Increased concentrations of soluble vascular cell adhesion molecule-1 and soluble CD40L in subjects with metabolic syndrome

IVÁN G. PALOMO[1], JULIO C. JARAMILLO[1], MARCELO L. ALARCÓN[1], CÉSAR L. GUTIÉRREZ[1], RODRIGO MOORE-CARRASCO[1], FABIÁN M. SEGOVIA[1], ELBA M. LEIVA[1], VERÓNICA E. MUJICA[1] GLORIA ICAZA[2] and NORA S. DÍAZ[2]

[1]Department of Clinical Biochemistry and Immunohematology, School of Health Sciences, [2]Institute of Mathematics and Physics, Universidad de Talca, Talca, Chile

Received November 24, 2008; Accepted February 16, 2009

DOI: 10.3892/mmr_00000125

Abstract. Metabolic syndrome (MS) is associated with a high incidence rate of cardiovascular disease. It is characterized by abdominal obesity, elevated blood pressure, atherogenic dyslipidemia [high LDL-c (low density lipoprotein cholesterol) and low HDL-c (high density lipoprotein cholesterol)] and insulin resistance or glucose intolerance. In the context of MS, alterations in the plasmatic levels of some soluble forms of cell adhesion molecules can appear, e.g., soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble E-selectin (sE-selectin) and soluble CD40L (sCD40L). The objective of this study was to compare the serum levels of sVCAM-1, sE-selectin and sCD40L in MS and non-MS groups and to associate these molecules with the diagnostic criteria of MS. A total of 185 non-smokers between 45 and 64 years of age were included. Of these, 93 corresponded to the MS group and the remaining 92 to a non-MS group (according to modified ATP III criteria). The serum concentration of sVCAM-1, sE-selectin and sCD40L was determined by commercial solid phase ELISA. The results were expressed as a median and interquartile range. The MS group showed high levels of sVCAM-1 (558.9 ng/ml; 481.3-667.6 ng/ml) compared with the non-MS group (405.2 ng/ml; 361.0-470.5 ng/ml) ($p<0.0001$). As well, the median level of sCD40L (3.0 ng/ml; 2.1l-11.7 ng/ml) was significantly higher in the MS group than that in the non-MS group (2.6 ng/ml; 2.3-3.4 ng/ml) ($p=0.0061$). sE-selectin levels did not differ significantly between the groups: 73.9 ng/ml (58.3-87.0 ng/ml) and 68.5 ng/ml (51.6-97.5 ng/ml) in the MS and non-MS group, respectively. In conclusion, the serum levels of sVCAM-1 and sCD40L, but not sE-selectin, were significantly higher in patients with MS than in subjects that did not present MS. MS may therefore increase the expression of cell adhesion molecules, probably through endothelial activation.

Correspondence to: Dr Iván Palomo, Department of Clinical Biochemistry and Immunohematology, Faculty of Health Sciences, Universidad de Talca, P.O. Box 747, Talca, Chile
E-mail: ipalomo@utalca.cl

Key words: cell adhesion molecules, metabolic syndrome, soluble vascular cell adhesion molecule-1, soluble CD40L, soluble E-selectin

Introduction

Metabolic syndrome (MS) is a cluster of cardiovascular risk factors including central obesity, hypertension, dyslipidemias and glucose intolerance (1). Clinically, it has reached epidemic proportions, and with an increase in the elderly population its incidence and prevalence will further multiply (2).

Visceral adipose tissue, an important feature in individuals with MS, produces a range of circulating molecules with pro-inflammatory and pro-atherosclerotic actions. Several of these adipokines, including tumour necrosis factor α (TNF-α) and interleukin 6 (IL-6), have been linked to alterations in endothelial functions (3).

An alteration that characterizes endothelial dysfunction involves the secretion of cellular adhesion molecules (CAMs) on the surface of endothelial cells (ECs), thus enabling the CAMs to bind leukocytes (4,5). VCAM-1 (vascular cell adhesion molecule-1) is not expressed at high levels on the endothelium, but can be regulated *in vitro* in response to TNF-α, IL-4 and interferon-γ (IFN-γ) cytokines that are synthesized by adipose as well as other tissues. Soluble VCAM-1 (sVCAM-1) is found in the serum of healthy people, but is observed at elevated levels in patients with autoimmune disease, infections and inflammatory processes (6), and in those with non-compensated hypertension (7). E-selectin is also expressed in ECs after activation by inflammatory cytokines such as IL-1ß and TNF-α (8). CD40L is expressed in leukocytes and ECs, among other cells (9). IL-1ß, TNF-α and IFN-γ can stimulate the synthesis and expression of CD40L in certain types of cells, among them platelets, as well as its liberation to serum. CD40L can be found in proinflammatory states, as observed in MS (10,11).

Table I. Characteristics of the population by gender.

	Metabolic syndrome group (n=93)		Non-metabolic syndrome group (n=92)		P-value	
	Men	Women	Men	Women	Men	Women
Age (years)	54.6±5.7	54.1±5.7	54.0±5.0	52.9±6.0	0.6113	0.2901
Glycemia (mg/dl)	124.8±44.8	110.1±41.8	96.9±32.7	91.5±24.0	0.0024	0.0076
Total cholesterol (mg/dl)	207.0±49.4	206.5±38.1	191.6±34.8	204.6±32.0	0.1034	0.7865
LDL-c (mg/dl)	111.7±28.3	123.0±31.3	111.7±31.6	116.7±28.0	0.3868	0.2856
HDL-c (mg/dl)	41.8±12.0	48.0±11.9	50.1±13.2	64.8±15.2	0.0044	<0.0001
Triglycerides (mg/dl)	262.0±197.5	176.2±64.1	159.6±99.9	114.3±40.8	0.0037	<0.0001
Waist circumference (cm)	105.0±10.1	98.7±10.1	93.4±8.8	86.3±11.3	<0.0001	<0.0001
Systolic blood pressure (mmHg)	143.6±15.3	138.5±20.0	132.4±17.4	127.9±19.6	0.0030	0.0077
Diastolic blood pressure (mmHg)	88.2±10.7	83.7±9.7	82.6±11.8	76.1±10.0	0.0283	0.0002
Body mass index (kg/m^2)	30.7±3.9	33.5±6.7	27.2±2.9	27.0±5.0	<0.0001	<0.0001

Data are expressed as the mean ± standard deviation. HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol.

These molecules have been proposed as potential markers of atherosclerosis. The soluble forms of CAMs may be shed from the endothelial surface and are structurally different from the molecules expressed on ECs, apparently due to the absence of the cytoplasmic domain, which remains in the cell of origin (12).

CAMs are likely to play an important role in the pathogenesis of atherosclerosis. Increased expression of CAMs has been found in atherosclerotic plaques, and soluble forms have been correlated with surface CAM expression (5).

The aim of this study was to evaluate the plasma concentrations of sVCAM-1, sE-selectin and sCD40L in patients presenting MS, as well as to elucidate the correlations between these molecules and studied parameters such as total cholesterol, high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), triglyceride, glucose, blood pressure, body mass index (BMI) and MS criteria.

Subjects and methods

Subjects. The study included 185 non-smokers aged 45 to 64 years of age. Of these, 93 (44 men and 49 women) had MS and 92 (37 men and 55 women) were non-MS subjects.

MS was defined by the modified Third Adult Treatment Panel (ATP III) (13). The high blood pressure criterion was defined as systolic blood pressure >130 and/or diastolic blood pressure >85 mmHg, or prescribed antihypertension medication. Central obesity was defined as a waist circumference >102 cm in men or >88 cm in women. Fasting serum glucose was ≥100 mg/dl. Low HDL-c was defined as HDL-c <40 mg/dl in men or <50 mg/dl in women. Hypertriglyceridemia was defined as a fasting triglyceride >150 mg/dl. Subjects who met 3 or more of these 5 criteria were classified as presenting MS.

Laboratory tests. sVCAM, sE-selectin and sCD40L concentrations were determined separately by solid phase ELISA (R&D Systems, Minneapolis, MN). In brief, microtiter plates precoated with monoclonal antibodies (anti-sVCAM-1, anti-sE-selectin and anti-sCD40L, respectively) were incubated with serum samples. Bound sVCAM-1, sE-selectin and sCD40L were revealed by incubation with a secondary antibody coupled to horseradish peroxidase. After washing, the substrate solution was added. Finally, absorbance was measured at 450 nm by the StatFax-2100 microplate reader (Awareness Technology Inc.).

Statistical analysis. Mean and standard deviation were used to describe normally distributed variables. When the variables were not normally distributed, median and interquartile ranges (IQR = 75th-25th percentile) were used. The response variables sE-selectin, sVCAM-1 and sCD40L were transformed since they are not normally distributed. The transformations used were natural logarithm for the sE-selectin variable, and inverse function for the sVCAM-1 and sCD40L variables. Covariance analysis was performed to evaluate the mean difference in serum levels of sE-selectin, sVCAM-1 and sCD40L in the MS and non-MS groups. To identify which MS diagnostic criteria were individually affecting serum levels of sE-selectin, sVCAM-1 and sCD40L, a multivariate regression analysis was performed. Finally, multiple linear regression analysis was carried out to evaluate the effect of the number of MS components on serum levels of sE-selectin, sVCAM-1 and sCD40L. All the adjusted models were controlled by age and gender. Diagnostic regression measures were used to evaluate normality and homocedasticity linear regression assumptions. SAS 9.1.3 and SPSS 14.0 software was used for statistical analysis. A significance level of 5% was used.

Results

The characteristics of the study population are presented in Table I.

The median serum concentration of sVCAM-1 was 405.2 ng/ml (IQR=109.5 ng/ml) in non-MS subjects and 558.9 ng/ml (IQR=186.3 ng/ml) in MS subjects.

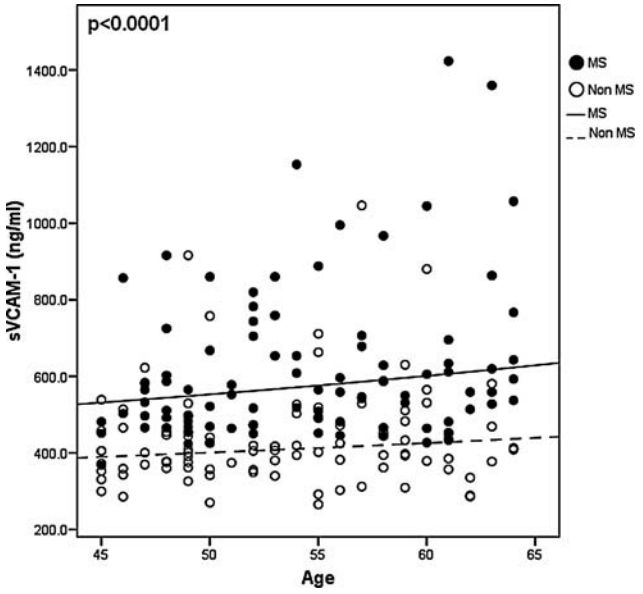

Figure 1. Concentrations of sVCAM in subjects with metabolic syndrome (MS) and in non-MS subjects by age.

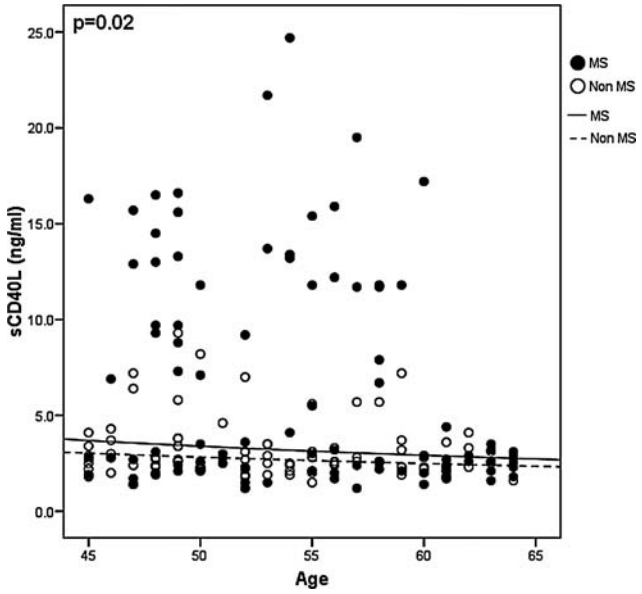

Figure 3. Concentration of sCD40L in metabolic syndrome (MS) and in non-MS subjects by age.

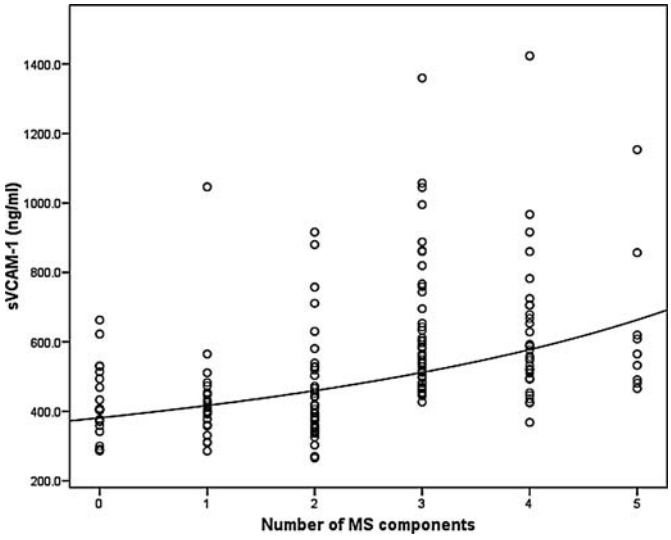

Figure 2. Relationship of sVCAM-1 concentration and number of MS criteria (n=185) for the mean age (53.8 years).

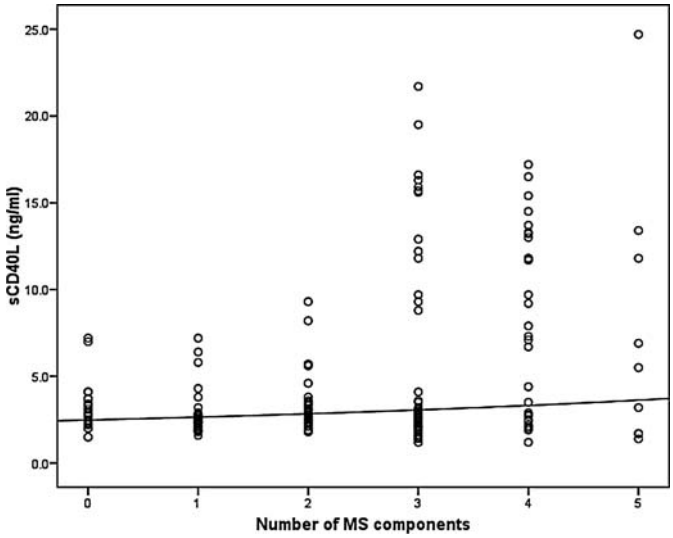

Figure 4. Relationship of sCD40L concentration and the number of MS criteria (n=185) for the mean age (53.8 years).

The inverse serum concentration of sVCAM-1 was associated with the presence of MS. When transforming the variable to its original scale, it was observed that sVCAM-1 serum levels were significantly higher in subjects with MS than in the non-MS subjects (Fig. 1) (p<0.0001), controlled by age. A significant correlation between inverse sVCAM-1 levels and age (p=0.0236) was found. No significant differences between gender were found. Inverse sVCAM-1 levels were associated with BMI (p=0.0360), diastolic blood pressure (p=0.01) and waist circumference (p<0.0001), and were controlled by age and gender. Inverse serum levels of sVCAM-1 were linearly and positively related to the number of MS criteria, as controlled by age (p<0.0001) (Fig. 2).

The median serum concentration of sCD40L was 2.6 ng/ml (IQR=1.1 ng/ml) in non-MS subjects and 3.0 ng/ml (IQR= 9.6 ng/ml) in MS subjects.

Inverse serum concentrations of sCD40L were associated with the presence of MS. When transforming the variable to its original scale, it was observed that sCD40L serum levels were significantly higher in subjects with MS than in the non-MS subjects (p=0.02) (Fig. 3). There were no differences in the serum concentrations of inverse sCD40L according to gender, and the concentrations decreased significantly with age (p=0.04).

Inverse sCD40L levels were not associated with MS variables. The inverse sCD40L concentration was linearly and positively related to the number of MS components, controlled by age (p=0.0061) (Fig. 4).

We did not find a significant difference in sE-selectin levels when comparing subjects with MS (73.9 ng/ml) (IQR=28.7 ng/ml) and non-MS subjects (68.5 ng/ml) (IQR=45.9 ng/ml). The natural logarithm of sE-selectin

levels was only associated with waist circumference (p=0.0011).

Discussion

MS is characterized by a number of cardiovascular risk factors (2). These include hyperglycemia, arterial hypertension, hypertriglyceridemia, low levels of HDL-c and increased waist circumference (14,15). The worldwide prevalence of MS is 20-30% in adults, and increases with age (16). MS is associated with a doubled relative risk of cardiovascular disease (17).

Subjects with MS present a proinflammatory state, which is distinguished by elevated levels of cytokines, such as TNF-α, IL-6 and acute phase reactants. These cytokines promote the expression of CAMs (18).

In this study, we found statistically significant differences between the serum levels of sVCAM-1 and sCD40L in the MS and non-MS groups, but not in the serum levels of sE-selectin. In several studies, sE-selectin and sVCAM-1 levels were found to exhibit higher levels in patients with hypertension than in control groups (19,20). However, other studies detected no difference in the levels of sE-selectin (21). This latter observation was confirmed in the present study.

Different new biomarkers of cardiovascular disease in patients with MS have been studied. For example, serum levels of sVCAM-1 were found to be higher in MS patients (17±5 ng/ml) than in a control group (13±4 ng/ml) (22). These patients had high plasmatic levels of inflammatory biomarkers (23). Additionally, in the first stage of atherosclerosis in primary hypertriglyceridemia, the patients exhibited higher sVCAM-1 levels (13.9±3.8 ng/ml) than the controls (5.6±4.5 ng/ml), $p<0.05$.

On the other hand, an association between the serum levels of sVCAM-1 and arterial blood pressure has been reported (24), and is corroborated by our study. This association could trigger the development of certain systemic failures, which could increase cardiovascular risk (7,25).

CD40L is the pro-inflammatory mediator expressed in activated platelets, of either membrane-bound or soluble form. Both forms interact with CD40 expressed on vascular cells, which is important in the cascade of inflammatory and proatherothrombotic functions (26).

In terms of sCD40L, Yan *et al* (27) showed sCD40L levels to be significantly higher in patients with hypertension than in a control group. Other studies have associated sCD40L with acute coronary syndromes (28), hypercholesterolemia (29), angina (30), recurrent myocardial infarction (31) and cardiovascular risk (32, 33).

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