The Relationship of Serum Soluble Fas Ligand (sFasL) Level with the Extent of Coronary Artery Disease

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Abstract

Fas/Fas ligand system contributes to the programmed cell death induced by myocardial ischemia. We investigated whether serum soluble Fas ligand (sFasL) level is independently related with the severity and extent of angiographically assessed coronary artery disease (CAD). We included 169 patients in this study. Two groups were formed based on the existence of a lesion on coronary angiography. First group included patients with normal coronary arteries (NCA; $n = 53$). Patients with atherosclerotic lesions were included in the second group ($n = 116$). We used the coronary vessel score (the number of the coronary arteries with a lesion leading to $\geq 50\%$ luminal obstruction) and the Azar score to determine the extent and the severity of CAD. Standard enzyme-linked immunosorbent assay kits were used to measure serum sFasL levels. The serum sFasL level was higher in patients with CAD than in patients with NCA (0.52 ± 0.23 mU/mL vs. 0.45 ± 0.18 mU/mL, $p = 0.023$). The sFasL level correlated with Azar score ($r = 0.231$, $p = 0.003$) and with coronary vessel score ($r = 0.269$, $p < 0.001$). In the multivariate analysis, we found that age (beta: 0.188, $p = 0.008$), gender (beta: 0.317, $p < 0.001$), diabetes mellitus (DM; beta: 0.195, $p = 0.008$), and sFasL level (beta: 0.209, $p = 0.003$) were independently related with Azar score. When we used coronary vessel score as the dependent variable, we found that age ($p = 0.020$), gender ($p < 0.001$), DM ($p = 0.006$), and sFasL level ($p = 0.001$) were independent predictors. Serum sFasL level is associated with angiographically more severe CAD. Our findings suggest that sFasL level may be a biochemical surrogate of severe coronary atherosclerosis.

Keywords

- soluble fas ligand
- ischemic heart disease
- atherosclerosis
- apoptosis

Coronary artery disease (CAD) is one of the most important causes of mortality and morbidity all over the world. Since coronary lesions progress in silence until a critical per cent of the coronary artery lumen becomes occluded, clinical presentation often occurs with an acute coronary syndrome (ACS), which has increased mortality and morbidity. Identification of biomarkers, which show the atherosclerotic burden in patients with stable angina, may provide risk stratification

and contribute to prevention of ACS or may prevent unnecessary invasive diagnostic strategies.

The process of programmed cell death—apoptosis—is an important mechanism in the pathophysiology of atherosclerosis. Atherosclerotic plaques include large numbers of apoptotic cells and related receptors, suggesting an important role for apoptosis in the existence and progression of atherosclerosis.^{1,2} Fas ligand (FasL) is a type II membrane protein

that induces apoptosis when it binds to its membrane receptor Fas.³ It is expressed by activated T lymphocytes, natural killer cells, and by certain organs and tissues as well as endothelial cells.^{4,5} The expression of FasL determines the immune-privileged status of these tissues, as the inflammation can be prevented by destruction of the activated inflammatory cells via FasL/Fas-mediated apoptosis invading these tissues.⁶

Ischemia can increase the expression of FasL. Thus, FasL contributes to the programmed cell death induced by myocardial ischemia.⁷ The membrane-bound FasL can be converted to a soluble form (sFasL) by the help of metalloproteinase.⁸ In hypertensive patients, sFasL level was found to be associated with carotid intima-media thickness.⁹ Shimizu et al found that sFasL increases in patients with ACS.¹⁰ This increase in FasL/Fas-mediated apoptosis in patients with ACS may be explained by that the patients have stable angina with stabilized plaques whereas high-risk patients did not, in which demand on this system may be increased to limit an excessive damage by the cytotoxic cells. Although these findings show a relationship between soluble Fas (sFas) and atherosclerosis, sFasL level has not been evaluated in patients with documented stable CAD.

The purpose of this study was to investigate whether a higher sFasL level is related with the angiographic extent and severity of obstructive coronary atherosclerosis in patients with stable CAD.

Methods

The local ethics review boards approved the study. Patients who presented to the outpatient clinic with stable angina pectoris and who underwent coronary angiography between June 2007 and January 2009 in Gazi University Hospital, were enrolled prospectively. The indication for angiography was based on the presence of typical angina pectoris or a positive stress test. Patients with ACS or unstable angina pectoris, chronic kidney disease (serum creatinine >1.4 mg/dL), chronic obstructive pulmonary disease, active malignancy, autoimmune disorder, thyroid disease, and a previous coronary artery revascularization either by coronary artery bypass graft operation or percutaneous coronary intervention were excluded from the study. Of 229 eligible patients, 169 patients gave informed consent and were included in the study. Baseline characteristics, which include presence of hypertension, diabetes mellitus (DM), smoking status, family history of CAD, lipid parameters, and active drug treatment were recorded during the direct interview with the patient before coronary angiography. Hypertension was defined as the active use of antihypertensive drugs or documentation of blood pressure more than 140/90 mm Hg. The measurements of systolic and diastolic blood pressure had been performed at visit 1 week before coronary angiography. DM was defined as fasting glucose levels over 126 mg/dL or glucose level over 200 mg/dL at any measurement or active use of antidiabetic drugs or insulin. Smoking was defined as current smoking. The family history for CAD was defined as a history of documentation of CAD or sudden death in a first-degree

relative before the age of 55 years for men and 65 years for women.

Standard selective coronary angiography with at least four views of the left coronary system and two views of the right coronary artery was performed using the Judkins technique. The coronary angiograms of the study patients were examined for CAD by two experienced interventional cardiologists who were totally blind to the results of the blood tests. The degree of the luminal obstruction caused by the atherosclerotic plaque was determined by visual assessment. The patients were divided into two groups according to the existence of lesions at coronary angiography. The first group included the patients with entirely normal coronary arteries (NCA) at coronary angiography ($n = 53$). All other patients who had CAD at coronary angiography were included in the second group (CAD group, $n = 116$).

For the analysis of the relation between multivessel disease and serum sFasL level, we determined the number of the diseased vessels according to the number of major coronary arteries having $\geq 50\%$ stenosis (coronary vessel score). We used the Azar score to determine the extent and the severity of the CAD, which was calculated according to the method defined by Azar et al.¹¹ According to this method, coronary artery anatomy was divided into nine segments: left main coronary artery, proximal left anterior descending (LAD) artery, mid LAD, distal LAD, proximal circumflex artery, distal circumflex artery, proximal right coronary artery, distal right coronary artery, and posterior descending artery. We gave 0 point if the segment had no lesion, 1 point if there was a lesion leading to obstruction of the vessel lumen by 1 to 49%, and 2 points if there was a lesion leading to the obstruction of the vessel lumen by 50 to 99%. If the vessel lumen was totally occluded, we gave 3 points and arbitrarily 1 point for each segment distal to the occlusion.

The blood samples for measurement of sFasL level were collected just before coronary angiography. Whole blood samples were centrifuged and the serum stored at -80°C in aliquots. Serum sFasL level was determined by using standard enzyme-linked immunosorbent assay kits (BioSource, Invitrogen Corp., Carlsbad, CA). The intra-assay coefficient of variations was 6.1%, and the interassay coefficient of variations was 7.0%.

Statistical Analysis

Continuous variables were given as mean $\pm$ SD; categorical variables were defined as percentage. Data were tested for normal distribution using the Kolmogorov-Smirnov test. The Student *t* test was used for the univariate analysis of the continuous variables and the χ^2 test for the categorical variables. Multivariate linear regression analyses with stepwise method were used to assess multivariate relations among various variables. All tests of significance were two-tailed. Statistical significance was defined as $p < 0.05$. The Statistical Package for the Social Sciences statistical software (SPSS 15.0 for Windows Inc., Chicago, IL) was used for all statistical calculations.

Results

The baseline clinical characteristics of the groups are shown in ►Table 1. The number of male patients was higher in the CAD group. The serum sFasL level was significantly higher in the CAD group (0.45 ± 0.18 mU/mL vs. 0.52 ± 0.23 mU/mL, $p = 0.023$). Other than sFasL level, serum creatinine level (0.88 ± 0.15 mg/dL vs. 1.01 ± 0.22 mg/dL, $p < 0.001$) was also significantly higher in patients with CAD. The high-density lipoprotein (HDL) level was higher in NCA group (51 ± 12 mg/dL vs. 45 ± 12 mg/dL, $p = 0.014$). As expected, the number of the patients who were under treatment of aspirin (acetylsalicylic acid, ASA)/clopidogrel, statin, and β -blocker was significantly higher in CAD group. In the univariate correlation analysis, we observed a positive correlation between sFasL level and Azar score ($r = 0.231$, $p = 0.003$) and between sFasL level and coronary vessel score ($r = 0.269$, $p < 0.001$). However, sFasL was not related with

the other study parameters. When we performed multivariate analysis to investigate the relation between sFasL and the Azar score as a measurement of the extent and the severity of the coronary atherosclerosis, we found that age (beta: 0.188, $p = 0.008$), gender (beta: 0.317, $p < 0.001$), DM (beta: 0.195, $p = 0.008$), and sFasL level (beta: 0.209, $p = 0.003$) were independently related with Azar score. In this model, when coronary vessel score was used as the dependent variable, gender ($p < 0.001$), DM ($p = 0.006$), and sFasL levels ($p = 0.001$) were found as the independent predictors (►Table 2). Furthermore, when we searched the effects of cardiovascular risk factors and the pharmacotherapy on sFasL levels, we found that hypertension and ASA/clopidogrel usage were related with increased sFasL levels as well as Azar score (►Table 3). In multivariate analysis, only ASA/clopidogrel usage (beta: 0.182, $p = 0.018$) and Azar score (beta: 0.202, $p = 0.008$) were found to be significantly related to sFasL (►Table 4).

Table 1 Baseline Characteristics of the Study Population

Parameters	NCA <i>n</i> = 53	CAD <i>n</i> = 116	<i>p</i> Value
Azar score	0 $\pm$ 0	4.87 $\pm$ 3.22	<0.001
Age (years)	55 $\pm$ 10	60 $\pm$ 10	0.003
Gender (male)	32%	65%	<0.001
BMI (kg/m ²)	28.7 $\pm$ 4.3	28.0 $\pm$ 4.2	NS
Hypertension	45%	60%	0.067
DM	17%	29%	0.088
Smoking (current)	30%	31%	NS
Family history of CAD	19%	16%	NS
Leukocytes (/mm ³)	7372 $\pm$ 1634	7873 $\pm$ 2341	NS
Hemoglobin (mg/dL)	14 $\pm$ 1.4	14 $\pm$ 1.5	NS
Platelets (/mm ³)	281 $\pm$ 77	246 $\pm$ 59	NS
Creatinine (mg/dL)	0.88 $\pm$ 0.15	1.01 $\pm$ 0.22	<0.001
Total cholesterol (mg/dL)	206 $\pm$ 35	200 $\pm$ 48	NS
HDL (mg/dL)	51 $\pm$ 12	45 $\pm$ 12	0.014
LDL (mg/dL)	130 $\pm$ 29	127 $\pm$ 40	NS
Triglyceride (mg/dL)	130 $\pm$ 61	152 $\pm$ 74	0.070
sFasL (mU/mL)	0.45 $\pm$ 0.18	0.52 $\pm$ 0.23	0.023
Medications			
ASA/clopidogrel	9%	28%	0.008
ACEI/ARB	36%	44%	NS
Statin	15%	31%	0.028
Beta blockers	21%	43%	0.005
CCB	11%	14%	NS
Nitrate	0%	3%	NS
OAD	9%	10%	NS

NCA, normal coronary arteries; CAD, coronary artery disease; BMI, body mass index; NS, not significant; DM, diabetes mellitus; HDL, high-density lipoprotein; LDL, low-density lipoprotein; sFasL, soluble Fas ligand; ASA, acetylsalicylic acid; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; CCB, calcium-channel blocker; OAD, oral antidiabetic drugs.

Table 2 Multivariate Analyses Using the Linear Regression Method^a

Variables	$\beta \pm SE$	Beta	<i>p</i> Value ^b	$\beta \pm SE$	Beta	<i>p</i> Value ^c
Age (years)	0.06 $\pm$ 0.02	0.188	0.008	0.02 $\pm$ 0.01	0.167	0.020
Gender (male)	2.2 $\pm$ 0.5	0.317	<0.001	0.62 $\pm$ 0.15	0.292	<0.001
DM	1.6 $\pm$ 0.6	0.195	0.008	0.49 $\pm$ 0.18	0.202	0.006
sFasL (mU/mL)	3.3 $\pm$ 1.1	0.209	0.003	1.1 $\pm$ 0.34	0.203	0.001
Constant	-3.6 $\pm$ 1.5	—	0.018	-0.5 $\pm$ 0.5	—	NS
R ²	0.206			0.195		

Note: The regression coefficients: Unstandardized coefficient, $\beta \pm SE$ (β coefficient $\pm$ standard error); standardized coefficient, Beta

^aLinear regression with stepwise method was used for multivariate analysis of independent variables including age, gender, hypertension (HT), DM, high-density lipoprotein (HDL), triglyceride, and sFasL levels, which were significantly different between NAC and CAD groups in univariate analysis. After exclusion of HT, creatinine, HDL, and triglyceride from model, linear regression with enter method were performed and then obtained results were presented.

^bCoronary atherosclerotic burden (determined by Azar score, *p* value).

^cThe number of coronary artery with significantly obstructive lesion (*p* value).

DM, diabetes mellitus; sFasL, soluble Fas ligand; NS, not significant.

Discussion

In this study, we aimed to investigate whether sFasL level is correlated with the angiographic extent and severity of obstructive coronary atherosclerosis in patients with stable CAD through the potential role of apoptosis on CAD. We found that sFasL level was significantly higher in patients with stable CAD compared with patients with normal coronary arteries at coronary angiography. Serum sFasL level was positively correlated with extent and severity of the CAD, which was determined by either Azar score or coronary vessel score. To the best of our knowledge, our study is the first one

to demonstrate a relationship between the extent of angiographic lesion and sFasL levels in patients with stable CAD.

Apoptosis or programmed cell death is a complex mechanism to control the tissue amount and architecture. This process is also shown to be effective in the development and progression of atherosclerosis. FasL is an important mediator in apoptotic cell death. It can bind Fas receptor and lead to inhibition of activated inflammatory cells within the atherosclerotic plaque.^{5,6} Certain immune system cells and vascular endothelial cells express FasL.¹² The membrane-bound FasL can be converted to a soluble form (sFasL) by metalloproteinases.⁸ Hence, the plasma level of

Table 3 Changes in sFasL Levels according to Traditional Cardiovascular Risk Factors and Medication Usage

Risk Factors	sFasL (mU/mL)		<i>p</i> Value
Age (years) (<45 vs. $\geq$ 45)	0.57 $\pm$ 0.27	0.57 $\pm$ 0.31	NS
Gender (female vs. male)	0.57 $\pm$ 0.31	0.57 $\pm$ 0.30	NS
Hypertension (absent vs. present)	0.52 $\pm$ 0.27	0.60 $\pm$ 0.33	0.019
DM (absent vs. present)	0.57 $\pm$ 0.31	0.59 $\pm$ 0.39	NS
Smoking, current (absent vs. present)	0.55 $\pm$ 0.30	0.60 $\pm$ 0.33	NS
Family history of CAD (absent vs. present)	0.56 $\pm$ 0.30	0.61 $\pm$ 0.35	NS
Medications			
ASA/clopidogrel (absent vs. present)	0.54 $\pm$ 0.27	0.65 $\pm$ 0.38	0.016
ACEI/ARB (absent vs. present)	0.56 $\pm$ 0.32	0.58 $\pm$ 0.30	NS
Statin (absent vs. present)	0.56 $\pm$ 0.29	0.62 $\pm$ 0.37	NS
Beta blockers (absent vs. present)	0.57 $\pm$ 0.30	0.58 $\pm$ 0.33	NS
CCB (absent vs. present)	0.57 $\pm$ 0.32	0.60 $\pm$ 0.23	NS
Nitrate (absent vs. present)	0.57 $\pm$ 0.31	0.56 $\pm$ 0.29	NS
OAD (absent vs. present)	0.57 $\pm$ 0.30	0.61 $\pm$ 0.3	NS

sFasL, soluble Fas ligand; NS, not significant; DM, diabetes mellitus; CAD, coronary artery disease; ASA, acetylsalicylic acid; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; CCB, calcium-channel blocker; OAD, oral antidiabetic drugs.

Table 4 Multivariate Analysis Using Linear Regression Method for Independent Predictors^a of sFasL Levels in Blood

Variables	$\beta \pm SE$	Standardized Coefficients	p Value
Hypertension, +	0.044 $\pm$ 0.033	0.099	0.188
ASA/clopidogrel usage, +	0.096 $\pm$ 0.040	0.182	0.018
Azar score	0.013 $\pm$ 0.005	0.202	0.008
Constant	412 $\pm$ 029	—	<0.001
R ²	0.099		

^aThe study parameters, which were significantly associated with sFasL in univariate analyses, were included in the multivariate analysis. sFasL, soluble Fas ligand; ASA, acetylsalicylic acid.

soluble form of FasL is mainly determined by the constitutive expression of FasL by the endothelial cells and their cleavage into plasma by metalloproteinases. Metalloproteinase activity increases in severe atherosclerosis.^{13,14} Thus, the compensatory protective effect of FasL in atherosclerosis may be further balanced by the increased conversion of FasL to the soluble form. This change in the form of FasL decreases its apoptotic capability and probably diminishes its protective effect against atherosclerosis progression.¹⁵ The finding of higher sFasL level in the patients with more severe and diffuse coronary atherosclerosis in our study is consistent with this mechanism. This finding is also in concordance with the previous findings. In hypertensive patients, the plasma level of sFasL was associated with increased carotid intima-media thickness.⁹ Thus, exaggeration in the conversion of FasL to its soluble form may be a risk marker for progression of CAD.

Several studies have shown that plasma level of sFasL is decreased in patients with higher cardiovascular risk.^{16,17} Statin treatment increased sFasL level.¹⁶ In our study, none of the cardiovascular risk factors seemed to have an independent effect on sFasL level in multivariate analysis. However, HDL levels in patients with normal coronary arteries were higher. This group had also higher sFasL level. Although this finding may be a reflection of the protective effect of higher HDL level against atherosclerosis, it may also be related with higher sFasL level detected in this group. The results of previous studies may be explained by the probable negative effect of the endothelial dysfunction on FasL expression by endothelial cells. The close relation between endothelial function and sFasL is confirmed by a recent study that showed a linear relation between forearm reactive hyperemia and sFasL level.¹⁸ In the light of these findings, expression of FasL seems to be closely associated with the correct functioning of endothelium. The severity of endothelial dysfunction may be variable in patients with atherosclerosis. Nitric oxide (NO) is the main determinant of the correct endothelial functioning, and may show different responses in different clinical pictures. Esaki et al demonstrated that Fas and FasL were expressed in the same areas that inducible form of nitric oxide synthase (iNOS) within the atherosclerotic plaque.¹⁹ Based on this finding, the investigators suggested that iNOS stimulates

Fas/FasL-mediated apoptosis for regression of atherosclerosis. The main enzyme responsible of NO synthesis is endothelial nitric oxide synthase (eNOS) in the healthy endothelium. In diseased states, inducible nitric oxide synthase (iNOS) activity increases and becomes the principle enzyme in the NO synthesis.²⁰ However, the NO synthesized by iNOS is different in quality than the NO synthesized by eNOS, and can show negative effects favoring the progression of atherosclerosis.²¹ The increased expression of Fas/FasL system in the areas with greater concentrations of iNOS may be the result of a compensatory response. In this way, the Fas/FasL system appears to exert a protective mechanism against atherosclerosis progression. On the other hand, as Fas system is a protective mechanism, its activation level should be correlated with the need for this protection. This means that if the progression of atherosclerosis is rapid, then necessity for Fas system increases. In previous studies, ischemia is shown to induce the expression of FasL.^{7,22}

In several studies, sFas level was found to correlate with coronary and peripheral atherosclerosis in patients with end-stage renal disease (ESRD).^{23,24} However, in Rotterdam Coronary Calcification study, which is a population-based study, the investigators could not show a significant relation between plasma sFas level and coronary/peripheral atherosclerosis.²⁵ The challenge was explained by the inclusion of a highly selected population in previous studies. The higher sFas level in patients with chronic renal failure in comparison to the general population may be the result of chronic inflammation in patients with ESRD. The use of sFasL level instead of sFas level, as an indicator of the severity of atherosclerosis, is more logical because sFasL is also expressed by vascular endothelium. In the study investigating the relation between Fas/FasL system and atherosclerosis, the investigators found a relation between carotid intima-media thickness and sFasL level but not sFas level.⁹

Our study is not the first study indicating a relation between sFasL level and coronary atherosclerosis. sFasL was demonstrated to increase in patients with ACS in a previous study.¹⁰ However, ACS is a strong indication for coronary angiography according to current guidelines and most of these patients undergo routine coronary angiography during the hospitalization period.^{26,27} Thus, usage of biomarkers indicating the angiographic existence and severity of coronary atherosclerosis do not add so much to clinical practice. In patients with stable angina pectoris, the decision for an invasive diagnostic test and treatment is more controversial. Thus, use of biomarkers such as sFasL may give an idea about the requirement of further diagnostic tests. Although our findings do not prove that usage of sFasL for this purpose is beneficial, it may pave the way for studies investigating the use of sFasL as a biochemical surrogate of severe coronary atherosclerosis to decide to more invasive diagnostic strategies.

In conclusion, our study showed that sFasL level was associated with higher coronary atherosclerotic burden and this relation is independent of traditional cardiovascular risk factors. Our results suggest that sFasL level may be a biochemical surrogate of severe coronary atherosclerosis.

This finding needs to be confirmed with further studies in larger patient populations.

Note

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