Serum Fas/FasL levels in dependence on clinical presentations of coronary disease and their relationship with risk factors

Koncentracije Fas/FasL u zavisnosti od kliničke prezentacije koronarne bolesti i njihov odnos sa faktorima rizika

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Abstract

**Background/Aim.** Ischemic heart disease is mostly a consequence of atherosclerosis. Besides the inflammation, the Fas/Fas ligand (FasL)/caspase death pathway is documented to be activated in atherosclerotic lesions. The aim of this study was to compare the values of soluble forms of Fas and FasL in patients with different presentations of coronary disease and to correlate Fas/FasL with risk factors.

**Methods.** We studied 30 patients with chronic stable angina pectoris (SAP), 27 with non-stable angina pectoris (NSAP), and 39 with acute ST-elevation myocardial infarction (STEMI) and 27 age-matched healthy volunteers (the control group). Serum Fas/APO-1 and FasL concentrations were determined using a commercially available enzyme-linked immunoassays (ELISA).

**Results.** Fas/APO-1 levels in the STEMI patients (6.981 ± 2.689 ng/mL) were significantly higher than Fas levels in the controls (5.092 ± 1.252 ng/mL, p < 0.01), but not significantly higher than Fas values in the SAP (5.952 ± 2.069 ng/mL) and the USAP patients (5.627 ± 2.270 ng/mL). Levels of FasL did not show any significant difference among the studied groups. In the SAP patients Fas/APO1 showed a significant positive correlation with high sensitivity C-reactive protein (hsCRP) (p < 0.05) and a negative correlation with high-density lipoprotein cholesterol (HDL-C) (p < 0.05), while FasL showed a significant positive correlation with low-density lipoprotein cholesterol (LDL-C) (p < 0.05). Fas levels between the patients having cholesterol within normal range and those whose cholesterol was above the normal range showed a significant difference (p < 0.05) only in the NSAP patients. Fas and FasL levels between the patients with hsCRP lower than 3.0 mg/L and those with hsCRP higher than 3.0 mg/L of the SAP group showed a significant differences (p < 0.001, p < 0.05, respectively). Strong correlation between Fas concentration and diabetes mellitus (p < 0.05) and FasL concentrations and both cholesterol (p < 0.01) and triglycerides (p < 0.01) in the NSAP patients was observed. The patients in the SAP group showed no strong correlation between Fas and FasL concentration and risk factors.

**Conclusions.** The obtained results showed that apoptotic process is dysregulated in the patients with ischemic heart disease. Interdependence between Fas and FasL and inflammatory and lipid markers as well as with cardiovascular risk factors was established.

**Key words:** myocardial ischemia; fas ligand protein; antigens, CD95; inflammation mediators; atherosclerosis; risk factors; cholesterol, LDL; cholesterol, HDL.

Apstrakt

**Uvod/Cilj.** Ischemijska bolest srca je najčešće posledica ateroskleroze. Pored inflamacije, u aterogenezi je dokazana aktivnost spoljašnjeg puta apoptoze (Fas/FasL/caspasa puta). Cilj ove studije bio je poredjenje nivoa Fas i FasL kod bolesnika sa različitim prezentacijom koronarne bolesti i korelisanje Fas/FasL s faktorima rizika. **Metode.** Ispitano je 30 bolesnika sa stabilnom anginom pektoris (SAP), 27 sa nestabilnom anginom pektoris (NSAP), 39 sa akutnim infarktom miokarda (AIM) i 27 zdravih osoba. Koncentracije Fas i FasL u serumu određivane su komercijalnim ELISA testovima. **Rezultati.** Koncentracije Fas kod bolesnika sa AIM (6,981 ± 2,689 ng/mL) bile su statistički značajno više od koncentracija Fas ispitanika kontrolne grupe (5,092 ± 1,252 ng/mL, p < 0.01) i statistički neznačajno više od Fas nivoa u SAP i NSAP grupi. Kod ispitivanih grupa nije bilo statistički značajne razlike u vrednostima FasL. Kod bolesnika sa SAP postojala je značajna pozitivna korelacija Fas i visoko senzitivnog C-
Introduction

Ischemic heart disease is mostly a consequence of atherosclerosis. Atherosclerosis represents a chronic inflammatory state which leads to the evolution of an uncomplicated atheromatous plaque into complex and vulnerable atheroma. A flared plaque inflammation is considered to be the source of intimal erosion and rupture and therefore of acute ischemia. Besides the inflammation, the Fas/Fas ligand (FasL)/caspase death pathway, involved in extrinsic apoptotic pathway, is documented to be activated in atherosclerotic lesions. Dysregulation of apoptosis within a vessel wall and upregulation of the Fas/FasL system contribute to the development of atherosclerosis. The effects of apoptosis during atherogenesis depend on the stage of the plaque, localization and the cell types involved. Both macrophages and smooth muscle cells as well as endothelial and blood borne cells, undergo apoptosis in atherosclerotic plaque.

The Fas ligand (FasL; CD95 or APO-1 ligand) is a cytokine that mediates apoptosis by binding to its receptor, Fas (CD95 or APO-1), leading to the activation of executor caspases. Fas is expressed almost ubiquitously in a variety of cells, including cardiomyocytes, whereas FasL is mainly expressed in natural killer cells, activated T cells and macrophages as well as in immune-privileged tissues of the eye and testes. Proteins secreted by cells implicated in atherosclerotic lesions, including soluble Fas (sFas) and soluble Fas ligand (sFasL), circulate in small, but detectable amounts.

Soluble Fas is generated by alternative messenger RNA splicing capable of encoding a soluble Fas molecule lacking the transmembrane domain, while sFasL is released in the serum from membrane-bound FasL, processed by a metalloprotease. It has been demonstrated that Fas and FasL are expressed in atherosclerotic lesions and the Fas/FasL system is related to the apoptotic and inflammatory responses present in atherosclerotic plaques. Measurement of circulating markers of inflammation may provide some insight into this process.

The first aim of this study was to compare the values of soluble forms of Fas and FasL in patients with different presentations of coronary disease and to correlate Fas/FasL with risk factors. The second aim of this study was to evaluate the diagnostic values of soluble forms of Fas and FasL in patients with stable angina pectoris (SAP), non-stable angina pectoris (NSAP) and acute myocardial infarction (AMI).

Methods

The study involved 96 patients with angina chest pain admitted to the Institute for Cardiovascular and Rheumatic Diseases “Niška Banja”. Among them, 30 patients (11 females and 19 males, aged 60.17 ± 11.78 years) had chronic SAP, 27 (15 females and 12 males aged 68.33 ± 8.75 years) had NSAP, and 39 (12 females and 27 males, aged 64.87 ± 9.03 years) had acute ST-elevation myocardial infarction (STEMI). Furthermore, SAP was defined as a typical exertional chest pain lasting from 1 to 15 minutes relieved by glyceryl trinitrate, electrocardiogram (ECG) changes (depression or elevation of ST-segment) in angina attack or with positive responses to exercise ECG and/or positive stress echocardiography testing. None of the patients from this group had a previous myocardial infarction in their history, as well as a cardiomyopathy, malignant arrhythmias or cardiac valve disease. All NSAP patients had angina chest pain at rest within the past 48 hours (class IIIB), typical changes in ECG (ST-segment changes, T-wave changes), negative cardiac enzymes and negative troponin I. Diagnosis of AMI was based on the ischemic symptoms (chest pain persisting longer than 30 minutes) and typical changes on the ECG at the admission and elevated troponin I levels. All of these patients had STEMI, which was defined as significant ST-elevation according to the current Guidelines of the European Society of Cardiology (new ST-segment elevation at J point with the cut-off points ≥ 0.2 mV in V1 through V3 and > 0.1 mV in other leads).

All patients gave the data about age, sex, risk factors: hypertension, diabetes mellitus, smoking, obesity (an obese person is every person with body mass index higher than 25 kg/m²), family history (family history are data about fathers’ or mothers’ appearance of AIM before 60 years of age), physical inactivity (physical inactive persons are ones who have way of life without any physical activity in their free time), cholesterol and triglyceride levels, and current therapy just after admission.

Venous blood samples from patients were taken within 24 hours after admission and standard analyses were performed on the same day. Sera for Fas and FasL were collected and stored in aliquots at -20° C until analysis.

We also tested 27 healthy volunteers (the control group). The controls were recruited from the Department for Blood Transfusion from blood bank donors. The volunteers did not have any history of hypertension, diabetes or ischemic heart disease.
disease. The patients and volunteers gave written informed consent before the study entry and the study was approved by the local Ethic Committee. All the patients were followed up for 1 year. Major clinical events during the follow-up were class III NSAP, non-fatal myocardial infarction and cardiac death.

Serum Fas/APO1 concentrations were determined using a commercially available immunoassay (ELISA kit, BioSource, Nivelles, Belgium) with sensitivity < 20 pg/mL.

Serum FasL concentrations were determined using a commercially available immunoassay (ELISA kit, BioSource, Nivelles, Belgium) with sensitivity < 0.1 pg/mL.

All other biochemical markers were estimated by an analyzer Olympus AU 400 (Olympus, Tokyo, Japan) and troponin I concentration by AxSYM analyzer (Abbott Laboratories, Abbott Park, IL, USA).

Statistical analysis was performed to identify the differences in serum concentrations of apoptotic markers, as well as to correlate their levels with standard biochemical markers and risk factors. The obtained data were tested out using analysis of descriptive (average, standard deviation) and analytical (Dunnett’ test-for multiple comparisons; Student’s non-paired t test) statistics. Linear regression analysis was used to assess the relationships between the studied apoptotic markers and risk factors. Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS) 15.0 computer program (SPCC Inc, Chicago, IL, USA).

Results

Baseline characteristics of patients and coronary risk factors are shown in Table 1. The NSAP patients had the highest prevalence of physical inactivity (100%) and hypertension (81.48%), higher than the SAP and STEMI patients. The SAP patients had a higher prevalence of smoking habits (50%) and the most STEMI patients had familiar history of coronary heart disease (51.28%).

Serum concentrations of apoptotic markers Fas and FasL are given in Figures 1 and 2. Fas levels in the STEMI patients had the highest prevalence of physical inactivity (100%) and hypertension (81.48%), higher than the SAP and STEMI patients. The SAP patients had a higher prevalence of smoking habits (50%) and the most STEMI patients had familiar history of coronary heart disease (51.28%).

Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>STEMI patients (n = 39)</th>
<th>NSAP patients (n = 27)</th>
<th>SAP patients (n = 30)</th>
<th>Controls (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), ŝ ± SD</td>
<td>64.87 ± 9.03</td>
<td>68.33 ± 8.75</td>
<td>60.17 ± 11.78</td>
<td>58.52 ± 5.60</td>
</tr>
<tr>
<td>Sex (male/female), n</td>
<td>27/12</td>
<td>12/15</td>
<td>19/11</td>
<td>12/15</td>
</tr>
<tr>
<td>Coronary risk factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>76.62†</td>
<td>81.48‡</td>
<td>66.67§</td>
<td>0</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>30.77†</td>
<td>18.52</td>
<td>26.67¶</td>
<td>0</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>41.03</td>
<td>29.63</td>
<td>50.00</td>
<td>25.6</td>
</tr>
<tr>
<td>Obesity (%)</td>
<td>17.95*</td>
<td>3.70 ¶</td>
<td>26.67</td>
<td></td>
</tr>
<tr>
<td>Family history (%)</td>
<td>51.28*</td>
<td>48.15</td>
<td>26.67</td>
<td>22</td>
</tr>
<tr>
<td>Physical inactivity (%)</td>
<td>92.31*</td>
<td>100.00</td>
<td>96.67§</td>
<td>39.5</td>
</tr>
</tbody>
</table>

*p < 0.05 STEMI vs controls; †p < 0.001 STEMI vs controls; ‡p < 0.001 NSAP vs controls; §p < 0.001 SAP vs controls; ¶p < 0.05 SAP vs controls; ||p < 0.05 SAP vs controls

STEMI – ST elevation myocardial infarction; NSAP – non-stable angina pectoris; SAP – stable angina pectoris

Fig. 1 – Serum Fas/APO-1 (ng/mL) values in patients with ischemic heart disease

SAP – stable angina pectoris; NSAP – non-stable angina pectoris; AIM – acute myocardial infarction

Fig. 2 – Serum FasL (ng/mL) values in patients with ischemic heart disease

SAP – stable angina pectoris; NSAP – non-stable angina pectoris; AIM – acute myocardial infarction
patients (6.981 ± 2.689 ng/mL) were significantly higher than Fas levels in the controls (5.092 ± 1.252 ng/mL, p < 0.01), but not significantly higher than Fas values in the SAP (5.952 ± 2.069 ng/mL) and NSAP patients (5.627 ± 2.270 ng/mL). Levels of FasL did not show any significant difference among the studied groups.

The correlation between apoptotic markers, Fas/Apo1 and FasL, and inflammatory and lipid markers was studied for each patient group. The significant correlation was observed only in the SAP patients. Fas/APO1 showed a significant positive correlation with the high sensitivity C-reactive protein (hsCRP) (p < 0.05) (Figure 3) and a negative correlation with high-density lipoprotein cholesterol (HDL-C) (p < 0.05) (Figure 4), while FasL showed a significant positive correlation with low-density lipoprotein cholesterol (LDL-C) (p < 0.05) (Figure 5).

All patient groups were divided into two subgroups by cholesterol levels (cholesterol levels in reference ranges 3.5–5.5 mmol/L (Cholesterol -) and cholesterol levels higher than reference values (Cholesterol +). Fas levels between these subgroups showed a significant difference (p < 0.05) only in NSAP patients (Table 2). In this group 51.85% of patients had hypercholesterolemia.

There are two subgroups in the SAP patient group according to hsCRP levels (hsCRP levels lower than 3.0 mg/L (hsCRP -), and hsCRP levels higher than 3.0 mg/L (hsCRP+). Fas and FasL levels between these subgroups showed a significant difference (p < 0.001, p < 0.05, respectively) (Table 3).

A strong correlations between Fas concentration and diabetes mellitus (p < 0.05), and FasL concentrations and both cholesterol (p < 0.01) and triglycerides (p < 0.01) in NSAP patients were observed. The SAP patients showed no strong correlation between Fas and FasL concentration and risk factors (Table 4).
AMI, but independently of the size of infarction. Serum Fas concentration increases in direct relation to the extent of necrosis, with sFas proteolytic release from necrotic cardiomyocytes. These findings may be explained by the fact that sFas is only increased in STEMI patients. This might be the result of different mechanisms.

Our results showed a significant increase in serum sFas levels in patients with end-stage renal disease 2. Our results showed a significant increase in serum sFas levels in patients with end-stage renal disease 2. Our results showed a significant increase in serum sFas levels in patients with end-stage renal disease 2. Our results showed a significant increase in serum sFas levels in patients with end-stage renal disease 2.

The Fas antigen is expressed on T and B cells, granulocytes, monocytes, and natural killer cells, particularly on activated cells. The Fas is cleaved by proteolytic enzymes produced by inflammatory cells and sFas is generated by alternative mRNA splicing 15. The supernatants containing sFas may contain sFasL in patients with myocarditis, chronic congestive heart failure and coronary artery disease, suggesting that the Fas/FasL system may contribute to the pathogenesis of cardiovascular disease 12–14.

Table 4

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Non-stable angina pectoris</th>
<th>Stable angina pectoris</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fas</td>
<td>FasL</td>
</tr>
<tr>
<td>Cholesterol -</td>
<td>6.18 ± 1.39</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>Cholesterol +</td>
<td>5.26 ± 2.53</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>TG -</td>
<td>6.18 ± 1.39</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>TG +</td>
<td>5.26 ± 2.53</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>DM -</td>
<td>6.08 ± 1.95</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>DM +</td>
<td>3.55 ± 0.23</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>Smoking -</td>
<td>5.83 ± 2.14</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>Smoking +</td>
<td>5.06 ± 1.31</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>Family history -</td>
<td>5.66 ± 2.29</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>Family history +</td>
<td>5.81 ± 1.81</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>Obesity -</td>
<td>5.72 ± 2.02</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>Obesity +</td>
<td>5.66 ± 2.29</td>
<td>0.08 ± 0.02</td>
</tr>
</tbody>
</table>

Discussion

This study shows that sFas levels are significantly increased only in STEMI patients compared to the controls while sFas levels in other patient groups are not significantly changed. Furthermore, sFasL levels were not significantly different among the studied groups. The results obtained from other studies in ischemic heart disease showed that sFas levels are increased and sFasL levels are decreased in subjects at high cardiovascular risk compared to healthy subjects. Several single-center studies demonstrated that sFas was elevated in patients with myocarditis, chronic congestive heart failure and coronary artery disease, suggesting that the Fas/FasL system may contribute to the pathogenesis of cardiovascular disease 12-14.

In our study sFas levels showed a positive correlation with hsCRP levels and a negative correlation with HDL-C levels in SAP patients. A positive correlation between FasL levels and LDL-C levels was also observed. Contrary to these findings, other authors showed that circulating sFas concentrations did not correlate with any lipid parameter analyzed at baseline, and the reduction observed in lipid values after atorvastatin treatment was not related to the change in sFas concentrations 20.

Inflammation plays a role in all stages of atherogenesis and some inflammatory markers, such as hsCRP, have been shown to be independent predictors of coronary heart disease. It is well known that CRP is a marker of systemic inflammation and may contribute actively to the development of the atherosclerotic lesions. Accumulation of CRP in early atherosclerotic lesions may precede the appearance of monocytes and native LDL co-incubated with CRP are taken up by macrophages. Likewise, CRP induces adhesion molecule expression (ICAM-1, VCAM-1, E-selectin) by human umbilical vein and coronary artery endothelial cells and can induce the production of the chemokine MCP-1, too. Statins can mediate this effect by inhibiting chemokine expression and reducing CRP plasma levels 19.

In our study sFas levels showed a positive correlation with hsCRP levels and a negative correlation with HDL-C levels in SAP patients. A positive correlation between FasL levels and LDL-C levels was also observed. Contrary to these findings, other authors showed that circulating sFas concentrations did not correlate with any lipid parameter analyzed at baseline, and the reduction observed in lipid values after atorvastatin treatment was not related to the change in sFas concentrations 20.

There is evidence that Fas/FasL interactions may be related to augmented proliferation and inflammatory response. In this sense, signals initiated by regulated Fas-associated death domain protein overexpression induce expression of monocyte-chemoattractant protein-1 and interleukin 8, and cause massive migration of macrophages in vivo, indicating that Fas and FasL act also as proinflammatory proteins. Univariate analyses demonstrated that circulating sFas levels are increased in patients with hypertension, diabetes or the metabolic syndrome compared with patients without these pathologies. Cigarette smoke is an important source of oxidants, including H2O2, and is thought to be a significant risk factor for chronic endothelial damage leading to atherosclerosis. Patients with familial combined hyperlipidemia or carotid atherosclerosis have decreased serum sFasL levels suggesting endothelial dysfunction. The role of apoptosis in vascular disease. J Pathol 2000; 190(3): 267–80.


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