Increase of Soluble and Leukocyte Surface Adhesion Molecules and Cytokines in Patients with Coronary Heart Disease

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This study dealt with the availability of the soluble adhesion molecules (sICAM-1, sVCAM-1) as well as leukocyte surface adhesion molecules (CD11b, CD54) and cytokines (TNF-a, IL-6), their correlation with severity of coronary heart disease and cardiovascular risk factors.

The main statistically significant ($p < 0.05$) findings were: 1) sICAM-1 and sICAM-1 levels were higher in acute coronary syndrome group compared with the normal levels in healthy subjects. Plasma concentration of sICAM-1 showed an increment in non-Q wave myocardial infarction (NQMI) group compared with unstable angina (UA) patients; 2) the increment of CD11b and CD54 expression on monocyte surface was noted in patients with acute coronary syndromes compared with stable angina and control groups; 3) there was a correlation between TNF-a and sVCAM-1 ($R = 0.4$) and sICAM-1 ($R = 0.3$). 4) analyzing the relation of adhesion molecules with cardiovascular risk factors was estimated that smoking and obesity influenced sICAM-1 levels. The mean concentration of sICAM-1 was higher in smoking patients and in patients with obesity. The increased expression of CD54 and CD11b on monocyte surface was influenced by hypertension. However, family history of coronary heart disease and patients' age did not significantly influence the concentrations of adhesion molecules and their expression.

These findings indicate that the immunological factors studied could be markers of atherosclerosis and of the severity of coronary heart disease.

Key words: atherosclerosis, adhesion molecules, inflammation, cytokines

INTRODUCTION

Inflammation in the vessel wall is now considered to play an essential role in the initiation, progression and erosion of atherosclerotic plaque, fissuring and eventual rupture. Morphological changes are preceded by dysfunction of endothelial cells, which produce adhesion molecules such as intercellular adhesion molecule-1 (sICAM-1), vascular cell adhesion molecule-1 (sVCAM-1) which interact with inflammatory cells through ligands on their surfaces. Atherosclerosis and its clinical complications are not solely characterizied by local inflammation (1). This study looked for the presence of circulating (sICAM-1, sVCAM-1) and peripheral blood leukocyte surface adhesion molecules (CD11b, CD54), their interactions with released key cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). A correlation with cardiovascular risk factors, clinical syndromes and severity of coronary heart disease was assessed. We hypothesized that these immunological factors could be markers of the presence and severity of atherosclerosis.

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MATERIALS AND METHODS

Study population

The study population consisted of patients with coronary heart disease (CHD) who were admitted to the Department of Cardiology at the Vilnius University Santariskiu Hospital. The study was carried out in 73 patients with CHD: 24 admitted with acu-

te non-Q wave myocardial infarction (NQMI), 24 – with unstable angina (UA) and 25 – with stable angina (SA). The presence of coronary lesions in all patients was confirmed by coronary angiography.

The study cohort comprised 62 men and 11 women (all in menopause) 60 ± 8.6 years old. The patients had the following cardiovascular risk factors: hypercholesterolemia – 58 patients (93.5%), obesity – 22 (35.5%), smoking – 44 (71%), hypertension – 27 (43.5%), family history of coronary heart disease – 23 patients (37%).

Patients with the history of infectious diseases, diabetes melitus and malignancy were excluded. Also we excluded patients treated with systemic steroids or non-steroid agents (except aspirin), because of their possible effect on inflammatory response.

Control values were determined in 20 healthy volunteers (aged 35 ± 11.4 years) without clinical signs of coronary heart disease. We preferred a younger control group because of the lower probability of asymptomatic atherosclerotic disease in this population.

Blood Sampling and Analytic Methods

Biochemical analyses were performed by individuals unaware of the clinical status of the patients. Venous blood was sampled from an antecubital vein within 6 hours of admission. For the measurement of sICAM-1, sVCAM-1, TNF-α and IL-6 concentrations, serum samples were stored at –20 °C until assessment.

sICAM-1 and sVCAM-1 were measured by commercially available enzyme-linked immunoabsorbent assay (R&D Systems, Mineapolis, MN, USA). TNF-α and IL-6 were measured by two-site sequential chemiluminescent immunometric assay (IMMULITE System, DPC, USA).

Cellular surface expression of the adhesion molecules CD11b and CD54 was measured by a direct immunofluorescence flow cytometry technique (Becton Dickinson Immunocytometry Systems, USA). All other variables were analyzed by routine methods at the Laboratory Diagnostics Center of the hospital.

Statistical Analysis

All statistical analyses were performed using the Statistica v5.5a statistical package. The results are presented as means ± SD. Variables were analyzed using two nonparametrical tests: Mann–Whitney U test and one-way analysis of variance (ANOVA). Correlations were performed by using the Spearman coefficient of correlation R. For all results, values of $P < 0.05$ were considered statistically significant.

RESULTS

Relation of soluble adhesion molecules availability with acute coronary syndrome. The sICAM-1 and sICAM-1 levels were statistically significantly higher ($p < 0.05$) in acute coronary syndrome groups (NQMI and UA) when compared with the normal levels of sICAM-1 (211 ± 95 ng/mL) and sVCAM-1 (553 ± ± 159 ng/mL), which were estimated by assay manufacturers during the reference range study from 105 healthy subjects' plasma samples for sVCAM-1 and for 130 plasma samples for sICAM-1.

The plasma concentration of sICAM-1 in our study showed a statistically significant ($p < 0.02$) difference between the UA and NQMI patients. The sVCAM-1 level was higher in NQMI group (Table 1).

Table 1. **Availability of soluble adhesion molecules in acute coronary syndrome groups**

Adhesion molecules	NQMI group	UA group	P value
sICAM-1 (ng/mL)	535 ± 228	390 ± 150	0.02
sVCAM-1 (ng/mL)	1327 ± 714	1054 ± 349	0.1

Relation of expression of adhesion molecules' availability on leukocyte surface with CHD subgroups. A statistically significant difference ($p < 0.0001$) was noted in the expression of CD11b and CD54 on monocyte surface in patients with acute coronary syndrome compared with SA and control group. The expression of these adhesion molecules on lymphocyte surface showed no significant difference between CHD subgroups (Table 2).

Table 2. **Expression of adhesion molecules on leukocyte surface in coronary heart disease patients and control group**

Adhesion molecules	NQMI group	UA group	SA group	control group
CD11b on monocytes (%)	90 ± 4	89 ± 7	81 ± 8	80 ± 4
CD11b on lymphocytes (%)	29 ± 9	30 ± 10	33 ± 13	33 ± 7
CD54 on monocytes (%)	49 ± 10	48 ± 11	34 ± 18	34 ± 11
CD54 on lymphocytes (%)	6 ± 3	7 ± 4	5 ± 3	4 ± 2

Relation of cytokines with acute coronary syndrome. The mean concentrations of IL-6 and TNF-α were higher in patients with NQMI compared with UA group, but they did not reach statistical significance (Table 3).

The correlation between the concentrations of cytokines and adhesion molecules was estimated. There was a correlation between TNF-α and sVCAM-1 (R = 0.4, P = 0.01) and sICAM-1 (R = 0.3, P = 0.06). A slight but not statistically significant correlation between IL-6 and adhesion molecules' concentrations was found.

Table 3. **Cytokines in acute coronary syndrome groups**

Cytokines	NQMI group	UA group	p value
IL-6 (pg/mL)	8.4 ± 6	6 ± 3	0.3
TNF-a (pg/mL)	9.4 ± 5	7.9 ± 5	0.29

Correlation of circulating adhesion molecules with leukocyte and monocyte count. Despite an insignificant correlation between circulating sICAM-1, sVCAM-1 and leukocytes count, there was a weak correlation between soluble adhesion molecules and monocyte count (R = 0.25 and R = 0.29).

Relation of adhesion molecules with cardiovascular risk factors in stable angina. Relation between plasma sICAM-1, sVCAM-1 concentration and expression of the CD11b and CD54 on leukocyte surface with cardiovascular risk factors such as smoking, hypertension, family history of coronary heart disease and obesity was analyzed in patients with SA. Patients with acute coronary syndrome were excluded because of the possible inflammatory effect on these parameters.

A significantly higher (P = 0.05) mean concentration of sICAM-1 was found in patients with obesity compared with normal weight group. Smoking influenced only the sICAM-1 level, too: the mean concentration of sICAM-1 was higher in smoking patients compared with non-smokers (496 ng/mL *vs.* 466 ng/mL, P = 0.6).

There were no statistically significant differences in circulating adhesion molecule levels in patients with and without hypertension, but the expression of CD54 and CD11b on monocyte surface was higher in patients with hypertension.

Family history of coronary heart disease and age did not significantly influenced the concentration and expression of adhesion molecules.

DISCUSSION

Evidence is now accumulating that systemic inflammation may play an important role in the progression of atherosclerosis and may be related to the risk for acute cardiovascular events (1). Current research suggests that inflammation may play a role in atherosclerotic process. Most forms of inflammation are accompanied with increased concentrations of markers such as acute phase reactants, released key cytokines and chemokines, expressed cellular adhesion molecules (2). These circulating markers have been proposed as potential indicators of underlying atherosclerotic disease and unstable plaques.

The present study examined the availability of soluble adhesion molecules (sICAM-1, sVCAM-1) as well as leukocyte surface adhesion molecules (CD11b,CD54) and released key cytokines (TNF-α and IL-6), their correlation with the severity of coronary heart disease and cardiovascular risk factors.

Since ICAM-1 and VCAM-1 are upregulated on activated endothelial surfaces such as overlying atherosclerotic plaques, advanced coronary atherosclerosis may influence circulating sICAM-1 and sVCAM-1 levels (3, 4). We hypothesized that a soluble form of sICAM-1 and sVCAM-1 could be a marker for the presence and severity of atherosclerosis.

Plasma concentration of sICAM-1 in our study showed a statistically significant ($p < 0.02$) difference between the UA and NQMI patients. The sVCAM-1 level was higher in NQMI group, but not statistically significantly. sICAM-1 and sICAM-1 levels were statistically significantly higher ($p < 0.05$) when compared with the normal levels. These findings may be the basis for the future evaluation of circulating VCAM-1 and ICAM-1 as potential serum markers for the presence of atherosclerosis. It is mentioned in the literature that additional prospective studies, particularly large-scale studies, will be required to prove that by estimating the circulating sVCAM-1 and sICAM-1 asymptomatic atherosclerosis can be detected and thus eventually serve as a diagnostic basis for early secondary prevention (5).

ICAM-1 and VCAM-1 favour a firm adhesion of monocytes to the vascular endothelium (6), so leukocytes that take part in inflammation may be potential risk factors for coronary atherosclerosis. That's why we analyzed the relation of sICAM-1 and sVCAM-1 levels with the count of leukocytes and monocytes. However, there was no significant correlation between plasma sICAM-1, sVCAM-1 levels and leukocytes, but there was a slight correlation between plasma sICAM-1, sVCAM-1 and monocyte count (R = 0.25 and R = 0.29). Therefore it should be noted that the increased sICAM-1, sVCAM-1 levels and circulating monocytes' count could be risk factors for coronary heart disease.

It is known that one of ICAM-1 ligands is Mac-1 (CD11b/CD18) (7). We analyzed the expression of CD11b (subunit of the Mac-1 integrin) on monocytes and lymphocytes in order to detect relations between these molecules. We were also interested in another adhesion molecule, CD54, which binds fibrinogen and may take part in the development of atherothrombo-

sis (8). A statistically significant ($p < 0.0001$) increment of the expression of CD11b and CD54 on monocytes in patients with acute coronary syndrome (non-Q-wave myocardial infarction and unstable angina) was noted in our study. These findings indicate that acute coronary syndrome is associated with leukocyte activation and may have a role in the pathogenesis of unstable plaque. So, increased expression of these antigens may be useful markers for the diagnosing, assessment and monitoring of CHD activity. Leukocyte activation is associated with increased adherence to endothelium (9). However, no significant correlation between circulating and cell surface adhesion molecules was found.

We measured IL-6 and TNF-α concentrations as markers of inflammatory activity in patients with acute coronary syndrome, as these cytokines have intense proinflammatory and procoagulant properties (10). The results presented in this article show that the mean concentrations of IL-6 and TNF-α were higher in patients with NQMI compared with UA group, but did not reach statistical significance. So, measurement of these cytokines may by useful in diagnosing and monitoring the CHD activity. There was a correlation between TNF-α and sVCAM-1 ($R = 0.4$, $P = 0.01$), sICAM-1 ($R = 0.3$, $P = 0.06$).

Analyzing the relation of adhesion molecules with cardiovascular risk factors we found that smoking and obesity influenced sICAM-1 levels. The mean concentration of sICAM-1 was higher in smoking and obese patients. The increased expression of CD54 and CD11b on monocyte surface was influenced by hypertension. However, family history of coronary heart disease and patients' age did not significantly influence the concentrations and expression of adhesion molecules.

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References

1. Koening W. Atherosclerosis involves more than just lipids: focus on inflammation. Eur Heart J Supplements 1999; Suppl 1: 19–26.
2. Ross R. Atherosclerosis – an inflammatory disease. N Engl J Med 1999; 340: 115–26.
3. De Caterina R, Basta G et al. Soluble vascular cell adhesion molecule-1 as a biohumoral correlate of atherosclerosis. Arteriosclerosis, Thrombosis and Vascular Biology 1997; 17: 2646–54.
4. Kamijikkoku S, Murohara T, et al. Acute myocardial infarction and increased soluble intercellular adhesion molecule-1: a marker of vascular inflammation and risk of early restenosis? Am Heart J 1998; 136 (2): 231–6.
5. Peter K, Nawroth P, Conradt Ch et al. Circulating vascular cell adhesion molecule-1 correlates with the extent of human atherosclerosis in contrast to circulating intercellular adhesion molecule-1, E-selectin, P-selectin, and thrombomodulin. Arteriosclerosis, Thrombosis and Vascular Biology 1997; 17: 505–10.
6. Springer TA. Adhesion receptors of the immune system. Nature 1990; 346: 425–34.
7. Meersschaert J, Furie MB. The adhesion molecules used by monocytes for migration across endothelium include CD11a/CD18, CD11b/CD18, and VLA-4 on monocytes and ICAM-1, VCAM-1, and other ligands on endothelium. J Immunol 1995; 154: 4099–112.
8. Albelda S et al. Adhesion molecules and inflammatory injury. FASEB J 1994; 8: 504–12.
9. Entman ML, Ballantyne CM. Inflammation in acute coronary syndrome. Circulation 1993; 22: 800–3.
10. Mendall MA. Inflammatory responses and coronary heart disease. BMJ 1998 Mar; 160; Issue 7136: 953–4.

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TIRPIŲ IR LEUKOCITŲ PAVIRŠINIŲ ADHEZIJOS MOLEKULIŲ BEI CITOKINŲ PADIDĖJIMAS LIGONIAMS, SERGANTIEMS KORONARINE ŠIRDIES LIGA

S a n t r a u k a

Tyrimo metu buvo nustatomos tirpios adhezijos molekulės (sICAM-1, sVCAM-1), leukocitų paviršiuje ekspresuojamos adhezijos molekulės (CD11b, CD54) bei uždegiminiai citokinai (TNF-α ir IL-6) ligoniams, sergantiems koronarine širdies liga. Įvertinti šių rodiklių pokyčiai ir koreliacija su KŠL aktyvumu bei aterosklerozės rizikos veiksniais.

Nustatyti šie statistiškai patikimi ($p < 0,05$) rodikliai: sICAM-1 ir sVCAM-1 koncentracija kraujo plazmoje bei CD11b ir CD54 ekspresija leukocitų paviršiuje buvo didesnė ligoniams, sergantiems ūmiu koronariniu sindromu, palyginti su sveikųjų grupe; sICAM-1 koncentracija buvo didesnė ligoniams, sergantiems (Q-) bangos miokardo infarktu, palyginti su nestabilios krūtinės anginos grupe; nustatyta koreliacija tarp TNF-α ir sICAM-1 bei sVCAM-1; analizuojant aterosklerozės rizikos veiksnių įtaką adhezijos molekulių pokyčiams, nustatytas ryšys su rūkymu, nutukimu ir hipertenzija.

Šie duomenys rodo, jog tirtieji rodikliai gali būti aterosklerozės ir koronarinės širdies ligos aktyvumo žymenimis.