

ORIGINAL PAPER

CIRCULATING VASCULAR CELL ADHESION MOLECULE-1 AND SUBCLINICAL ATHEROSCLEROSIS

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SUMMARY

Background: It has been suggested that elevated levels of soluble forms of vascular cell adhesion molecule-1 (sVCAM-1) may be an index of endothelial activation or even a molecular marker of early atherosclerosis.

The **aim** of this study was to determine plasma levels of VCAM-1 in a group of young healthy adults and to evaluate correlations between VCAM-1, subclinical atherosclerosis and cardiovascular risk factors.

Methods: Study was conducted on 49 young (aged 20-40 years, 40 men) healthy adults with cardiovascular risk factors. Cardiovascular risk factors, serum VCAM-1, flow mediated dilation (FMD) for evaluation of endothelial function and carotid-intimae-media-thickness (CIMT) were determined in all subjects.

Results: Mean values for VCAM-1 were $535,69 \pm 77,64$ ng/ml. Serum levels of VCAM-1 correlated with age ($r=0,35$, $p=0,01$), blood glucose ($r=0,43$, $p=0,01$), systolic blood pressure ($r=0,28$, $p=0,04$) and also strongly correlated with CIMT ($r=0,64$, $p<0,0001$) and inversely with FMD ($r=-0,62$, $p<0,0001$). In multiple regression analysis CIMT ($r=0,64$, $p=0,019$) and FMD ($r=-0,61$, $p=0,029$) remained independent predictors of circulating levels of VCAM-1.

Conclusions: Serum VCAM-1 concentrations correlate with markers of subclinical atherosclerosis. VCAM-1 can be useful as a serum marker for subclinical atherosclerosis and can serve as a diagnostic basis for early secondary prevention.

Abbreviations: BMI=body mass index, CIMT = carotid-intimae-media-thickness, DBP=diastolic blood pressure FMD = flow mediated dilation, SBP=systolic blood pressure sVCAM-1 = vascular cell adhesion molecule-1

Key words: flow mediated dilation, intimae-media thickness, subclinical atherosclerosis, VCAM-1, young adults

RÉSUMÉ

La molécule 1 d'adhésion cellulaire dans la circulation vasculaire et l'athérosclérose sous-clinique

Contexte: Il a été suggéré que des niveaux élevés de formes solubles de la molécule 1 d'adhésion cellulaire vasculaire (sVCAM-1) peuvent être un indice d'activation endothéliale ou même un marqueur moléculaire de l'athérosclérose précoce.

Le **but** de cette étude était de déterminer les niveaux plasmatiques de VCAM-1 dans un groupe de jeunes adultes en bonne santé et d'évaluer les corrélations entre la VCAM-1, l'athérosclérose sous clinique et les facteurs de risque cardiovasculaires.

Méthodes: Nous avons évalué 49 jeunes (âgés de 20 à 40 ans, 40 hommes) adultes sains avec des facteurs de risque cardiovasculaire. Les facteurs de risque cardiovasculaire, le sérum VCAM-1, le flux médié par dilatation (FMD) pour l'évaluation de la fonction endothéliale et l'épaisseur de l'artère carotide intima-media (CIMT) ont été déterminés chez tous les sujets.

Résultats: Les valeurs moyennes de la VCAM-1 ont été $535,69 \pm 77,64$ ng/ml. Les taux sériques de VCAM-1 corrélés avec l'âge ($r=0,35$, $p=0,01$), la glycémie ($r=0,43$, $p=0,01$), la pression artérielle systolique ($r=0,28$, $p=0,04$) et également fortement corrélée avec la CIMT ($r=0,64$, $p<0,0001$) et inversement avec FMD ($r=-0,62$, $p<0,0001$). Dans une analyse de régression multiple CIMT ($r=0,64$, $p=0,019$) et FMD ($r=-0,61$, $p=0,029$) sont demeurés des prédicteurs indépendants de niveaux circulants de VCAM-1.

Conclusions: Les concentrations de la VCAM dans le sérum sont corrélées avec les marqueurs de l'athérosclérose sous-clinique. VCAM-1 peut être utile comme marqueur de sérum pour l'athérosclérose sous-clinique et peut servir de base de diagnostic pour le début de la prévention secondaire.

Mots clés: le flux médié par dilatation, intima-media carotide épaisseur, l'athérosclérose sous clinique, molécule-1 vasculaire d'adhésion cellulaire, jeunes adultes

INTRODUCTION

Cellular adhesion molecules are expressed on the endothelial cell membrane and mediate the adhesion and migration of leukocytes that play an important role in early atherogenesis. Pathological studies have shown increased cellular adhesion molecules expression in several components of the atherosclerotic plaque (1, 2). Circulating soluble forms of adhesion molecules have been described that are probably generated by a cleavage at a site close to the membrane insertion. While cell surface activity of adhesion molecules appears critical in the development of atherosclerotic lesions, the value of plasma levels of soluble adhesion molecules in the prediction of the extent of atherosclerotic disease is still under debate since published studies differ in their findings. This limited consensus in the literature may be explained either by unrecognized confounding factors or perhaps by unpredictable relationship between cell surface expression and activity of cellular adhesion molecules and their shedding into the plasma (3, 4). Raised plasma soluble cellular adhesion molecules levels have been found in a variety of pathological conditions, in patients with ischemic heart disease, atherosclerosis, hyperlipidemia, diabetes. Soluble vascular cell adhesion molecules (VCAM-1) have been related to the extent of atherosclerosis, established angiographically in multiple vascular beds in patients with peripheral arterial disease (5). In various populations soluble CAMs has been positively related to common carotid intima-media thickness (6, 7, 8). Elevated circulating VCAM-1 levels have been reported in patients with atherosclerotic aorta compared with asymptomatic control subjects (9). Thus there are reports supporting that serum levels of circulating adhesion molecules may provide information on atherosclerosis.

Atherosclerosis is a chronic, progressive, inflammatory disease with a long asymptomatic phase. While the disease is still in a subclinical stage, however the presence of atherosclerosis can be identified non-invasively by measuring the combined thickness of the intima and medial layers, usually measured in the common carotid artery with B-mode ultrasonography. Carotid intima-media thickness (IMT) represents a marker of structural atherosclerosis often used in epidemiological studies as a surrogate for early atherosclerosis and is correlated with cardiovascular risk factors (10), the severity of coronary atherosclerosis (11) and is a strong predictor of cardiovascular events (12).

Endothelial dysfunction is an early event of atherosclerosis that precedes structural atherosclerotic changes in the vascular wall and can be determined non-invasively by an ultrasound technique that measures the brachial artery flow-mediated dilatation (FMD). Brachial FMD is correlated with coronary endothelial function as tested by invasive methods (13, 14). An impaired FMD is related to the prevalence and extent of coronary atherosclerosis (15) and predicts cardiovascular events (16, 17).

The aim of this study was to determine plasma levels of VCAM-1 in a group of young healthy adults and to evaluate

the correlations between VCAM-1, subclinical atherosclerosis and cardiovascular risk factors. Subclinical atherosclerosis was assessed by determining endothelial dysfunction evaluated with brachial flow mediated dilatation and carotid intima-media thickness.

METHODS

Study was conducted on 49 young (aged between 20-40 years, 40 men) healthy adults with cardiovascular risk factors. Informed consent of the subjects has been obtained and study was approved by CNCSIS- a council of scientific research of higher studies in Romania. All subjects were completely evaluated by clinical examination with determination of age, sex, smoking status, height, weight and body mass index (BMI), systolic and diastolic blood pressure (BP). Fasting blood samples were recorded for determination of blood glucose, lipid profile (LDL, HDL cholesterol, triglycerides) and serum VCAM-1. EDTA anticoagulated samples were obtained for sVCAM-1 determination. Blood was then centrifuged for 20 minutes at 2500 rpm and aliquots were stored at -70 C. Serum VCAM was measured with ELISA based on purified proteins and polyclonal antibodies (R&D Systems) according to the manufacturer's recommendations. Reported sensitivity of the assays for sVCAM-1 is < 2.

FMD for evaluation of endothelial function and CIMT were determined in all subjects. Each subject underwent a detailed ultrasound evaluation of the carotid arteries. These examinations were performed using an Acuson with a 7, 5 MHz linear transducer. Images were obtained with the patients in the supine position with the neck mildly extended and the head rotated contra laterally to the side. Longitudinal, lateral and anterior oblique views of the distal 10 mm of the right and left common carotid arteries were obtained. For the purpose of statistical analysis right and left measurements were averaged. Plaques were defined by the presence of focal severe (IMT > 2 mm) wall thickening, wall irregularities and calcifications.

Vascular ultrasound scans were performed according to the method described by Celermajer et al (18) for non-invasive determination of endothelial dysfunction. Ultrasound images were recorded with an Acuson with a transducer of 7, 5 MHz and were registered on videotape. Flow increase was induced by inflation of a sphygmomanometer placed 10 cm above the elbow to 300 mm Hg for 4 minutes followed by decompression. Diameter of right brachial artery was measured basally and 60 seconds after cuff deflation, at 5 cm above the elbow. FMD was estimated by the changes of brachial artery diameter during reactive hyperaemia, expressed as percentage change relative to average baseline scan.

Statistical analysis was done with Statistica six sigma and Medcalc. Demographic data and risk factors are expressed as mean \pm standard deviation. The correlates for sVCAM-1 were studied by regression techniques. Multiple regression was used to examine the relationship between dependent variable sVCAM-1 and several clinical and laboratory independent variables.

RESULTS

Patient characteristics

Baseline characteristics of studied patients are recorded in Table 1. Mean values for VCAM-1 were 535, 69 ± 77, 64 ng/ml. Mean values of carotid IMT were 0, 49 ± 0, 02 mm (95% CI 0, 48 to 0, 50). Mean values of FMD were 7, 62 ± 4, 67% (95% CI 6 to 8).

Unadjusted associations between sVCAM-1 levels, sub-clinical atherosclerosis and cardiovascular risk factors

Serum levels of VCAM-1 correlated significantly with age ($r=0,35$, $p=0,01$; 95%CI for r 0,08335 to 0,5794) (Fig. 1); blood glucose ($r=0,43$, $p=0,001$; 95%CI for r 0,1796 to 0,6408) (Fig. 2) and systolic blood pressure ($r=0,28$, $p=0,04$; 95% CI for r 0,003612 to 0,5238)(Fig. 3).

Serum VCAM-1 did not correlate with: sex, smoking status, BMI, lipids (LDL-cholesterol, HDL-cholesterol, triglycerides), diastolic blood pressure ($p=NS$).

Serum VCAM-1 also strongly correlated with markers

Table 1 - Baseline characteristics of the study population

Variable	Values
age	33,93 ± 7,03 y
sex	40 male/9 female
BMI	29,89 ± 4,36
SBP	132,92 ± 17,47 mmHg
DBP	77,3 ± 8,67 mmHg
Blood glucose	86,36 ± 13,05 mg/dl
LDL-cholesterol	131,08 ± 18,79 mg/dl
HDL-cholesterol	39,77 ± 7,68 mg/dl
Triglycerides	149,30 ± 21,27 mg/dl
sVCAM-1	535,69 ± 77,64 ng/ml
FMD	7,62 ± 4,67 %
Carotid IMT	0,49 ± 0,02 mm

Legend: BMI=body mass index, SBP=systolic blood pressure, DBP=diastolic blood pressure, sVCAM-1=serum vascular cell adhesion molecules, FMD=flow mediated dilation, IMT=intimae-media thickness. Data are means ± SD

of subclinical atherosclerosis: directly with carotid IMT ($r= 0, 64$, $p<0, 0001$; 95% CI for r 0, 4409 to 0, 7823) (Fig. 4) and inversely with FMD ($r= - 0, 62$, $p<0, 0001$; 95% CI for r - 0, 7664 to - 0, 4084) (Fig. 5).

Risk factors adjusted associations between sVCAM-1 levels and subclinical atherosclerosis

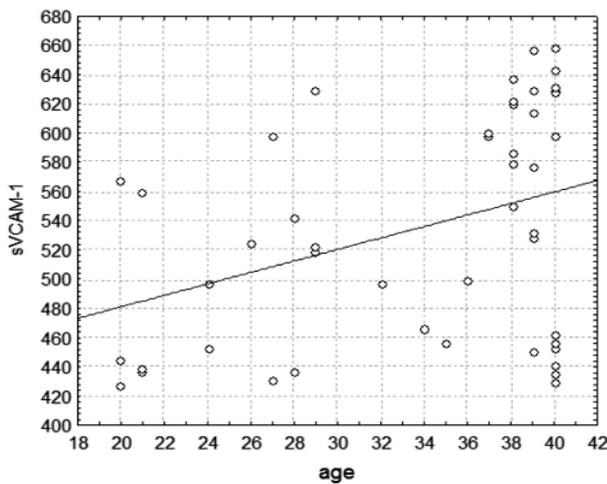


Figure 1 - Correlation VCAM -age

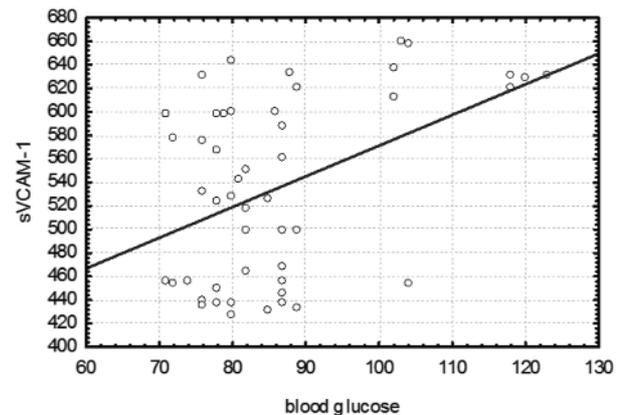


Figure 2 - Correlation VCAM -glycaemia

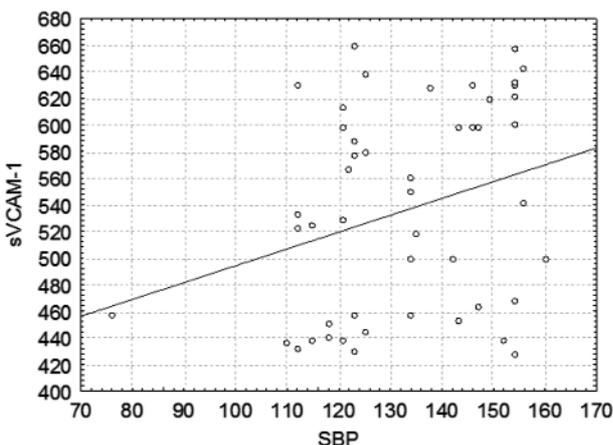


Figure 3 - Correlation VCAM-SBP

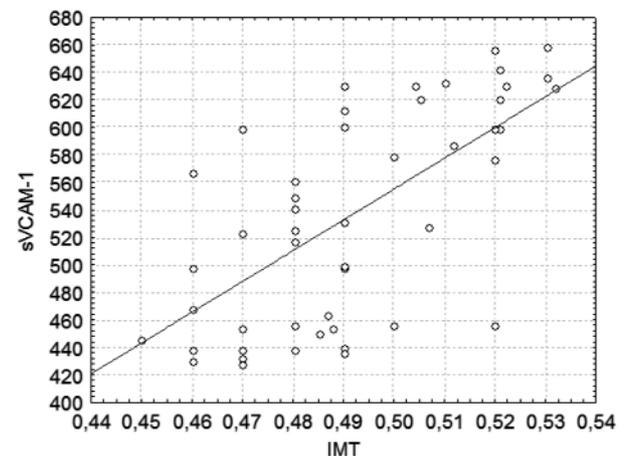


Figure 4 - Correlation VCAM-IMT

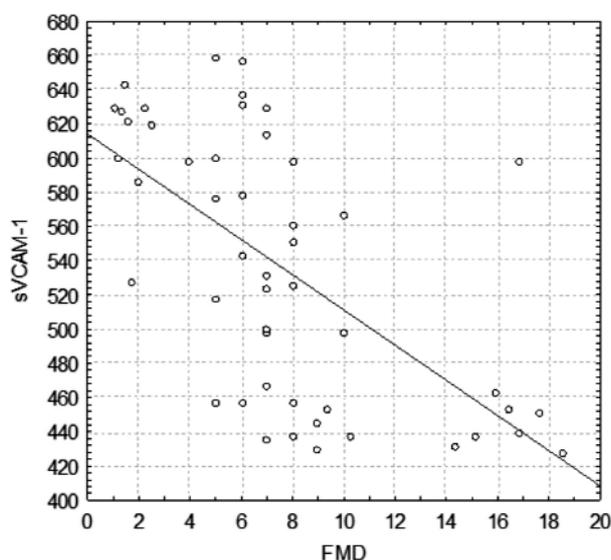


Figure 5 - Correlation VCAM-FMD

In multiple regression analysis, after adjustment for potential confounding risk factors only carotid IMT ($r=0,64$, $p=0,019$) and FMD ($r=-0,61$, $p=0,029$) remained independent predictors of circulating levels of VCAM-1 (R^2 adjusted= $0,48$; F ratio= $10,11$, significance level $p<0,001$) (Fig. 6).

DISCUSSIONS

In our study serum VCAM significantly correlated with subclinical atherosclerosis. There was a strong correlation between serum VCAM and carotid IMT a surrogate marker of subclinical atherosclerosis but also between serum VCAM and endothelial dysfunction an early event of atherosclerosis that precedes structural atherosclerotic changes assessed as flow mediated dilation of the brachial artery. So our data support the concept that circulating VCAMs could be used as a serum marker for subclinical atherosclerosis.

Several studies have reported significant associations of sVCAM-1 (5, 7) or both sVCAM-1 and ICAM (8) with measures of atherosclerosis.

A significant correlation was found between sVCAM-1 and the extent of atherosclerosis as assessed by angiography. Furthermore, circulating VCAM-1 could be used to indicate stages of atherosclerosis with a high degree of statistical significance (5).

Caterina et al (7) demonstrated a significant association between soluble VCAM-1 and carotid atherosclerosis in a small group of hypertensive patients. Rohde et al (8) performed a cross-sectional survey of 92 patients and observed that sVCAM-1 were significantly correlated with mean IMT of the common carotid artery and carotid bifurcation and further support that systemic inflammation may have a role in atherosclerotic lesion development. The association of sVCAM-1 with carotid IMT emphasizes the role of these proteins in early phases of atherogenesis.

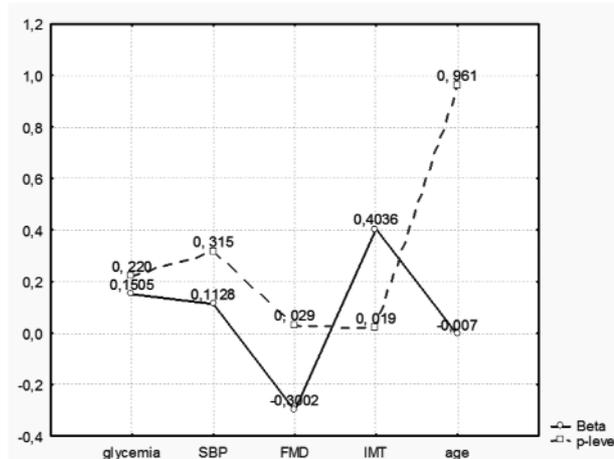


Figure 6 - Correlation VCAM in multiple regression analysis

In the Rotterdam study a population-based cohort study of men and women aged ≥ 55 years sVCAM-1 was not significantly associated with any of the measures of atherosclerosis (the ankle-arm index, carotid IMT and plaques, aortic calcifications) (19).

Also two studies that measured levels of soluble CAMs in patients with peripheral vascular disease found contradictory results (5, 20). The study performed by Peter K. et al (5) found significant correlation between sVCAM-1 and the extent of atherosclerosis as assessed by angiography. Another study did not reveal significant differences between symptomatic peripheral arterial vascular disease patients and asymptomatic patients regarding sVCAM-1 levels (20). But asymptomatic patients may have asymptomatic atherosclerosis and on the other hand symptomatic patients may have a low atherosclerotic burden. Nevertheless in comparison between patients with proven advanced atherosclerosis and patients demonstrating no symptoms of peripheral arterial vascular disease or abnormalities on physical examination a significant difference in sVCAM-1 level can be demonstrated. Moreover two asymptomatic control groups differing in age and thus probably differing in their atherosclerotic burden revealed significant differences in sVCAM-1 levels. Thus, sVCAM-1 level may indicate hitherto asymptomatic atherosclerosis.

Nakai et al (9) observed a higher sVCAM-1 in 13 patients with atherosclerotic aortic disease compared with 40 healthy volunteers and also found a correlation between VCAM-1 mRNA expression and the concentration of circulating VCAM-1 which is important because this result suggests that the circulating VCAM-1 level can be used as an indicator of VCAM-1 expression in atherosclerotic plaques.

Soluble VCAM-1 has a strong predictive value in patients with atherosclerotic lesions in several studies (21, 22, 23) but De Lemos et al found no evidence of an association between sVCAM-1 levels and the risk of future myocardial infarction in a large cohort of apparently healthy men (24).

A sub study of the Atherosclerosis Risk in Communities (ARIC) (6) failed to demonstrate differences in sVCAM-1 levels among patients with carotid atherosclerosis, incident coronary heart disease and control subjects. Rohatgi et al (25)

in the Dallas heart study found that endothelial cell-selective adhesion molecule (sESAM) was independently associated with prevalent coronary calcium, abdominal aortic wall thickness, and aortic compliance. In contrast, no independent associations were observed between sICAM-1 or sVCAM-1 and any of the atherosclerosis phenotypes.

It is not clear what accounts for the diversity of the associations found between sCAMs and atherosclerosis. The populations that have been studied varied substantially in the degree of atherosclerosis and atherosclerosis has been measured at different sites of the arterial tree. Soluble fractions of different CAMs may not be operative in populations in whom the atherosclerotic burden is less accentuated.

Regarding the correlation between serum VCAM-1 and different types of dyslipidemia we found no statistically significant correlation in the present study. Numerous reports document the role of vascular adhesion molecules in the development and progression of atherosclerosis and are now recognized as a critical factor in disease initiation and progression (26). These findings suggest an important role of VCAM-1 in atherosclerosis and may serve as a basis for further evaluation of sVCAM-1 as a potential serum marker for atherosclerosis.

CONCLUSIONS

Serum concentrations of circulating VCAM-1 correlate with markers of subclinical atherosclerosis: carotid intima-media thickness and of endothelial dysfunction (flow mediated dilation). Serum VCAM-1 can probably be useful as a serum marker for subclinical atherosclerosis and can serve as a diagnostic basis for early secondary prevention.

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