal blood pressure levels, physical examination findings, electrocardiogram, echocardiogram, chest radiogram, and laboratory test results (including urinalysis; 24-hour microalbu-

minuria; and levels of serum cholesterol, triglycerides, electrolytes, creatinine, and creatinine clearance), *fundus oculi*, and echo Doppler study of major arteries.

Clinical evaluation included blood pressure measurement, physical examination, chest radiograph, 12-lead electrocardiogram.

Blood pressure was measured on the left arm after five minutes of relaxation, using a standard mercury sphygmomanometer. Hypertension was diagnosed according to 2007 European Guidelines for the Management of Arterial Hypertension (6).

Atherogenic dyslipidemia was defined as low plasma high-density lipoprotein cholesterol and elevated triglycerides, total cholesterol and LDL cholesterol.

Blood samples for serum cholesterol, serum triglycerides, and lipoprotein fractions were drawn after a fasting period of 10 to 12 hours. Cholesterol and triglyceride levels were determined by enzymatic techniques (7, 8). LDL cholesterol was calculated as described by Friedewald et al (9).

Endothelin-1 was measured by a commercially available sandwich ELISA (DRG Diagnostics, DRG Instruments, GmbH, Germany) (normal values 1-3 pg/mL).

Endothelial function was assessed by means of flow – mediated vasodilatation on brachial artery, using B – mode ultrasonography (ALOKA ProSound 4000, with 7.5 MHz linear transducer). Before the FMD determination, the patients were relaxed in a stable room temperature between 20 – 25 ºC; smoking was prohibited. The diameter of the brachial artery was measured from the anterior to the posterior interface between the media and adventitia at a fixed distance, synchronized with the R-wave peaks on the electrocardiography (D_i). Then, ischemia was induced by inflating the pneumatic cuff to a pressure 50 mmHg above the systolic one, in order to obliterate the brachial artery. After 5 minutes, the cuff was deflated and arterial diameter was measured at 60 seconds after deflation (D_f). FMD was calculated with the formula:

$$FMD = [(D_f - D_i)/D_i] \times 100.$$

Carotid IMT was measured by high-resolution B-mode ultrasonography with an ultrasonographyc apparatus (ALOKA ProSound 4000, with 7.5 MHz linear transducer). The image was focused on the posterior wall of the left carotid artery. A minimum of 4 measurements of the common carotid posterior wall were taken 10 mm proximal to the bifurcation to derive mean carotid IMT. We analyzed the maximum thickness of the intima-media complex as carotid IMT (normal values < 0.9 mm).

Continuous variables were expressed as means ± SD. Means were compared using variance analysis or the Student t-test. Pearson's correlation was used to test bivariate correlations and results were verified using the non-parametric Spearmans's rank correlation test. Statistical significance was defined as two–sided $p < 0.05$. All statistical analyses were performed using Excel Microsoft Office 2003.

The procedures followed were in accordance with the ethical standards of the Hospital Ethics Committee and with the Helsinki Declaration of 1975, as revised in 2000.

Results

The baseline characteristics of the subjects, the mean values of EI-1, FMD and IMT are summarized in *Table 1*.

The most elevated concentrations of endothelin-1 were found in coronary artery disease patients (25 ± 5.42 pg/ml), compared to atherogenic dyslipidemia (19 ± 5.63 pg/ml), hypertensive patients (16.8 ± 5.16 pg/ml) and control subjects (7.2 ± 2.53 pg/ml).

We found significantly decreased mean values of brachial FMD in coronary artery disease patients (5.44 ± 2.50 %), compared to hypertensive patients (7.67 ± 2.53 %), atherogenic dyslipidemia patients (10 ± 4.19 %), and control subjects (13 ± 1.47 %) ($p < 0.001$).

Significantly increased mean values of carotid IMT were observed in coronary artery disease patients (1.60 ± 0.18 mm), compared to ath-

Table 1. Baseline characteristics, ET-1, IMT and FMD of the studied patients (mean ± SD)

Characteristics	Control subjects	Coronary artery disease patients	Hypertensive patients	Atherogenic dyslipidemia patients
Patients (n)	12	32	12	12
Age (y)	56 ± 4.25	58 ± 3.15	55 ± 4.75	53 ± 5.68
Male gender (%)	68	71	60	68
Systolic BP (mmHg)	114 ± 6.08	149 ± 15.96	147 ± 10.10	120 ± 11.17
Diastolic BP (mmHg)	68 ± 5.84	90 ± 10.23	88 ± 7.78	72 ± 8.88
TC (mg/dL)	180 ±11.28	253 ± 19.67	200 ± 12.79	244 ± 21.53
LDL cholesterol (mg/dL)	115 ± 5.82	177 ± 13.62	122 ± 22.81	132 ± 20.94
HDL cholesterol (mg/dL)	50 ± 6.40	27 ± 2.05	49 ± 10.74	33 ± 8.90
Triglycerides (mg/dL)	98 ± 27.09	242 ± 69.35	176 ± 74.13	295 ± 92.79
Endothelin-1 (pg/mL)	7.2 ± 2.53	25 ± 5.42	16.8 ± 5.16	19 ± 5.63
IMT (mm)	0.7 ± 0.24	1.60 ±0.18	0.89 ± 0.26	0.94 ± 0.25
FMD (%)	13 ± 1.47	5.44 ± 2.50	7.67 ± 2.53	10 ± 4.19

Table 2. Correlation between ET-1, IMT and FMD in the studied group

ET-1	FMD		IMT	
	r	p	r	p
Control group	-0.84	<0.001	-0.14	0.03
Coronary artery disease group	-0.81	<0.001	0.57	0.04
Hypertensive group	-0.82	<0.001	0.10	0.08
Atherogenic dyslipidemia group	-0.85	<0.001	-0.10	0.06

erogenic dyslipidemia patients (0.94 ± 0.25 mm), hypertensive patients (0.89 ± 0.26 mm), and control subjects (0.7 ± 0.24 mm) ($p < 0.001$).

A significant negative correlation was found between ET-1 and brachial FMD in coronary artery disease patients ($r = -0.81$, $p < 0.001$) (*Figure 1*) as well as in hypertensive patients ($r = -0.82$, $p < 0.001$), atherogenic dyslipidemia patients ($r = -0.85$, $p < 0.001$), and control subjects ($r= -0.84$, $p < 0.001$) (*Table 2*).

A significant positive correlation between endothelin-1 and carotid IMT was found only in coronary artery disease patients ($r = 0.57$, $p<0.001$) (*Figure 2*). We did not find significant correlation between ET-1 and carotid IMT in hypertensive patients ($r = 0.10$, $p=0.08$), atherogenic dyslipidemia patients ($r = - 0.10$, $p=0.06$), and control subjects ($r = -0.14$, $p=0.03$) (*Table 2*).

Discussions

It has been widely reported that the reciprocal regulation of NO and ET-1 is vital for the maintenance of vascular tone, antithrombotic and antiatherogenic properties of the endothelium, as well as playing a key role in counteracting inflammatory events (10, 11).

Classically, endothelial dysfunction has been considered to be the result of a decrease in NO. The question now arises whether the decrease in NO is secondary to an increase in ET-1. Indeed, in addition to being a potent en-

dothelial-derived constrictor of vascular smooth muscle, ET-1 may induce endothelial dysfunction by decreasing NO bioavailability. Recent data show that endothelium-restricted overexpression of ET-1 causes endothelial dysfunction and a decrease in NO (12). Several studies showed that ET-1 levels were higher in patients with angina and angiographically normal coronary arteries than in control patients (13-15). Hypercholesterolemic patients also present increased circulating levels of the vasoconstrictor ET-1 and enhanced activity of ET receptors (16). Similar to these studies, our results showed increased plasma concentrations of ET-1 in patients with coronary artery disease, arterial hypertension and atherogenic dyslipidemia as opposed to control subjects.

In our study, endothelial dysfunction resulting from reduced NO bioavailability is reflected by a diminished NO-dependent flow-mediated dilation response in patients with coronary artery disease, arterial hypertension and atherogenic dyslipidemia.

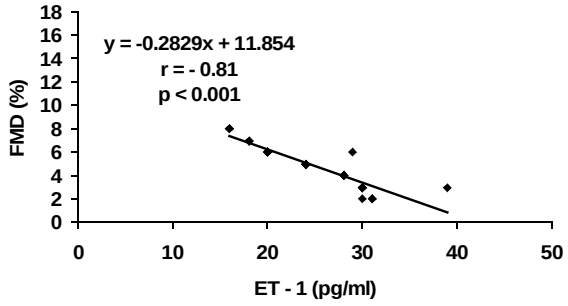

Figure 1. Correlation between ET-1 and FMD in coronary artery disease patients

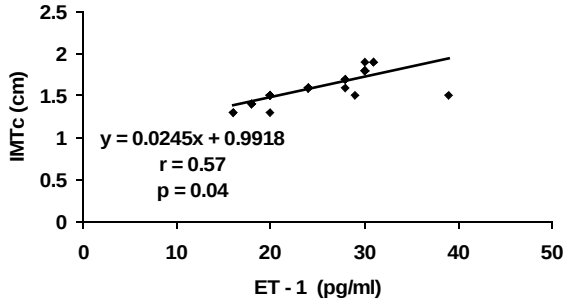

Figure 2.Correlation between ET-1 and IMT in coronary artery disease patients

Carotid IMT gives information on atherosclerosis extent and, as such, can be very useful in individual patient's CV risk assessment (17). In our study, patients with coronary artery disease, atherogenic dyslipidemia and arterial hypertension presented increased values of carotid IMT, a sign of a subclinical atherosclerosis. The significant correlation between ET-1 and carotid IMT noted in coronary artery disease patients suggests a more severe endothelial dysfunction in these patients compared to the other presently investigated groups.

Conclusion

We can consider that endothelin-1 is a key player in endothelial dysfunction. These results, which need to be confirmed using larger groups, suggest that evaluating carotid IMT and brachial FMD together with the measurement of plasma concentration of ET-1 can provide distinct, independent information about the complex atherosclerotic process in patients with different cardiovascular pathologies.

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