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Endothelial dysfunction as assessed by flow-mediated dilation in patients with cardiac syndrome X: role of inflammation

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Abstract. – *Background:* **Endothelial dysfunction, reduced coronary flow reserve and increased markers of inflammation are detectable in cardiac syndrome X (CSX). In this study we investigated the relation between inflammation and systemic endothelial function in CSX patients.**

Methods: **We studied 42 CSX patients (55±6 years, 14 men) and 20 healthy subjects (52±7 years, 9 men). Systemic endothelial function was assessed by flow-mediated dilation (FMD) of the brachial artery after 5-minute of forearm cuff inflation. Serum C-reactive protein (CRP) was measured by a high-sensitivity method.**

Results: **FMD was significantly lower in CSX patients compared to controls (4.8±4.4 vs. 13.7±4%, $p<0.001$), whereas CRP levels were higher in CSX patients than in controls (2.7±2.4 vs. 0.7±0.4 mg/L, $p=0.001$). In CSX patients FMD showed a significant inverse correlation with CRP levels, even after adjustment for potentially confounding variables (r=-0.34, $p=0.006$).**

Conclusion: **An impaired FMD is detectable in CSX patients, suggesting a generalized abnormality in vascular function. Subclinical inflammation seems to play a significant role in the impairment of endothelium-dependent vasodilator function of these patients.**

Key Words:

Syndrome X, Endothelial dysfunction, Inflammation.

Introduction

Coronary microvascular dysfunction has been shown to be a major component of cardiac syndrome X (CSX) and is characterized by an impairment of both endothelium-dependent and endothelium-independent vasodilator function[1-3]. Abnormal endothelium-dependent vasodilation has been reported in some studies in the peripheral circulation of CSX patients, suggesting a more general vascular disorder in at least a subgroup of these patients[4,5].

Recent findings have suggested that increased low grade inflammation may play a pathogenetic role in CSX[6,7], and a recent study has shown a relation between serum CRP levels and endothelium-dependent coronary microvascular dilation in a group of patients with normal coronary arteries who underwent coronary angiography because of chest pain[8]. However, whether inflammation has any relation with endothelial dysfunction in typical CSX patients is still unknown.

Subjects and Methods

Subjects

We studied 42 patients with classical CSX (mean age 54.8±6 years, 14 men). All patients had a history of effort angina, ST-segment depression associated with angina during exercise stress test, and smooth coronary arteries at angiography. Coronary artery spasm was excluded according to intracoronary or systemic ergonovine test in patients who referred angina episodes also at rest. A mild hypertension was present in 26 (62%) patients, but left ventricular hypertrophy was excluded by echocardiography in all patients. Other cardiac or systemic disease were carefully excluded, according to clinical history, physical examination, routine laboratory tests and two-dimensional and Doppler echocardiography.

A group of 20 apparently healthy volunteers (mean age 52.1±7 years, 9 men) served as controls. These subjects were enrolled from the non-medical staff of our Hospital and were selected to be comparable to CSX patients as to age and gender. Their

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clinical history excluded any clinically relevant disease, their physical examination was normal, and all had normal routine laboratory tests, standard 12 leads ECG, exercise stress test and two-dimensional and Doppler echocardiography.

There was no evidence of any acute or chronic inflammatory disease both in patients and controls. The study complies with the Declaration of Helsinki and was approved by the institutional Ethics Commitee. All subjects enrolled gave their informed consent for participation in the study.

Peripheral Endothelial Function Study

All subjects included in the study underwent non invasive assessment of systemic endothelium-dependent and endothelium-independent vasodilation in a peripheral artery, according to published guidelines (9). The tests were all performed in the morning by the same experienced sonographer who was blinded to the results of the other tests. Briefly, after withdrawing all potential vasoactive substances (including drugs, caffeine, tobacco) for 4 half-lives, and overnight fast, subjects were positioned supine in a quiet, temperature-controlled room (22-24°C), with the right arm in a comfortable position for imaging the brachial artery. After 15 minutes of rest, the right brachial artery was visualized in the longitudinal plane 2 to 15 cm proximally to the antecubital fossa with a 7.5 MHz linear array transducer connected to an IU22 ultrasound machine (Philips Medical Systems, Monza, Italy). Depth and gain were selected to optimally identify the anterior and posterior intimal interface between the lumen and vessel wall on 2D grayscale images. Baseline images were then obtained. Blood flow was measured from the pulsed wave Doppler signal with an incidence angle of 70°. After obtaining basal measures, a sphygmomanometer cuff was applied on the forearm and inflated to 250 mmHg for 5 minutes, causing forearm ischemia and consequent dilation of downstream resistance vessels through autoregulatory mechanisms. Forearm blood flow was measured during the first 15 seconds after cuff deflation and arterial image acquisitions were performed between 60 and 90 seconds after cuff deflation. After at least 15 minutes of rest, new images and flow measurement were obtained both at baseline and 3 minutes after the administration of 0.4 mg of sublingual nitroglycerin. Mean arterial diameter was calculated from 3 consecutive measures performed from the anterior to the posterior lumen-intima interface at end-diastole (R-wave on the electrocardiogram). Flow-mediated dilation (FMD), reflecting endothelium-dependent vasodilation, and nitroglycerine-mediated dilation (NMD), reflecting endothelium-independent vasodilation, were both expressed as percentage increase in post-stimulus diameter compared to the baseline diameter.

CRP Measurement

A venous blood sample was collected before ultrasonographic investigation in all patients and serum samples were frozen at –80°C until assayed. Subclinical inflammation was assessed by measuring C-reactive protein levels with a high-sensitivity nephelometric method (Behring Nephelometric 100 Analyzer, Scoppito, Italy) with a detection limit of 0.05 mg/L.

Statistical Analysis

According to Kolmogorov-Smirnov test, all variables showed a distribution not significantly different from normal in this study, including CRP levels. Thus, continuous variables were compared by unpaired t-test, whereas proportions were compared by Fisher exact test. Correlations were assessed by Pearson's correlation coefficient. The independent association of serum CRP levels with FMD was assessed in multivariate linear regression models, in which all cardiovascular risk factors were included as potential confounding variables. Data are reported as means ± standard deviation, unless differently indicated. A p value <0.05 was considered as statistically significant. The software SPSS 12.0.2 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

Results

Clinical and Laboratory Data

The main clinical characteristics of patients and controls are summarized in Table I. According to selection criteria, there were no differences between groups in age and gender. Cardiovascular risk factors, however, also did not differ significantly between the 2 groups, although they tended to be more frequent in CSX patients. In contrast, serum CRP levels were found to be significantly higher in CSX patients than in controls (2.75±2.4 vs. 0.74±0.4 mg/L, p=0.001).

Endothelial Function

Basal brachial artery diameter was similar in CSX patients and in controls (3.5±0.7 vs. 3.2±0.6

Table I. Main clinical characteristics of subjects enrolled in the study.

	Syndrome X patients (n = 42)	Healthy controls (n = 20)	P
Age (years)	55 ± 6	52 ± 7	0.14
Sex (M:F)	14:28	9:11	0.37
Hypercholesterolemia	24 (57.1%)	7 (35%)	0.10
Hypertension	26 (62%)	11 (55%)	0.6
Glucose intolerance	3 (7%)	0	0.22
Familiarity for CVD	20 (55.5%)	8 (40%)	0.36
Active smoking	5 (33.3%)	3 (13.6%)	0.73

mm, p=0.27). Compared to baseline, the increase of forearm blood flow velocity during hyperemia did not differ significantly between the two groups (59±23% vs. 69±39%, respectively; p=0.26).

Endothelium-dependent vasodilation of the brachial artery in response to hyperemia, however, was significantly lower in CSX patients than in controls (4.8±4.4% vs. 13.7±4%, p<0.001, Figure 1). In contrast, no difference was found between the two groups in the vasodilator response of brachial artery diameter to nitroglycerin (22±9% vs. 22±7%, respectively, p=0.87, Figure 1). The differences between the 2 groups in FMD persisted highly significant (p<0.001) after adjustment for all cardiovascular risk factors, including serum CRP levels.

Inflammation and Endothelial Function

A significant correlation in CSX patients was found between serum CRP levels and FMD (r= –0.34, p=0.006, Figure 2), and peripheral FMD was significantly lower in patients with serum CRP levels >3 mg/L compared to those with CRP levels <3 mg/L (4.5±5% vs. 9±6%, p=0.01). Conversely, no correlation was found between CRP levels and nitroglycerin-mediated brachial artery dilation (r= –0.10, p=0.44), which was also similar in CSX patients with CRP levels >3 mg/L or <3 mg/L (21±5% vs. 22±9%, p=0.62).

Serum CRP levels were the only independent variable predictive of impaired FMD (p=0.015), together with female gender (p=0.014).

Discussion

In this study we show that peripheral FMD was significantly impaired in patients with CSX, suggesting a more generalized disorder of vascular function in these patients. Furthermore, our data suggest that subclinical inflammation plays a significant role in this vascular abnormality.

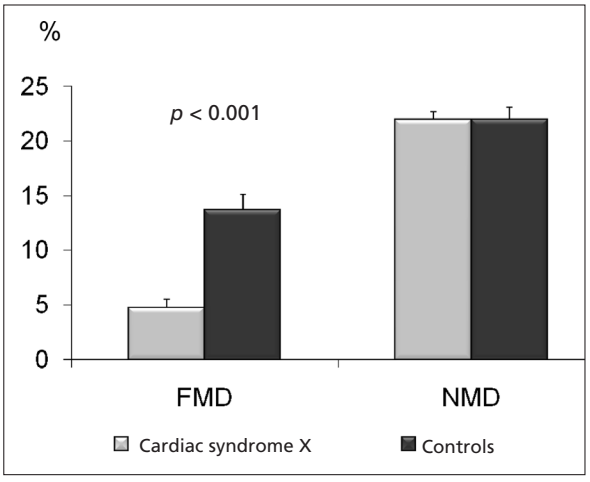

Figure 1. Dilation of the right brachial artery in response to hyperemia (flow-mediated dilation, left) and in response to sublingual nitroglycerin (NTG) (*right*) in cardiac syndrome X (CSX) patients and in controls. Data are means ± standard deviations and are expressed as percentages.

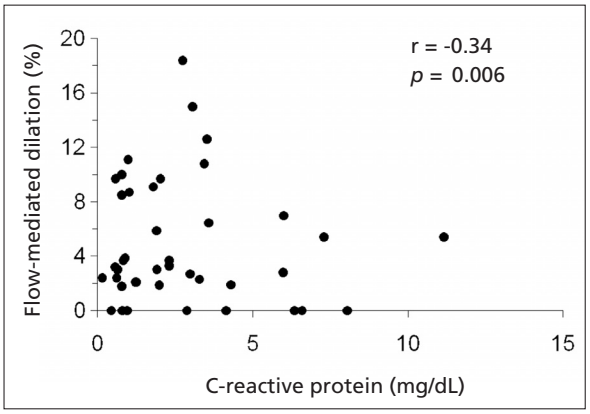

Figure 2. Relation between peripheral flow-mediated dilation of the right brachial artery and systemic inflammation, as assessed by serum C-reactive protein levels, in cardiac syndrome X patients.

CSX patients have repeatedly been found to present evidence of coronary microvascular dysfunction, which may involve an impairment of both endothelium-dependent and endothelium-independent dilatation[1-3]. However, there is controversy about whether the vascular disorder is limited to coronary circulation or is more generalized.

Previous studies already showed reduced peripheral FMD in CSX patients, compared to healthy control subjects[4,5,10,11]. Furthermore, in a recent study, impaired peripheral FMD in CSX patients was associated with a greater evidence of stress-induced perfusion defects on myocardial scintigraphy, suggesting some pathophysiological link between coronary microvascular dysfunction and general endothelium dependent vascular function[12].

The underlying mechanisms responsible for the endothelium-dependent vascular dysfunction in CSX patients, however, remain poorly known, although they are likely to be multiple and different in individual patients.

In previous studies increased evidence of subclinical inflammation has been found in CSX patients, compared to healthy controls[6,7]. Furthermore, data obtained in a population of CSX-like patients (i.e., angina with normal coronary arteries) showed a significant correlation between CRP serum levels and impairment of coronary microvascular endothelial function, as assessed by intracoronary Doppler recordings during acetylcholine administration[8]. In a recent study we have shown as endothelium-dependent and endothelium-independent coronary microvascular dysfunction cannot be reliably predicted by cardiovascular risk factors, including serum CRP levels[13]. This is the first study that correlates PCR levels and peripheral endothelial function in patients affected by CSX.

In conclusion, in this study we provide a direct evidence of a relation between peripheral impairment of FMD and CRP serum levels, further supporting the hypothesis of a pathogenetic role of low grade inflammation in the vascular abnormalities of CSX patients. CRP level was the only variable found to be significantly associated with impaired FMD in our patients.

References

1) Lanza GA, Crea F. Primary coronary microvascular dysfunction: Clinical presentation, pathophysiology, and management. Circulation 2010; 121: 2317-2325.

2) Egashira K, Inou T, Hirooka Y, Yamada A, Urabe Y, Takeshita A. Evidence of impaired endothelium-dependent coronary vasodilation in patients with angina pectoris and normal coronary angiograms. N Engl J Med 1993; 328: 1659-1664.

3) Chauhan A, Mullins PA, Taylor G, Petch MC, Schofield PM. Both endothelium-dependent and endothelium-independent function is impaired in patients with angina pectoris and normal coronary angiograms. Eur Heart J 1997; 18: 60-68.

4) Sax FL, Cannon RO 3rd, Hanson C, Epstein SE. Impaired forearm vasodilator reserve in patients with microvascular angina. Evidence of a generalized disorder of vascular function? N Engl J Med 1987; 317: 1366-1370.

5) Lekakis JP, Papamichael CM, Vemmos CN, Voutsas AA, Stamatelopoulos SF, Moulopoulos SD. Peripheral vascular endothelial dysfunction in patients with angina pectoris and normal coronary arteriograms. J Am Coll Cardiol 1998; 31: 541-546.

6) Cosin-Sales J, Pizzi C, Brown S, Kaski JC. C-reactive protein, clinical presentation, and ischemic activity in patients with chest pain and normal coronary angiograms. J Am Coll Cardiol 2003; 41: 1468-1474.

7) Lanza GA, Sestito A, Cammarota G, Grillo RL, Vecile E, Cianci R, Speziale D, Dobrina A, Maseri A, Crea F. Assessment of systemic inflammation and infective pathogen burden in patients with cardiac syndrome X. Am J Cardiol 2004; 94: 40-44.

8) Teragawa H, Fukuda Y, Matsuda K, Ueda K, Higashi Y, Oshima T, Yoshizumi M, Chayama K. Relation between C reactive protein concentrations and coronary microvascular endothelial function. Heart 2004; 90: 750-754.

9) Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R; International Brachial Artery Reactivity Task Force. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery. A report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002; 39: 257-265.

10) Li AH, Lee BC, Chen KC, Weng CS, Chu SH. Brachial artery flow-mediated vasodilation in patients with cardiac syndrome X. Angiology 2008; 59: 581-586.

11) Lekakis JP, Papamichael CM, Vemmos CN, Voutsas AA, Stamatelopoulos SF, Moulopoulos SD. Peripheral vascular endothelial dysfunction in patients with angina pectoris and normal coronary arteriograms. J Am Coll Cardiol 1998; 31: 541-546.

12) Masci PG, Laclaustra M, Lara JG, Kaski JC. Brachial artery flow-mediated dilation and myocardial perfusion in patients with cardiac syndrome X. Am J Cardiol 2005; 95: 1478-1480.

13) Sestito A, Lanza GA, Di Monaco A, Lamendola P, Careri G, Tarzia P, Pinnacchio G, Battipaglia I, Crea F. Relation between cardiovascular risk factors and coronary microvascular dysfunction in cardiac syndrome X. J Cardiovasc Med (Hagerstown). 2010 Oct 16. [Epub ahead of print].