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Soluble Endothelial Cell-Selective Adhesion Molecule and Incident Cardiovascular Events in a Multi-Ethnic Population

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Abstract

Background—Cell adhesion molecules are key regulators of atherosclerotic plaque development, but circulating levels of soluble fragments, such as intercellular adhesion molecule (sICAM-1) and vascular cell adhesion molecule (sVCAM-1), have yielded conflicting associations with atherosclerotic cardiovascular disease (ASCVD). Endothelial cell-selective adhesion molecule (ESAM) is expressed exclusively in platelets and endothelial cells, and soluble ESAM (sESAM) levels have been associated with prevalent subclinical atherosclerosis. We therefore hypothesized that sESAM would be associated with incident ASCVD.

Methods—sESAM, sICAM-1, and sVCAM-1 were measured in 2,442 participants without CVD in the Dallas Heart Study, a probability-based population sample age 30–65 enrolled between 2000–2002. ASCVD was defined as first myocardial infarction, stroke, coronary revascularization, or CV death. A total of 162 ASCVD events were analyzed over 10.4 years.

Results—Increasing sESAM was associated with ASCVD, independent of risk factors (HR Q4 vs. Q1: 2.7, 95% CI 1.6–4.6). Serial adjustment for renal function, sICAM-1, VCAM-1, and prevalent coronary calcium did not attenuate these associations. Continuous ESAM demonstrated similar findings (HR 1.31 95% CI 1.2–1.4). Addition of sESAM to traditional risk factors improved discrimination and reclassification (delta c-index: $p=0.009$; IDI $p=0.001$; NRI = 0.42, 95% CI 0.15–0.68). Neither sICAM-1 nor sVCAM-1 was independently associated with ASCVD.

Conclusions—sESAM but not sICAM-1 or sVCAM-1 levels are associated with incident ASCVD. Further studies are warranted to investigate the role of sESAM in ASCVD.

Keywords

Adhesion Molecules; Biomarkers; Atherosclerosis

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Introduction

Atherosclerotic cardiovascular disease (ASCVD) is caused by lipid deposition within the arterial wall, chronic inflammation, and thrombosis. Endothelial dysfunction is associated with all of these pathways and is promoted by the migration and recruitment of circulating leukocytes across the vascular endothelium into the intimal space.¹ This process is facilitated by cellular adhesion molecules expressed on the endothelial cell surface, including intracellular-cell adhesion molecule-1 (ICAM-1),² vascular cell adhesion molecule-1 (VCAM-1),² platelet cell adhesion molecule-1 (PECAM-1),³ and P-selectin.⁴ Studies correlating circulating levels of soluble fragments of these CAMs have yielded conflicting associations with ASCVD.^{5–8}

Endothelial cell-selective adhesion molecule (ESAM) is known to be exclusively expressed in the endothelial tight junctions and platelets, and is thought to play a key role in leukocyte extravasation in the setting of endothelial injury or inflammation.^{9,10} Animal models lacking ESAM display a reduction in the size and complexity of atherosclerotic lesions.¹¹ We previously demonstrated in a population-based cohort that increasing soluble ESAM (sESAM) levels, but not sICAM-1 or sVCAM-1, independently associated with prevalent coronary and peripheral atherosclerosis.¹² This was the first study of sESAM at a population level; however, it was limited by the cross-sectional study design and use of subclinical measures.

Therefore, given the consistency of the preclinical and clinical evidence supporting a role for ESAM in atherosclerosis, we hypothesized that increased levels of sESAM would be associated with clinical ASCVD events. We sought to test this hypothesis by investigating the association between baseline levels of sESAM and incident cardiovascular events in a population-based cohort free from cardiovascular disease.

Methods

The Dallas Heart Study is a probability-based population cohort of Dallas County residents, with intentional oversampling of Blacks to account for 50% of the cohort.¹³ A total of 2,971 participants age 30–65 completed in-home medical history, physical exam, and blood collection as well as on-site atherosclerosis imaging. After excluding those without full risk factor assessment, coronary calcium imaging, or sESAM measurement as well as those with a history of cardiovascular disease (CVD), the current study includes 2,442 participants. This study was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center and conducted in accordance with institutional guidelines; all participants gave written informed consent.

Information including demographics, anthropometric measurements, and other variable definitions have been previously described.¹³ Gender, race/ethnicity, smoking, and all medication use were self-reported. Hypertension was defined as an average systolic blood pressure of ≥ 140 mm Hg, diastolic pressure ≥ 90 mm Hg, or use of an antihypertensive medication. Diabetes was defined as a fasting glucose level > 126 mg/dL, nonfasting glucose of > 200 mg/dL, or self-report diabetes paired with the use of any glucose lowering

medication. Body mass index (BMI) was calculated based on measured height and weight. History of cardiovascular disease (CVD) was defined as a self-reported history of myocardial infarction (MI), coronary revascularization, congestive heart failure (CHF), or stroke.

Coronary artery calcium (CAC) assessment was performed as previously described¹⁴, and the mean Agatston score from two sequential electron beam computed tomography (EBCT) scans was used. Prevalent CAC was defined as a mean score of > 10 Agatston units as described in a previous study.¹⁴

Venous blood was collected in standard blood collection tubes containing citrate EDTA and samples were maintained at 4 °C for 4 h then subsequently centrifuged (1430 g for 15 minutes) at 4 °C. The samples were then removed and frozen at -80 °C until assays were performed. sESAM, sICAM-1, and sVCAM-1 were all measured from the same sample (thawed frozen plasma) at Alere San Diego, Inc. (sESAM on a proprietary device platform with minimum detection limit = 0.25 ng/mL; within-assay CV 13%; sICAM-1 and sVCAM-1 on a Luminex 200 reader and modified paramagnetic Luminex beads from Radix Biosolutions using a competitive assay [sICAM-1: minimum detection limit = 100 ng/mL; within-assay CV 16%; sVCAM-1: minimum detection limit 200 ng/mL; within-assay CV 17%]). All assays were performed by individuals who were blinded to all of the clinical data. Samples were all thawed once for aliquoting prior to biomarker measurement. The following analytes have had their methods of measurement described previously: high-sensitivity C-reactive protein (hsCRP),¹⁵ N-terminal pro-B-type natriuretic peptide (NT-proBNP),¹⁶ cystatin C¹⁷, and cardiac troponin T (cTNT)¹⁸.

The primary end point was a composite ASCVD outcome, defined as first nonfatal myocardial infarction, stroke, percutaneous coronary intervention, coronary artery bypass grafting, or cardiovascular death. Total CVD included all ASCVD events as well as hospitalizations for heart failure and atrial fibrillation and peripheral artery revascularization. All non-fatal end points were adjudicated by two cardiologists who were unaware of the measurements of sESAM after reviewing hospital records using clinical criteria.¹⁹ Stroke did not include transient ischemic attack. Heart failure, atrial fibrillation, and peripheral vascular disease were defined as hospitalization for these conditions and adjudicated using clinical criteria. The National Death Index was used to determine vital status for all the participants through December 31, 2011. Death from cardiovascular causes was defined according to the *International Classification of Diseases, 10th Revision*, codes I00 to I99. Subjects participated in a detailed health survey regarding interval nonfatal cardiovascular events on an annual basis. Furthermore, quarterly assessments for hospital admissions using the Dallas-Fort Worth Hospital Council Data Initiative database were tracked, which includes hospital claims for 77 hospitals across 28 counties in North Texas and represents 90% of the health care market volume in this region¹⁹.

Demographic and clinical variables were compared across quartiles of sESAM, VCAM, and ICAM using the Jonckheere-Terpstra trend test. Correlations with continuous markers were assessed with Spearman coefficients. Cox proportional hazards models were used to assess the association between increasing sex- and race-specific quartiles of sESAM and the time

to first event for ASCVD and total CVD. Multivariable models included age, diabetes, hypertension, current smoking, total cholesterol, high-density lipoprotein cholesterol, log triglycerides, body mass index, and statin use. The same models were used to assess sICAM and sVCAM as predictors. Models were serially adjusted for eGFR (model 2), sICAM and sVCAM (model 3), and then CAC (log CAC+1) (model 4). The proportional hazards assumption was met for all models. The ability of sESAM to improve risk prediction of ASCVD beyond traditional risk factors (including age, sex, race, hypertension, diabetes, current smoking, BMI, hypercholesterolemia, low HDL, triglycerides, and statin use) was analyzed with the use of multiple metrics of biomarker performance,²⁰ including discrimination (Harrell's C-statistic),²¹ calibration, and reclassification (integrated-discrimination-improvement index²² and category-less net reclassification index²³). Calibration was assessed via the modified Nam-D'Agostino chi-squared statistic for censored data,²⁴ showing good calibration for models including sESAM: (ASCVD endpoint: the chi-squared statistic is 14.2, p-value = 0.12; global CVD endpoint: the chi-squared statistic is 13.2, p-value = 0.15). All probabilities of events were derived from the Cox models. Sensitivity analyses were performed by analyzing sESAM as a continuous variable and by investigating the association of sESAM with each component of the primary end point separately. Two-sided P values of 0.05 or less were considered statistically significant. All statistical analyses were performed using SAS Version 9.3 (SAS Institute).

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Results

A total of 2442 participants were included in this study, with a median age of 44 years [IQR 36–51]; 57% were women, 49% were black. As reported previously, increasing sESAM was associated with all of the traditional cardiac risk factors except for current smoking (Table 1) and strongly correlated with more severe renal dysfunction¹². sESAM was only weakly correlated with sICAM-1 and sVCAM-1 ($\rho < 0.30$) whereas sICAM-1 and sVCAM-1 were highly correlated with each other ($\rho = 0.60$; $p < 0.0001$).¹² sESAM was weakly correlated with several inflammatory markers, including MCP-1, IL-18, hs-CRP, and LP-PLA2 mass (Table 2). The correlation between sESAM and D-dimer qualitatively differed by sex and race/ethnicity (Table 2).

A total of 162 first ASCVD events were identified over a median follow up period of 10.4 years. In unadjusted analyses, the highest versus lowest quartile of sESAM was associated with increased rates of incident ASCVD (13.4% in Q4 vs. 3.1% in Q1; HR 4.65 (95% CI 2.82–7.67; Figure 1). This association remained significant when adjusted for traditional risk factors (HR 2.73, 95% CI 1.64–4.57; Figure 1). Serial adjustment for eGFR, sICAM, and sVCAM, hsCRP, and CAC did not attenuate this association (fully adjusted HR 2.90, 95% CI 1.59, 5.30; $p = 0.0005$ Figure 1). In addition, serial adjustment for NT-proBNP and hs-TNT together also did not change the associations (fully adjusted HR for ASCVD: 2.30, 95% CI 1.37, 3.87; $p = 0.002$; total CVD: HR 1.96, 95% CI 1.25, 3.06; $p = 0.003$). Similar

findings were seen for total CVD (Figure 2). Sensitivity analyses utilizing continuous sESAM yielded similar results (Table 3).

Analyzing the individual component end points of ASCVD separately, quartile 4 vs. 1 of sESAM was most strongly associated with CV death, followed by non-fatal MI and PCI/CABG (Figure 3). The weakest association was with incident non-fatal stroke. Adding sESAM categories to traditional risk factors improved the C-statistic (0.828 to 0.844; $p=0.009$), IDI (0.017; $p=0.001$) and category-less NRI (0.42, 95% CI 0.15–0.68) for ASCVD in well-calibrated models. In contrast to sESAM, sVCAM-1 and sICAM-1 did not associate with incident ASCVD or CVD (Figure 4).

Discussion

In this multiethnic, population-based cohort, we found significant associations between sESAM, a novel cellular adhesion molecule, and incident CV events. These findings were independent of renal function and prevalent CAC, suggesting that sESAM imparts additional information beyond kidney or calcific coronary disease for CVD. Of the three circulating soluble cellular adhesions molecules studied, sESAM but not sICAM or sVCAM was associated with CVD, highlighting differential associations between specific circulating adhesion molecules and CVD.

Chronic inflammation plays a key role in the development of ASCVD.¹ Adhesion molecules facilitate monocyte recruitment to the endothelium and migration across the endothelial surface into the intima.⁵ Unlike other cellular adhesion molecules that are expressed in multiple cell types, ESAM is exclusively expressed in endothelial tight junctions and platelets and promotes leukocyte diapedesis across the endothelium.^{10,25} Animal studies support a role of ESAM in the development of atherosclerotic plaque.¹¹ In a previous study, circulating sESAM levels were associated with several markers of prevalent atherosclerosis and vascular disease including CAC, aortic wall thickness, and aortic compliance, while sICAM-1 and sVCAM-1 did not.¹² The present study establishes the association between baseline levels of sESAM and incident CV events. Independent of traditional risk factors and renal function, increasing levels of sESAM associated with increased risk of both ASCVD and total CVD. The relationships were strongest for CV death and coronary events and weakest for stroke, suggesting a more specific cardiac phenotype in this cohort.

CAC is proportional to the burden of coronary atherosclerosis and robustly associates with incident ASCVD, including in the cohort studied here.^{26–28} sESAM correlates with CAC;²⁸ however, its associations with incident ASCVD and CVD were not attenuated when adjusted for CAC. This suggests that calcific coronary atherosclerosis in this cohort does not fully explain the association between sESAM and CV events. Given the younger age of this study population, perhaps the association between sESAM and incident events is mediated by non-calcified arterial plaque with increased inflammation and thrombotic risk. Further studies in older cohorts may help clarify the relationship between sESAM, coronary atherosclerosis burden, and CVD.

A recent report in patients with baseline stable coronary disease revealed similar findings that sESAM levels were associated with incident cardiovascular endpoints, including myocardial infarction, heart failure, and death.²⁹ In contrast to our study, however, adjustment for renal function as measured by eGFR attenuated this association. This discordance may be due to differences in study population as well as the possibility that increased sESAM levels in higher risk cohorts may be more confounded by worsening renal disease. Our report is the first to assess the association between sESAM and incident events in a low-risk population free of disease and cardiovascular treatment and the first to compare sESAM and other CAMs with regard to ASCVD risk prediction.

Lastly, association with CVD beyond traditional risk factors does not necessarily imply meaningfully improvement in risk prediction as assessed by performance metrics such as discrimination and reclassification. It is interesting that in our study sESAM not only associated with CVD but also improved discrimination and reclassification indices when added to traditional risk factors, suggesting further studies could be performed to determine clinical relevance in measuring circulating sESAM levels.

Limitations include a relatively small number of events. Also, blacks comprised 50% of the study design, which may limit generalizability to other race/ethnic groups. Furthermore, our study did not adjust for emerging or novel markers, which could be explored in future studies. Finally, we performed only a single measurement of cellular adhesion molecules, so cannot determine whether dynamic changes in these biomarkers provide incremental information over the single baseline measurement.

In summary, increasing levels of sESAM associated with increased risk of CVD independent of traditional risk factors, renal function, and CAC and improved risk prediction indices. Future studies are warranted to extend these findings in other cohorts with differing ages and ethnicities and also to investigate whether sESAM mediates the association with CVD.

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Abbreviations

ESAM	endothelial cell-selective adhesion molecule
ICAM	intercellular adhesion molecule
VCAM	vascular cell adhesion molecule
ASCVD	atherosclerotic cardiovascular disease
CAC	coronary artery calcium
CVD	cardiovascular disease

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Figure 1. Univariable and Multivariable-adjusted Associations of sESAM and ASCVD (n = 162) Hazard ratios derived from Cox proportional hazards models for quartile 4 vs. 1 of sESAM. Traditional risk factors include age, sex, race, hypertension, diabetes, current smoking, BMI, hypercholesterolemia, low HDL, triglycerides, statin use. eGFR: estimated glomerular filtration rate; CAC: coronary artery calcium; sICAM: soluble intercellular adhesion molecule; sVCAM: soluble vascular cell adhesion molecule. ASCVD: first nonfatal and fatal MI, stroke, PCI, CABG, or CV Death.

Figure 2. Univariable and Multivariable-adjusted Associations of sESAM and Total CVD (n = 201)

Hazard ratios derived from Cox proportional hazards models for quartile 4 vs. 1 of sESAM. Traditional risk factors include age, sex, race, hypertension, diabetes, current smoking, BMI, hypercholesterolemia, low HDL, triglycerides, statin use. eGFR: estimated glomerular filtration rate; CAC: coronary artery calcium; sICAM: soluble intercellular adhesion molecule; sVCAM: soluble vascular cell adhesion molecule. CVD: first nonfatal and fatal MI, stroke, PCI, CABG, CV Death, hospitalization for heart failure or atrial fibrillation.

Figure 3. Hazard Ratios of sESAM and Individual ASCVD Endpoints (n = 162)

Hazard ratios derived from Cox proportional hazards models for quartile 4 vs. 1 of sESAM adjusted for traditional risk factors and eGFR. ASCVD: atherosclerotic cardiovascular disease; CV Death: death from cardiovascular causes; MI: Myocardial Infarction; PCI/CABG: percutaneous coronary intervention/coronary artery bypass grafting; CVA: cerebrovascular accident (stroke).

Figure 4. Hazard ratios of sESAM, sVCAM, and sICAM for ASCVD (n = 162) and Total CVD (n = 201)

Hazard ratios derived from Cox proportional hazards models for quartile 4 vs. 1 adjusted for traditional risk factors and eGFR. sVCAM: soluble vascular cell adhesion molecule; sICAM: soluble intercellular adhesion molecule. ASCVD: first nonfatal and fatal MI, stroke, PCI, CABG, or CV Death. Total CVD: ASCVD, hospitalization for heart failure or atrial fibrillation.

Table 1

Demographics and Risk Factors Across Quartiles of sESAM

Variable sESAM (ng/mL)	Q1 3.49–26.9	Q2 26.9–33.7	Q3 33.8–42.4	Q4 42.5–145.0	P Trend
Age	40 (34,48)	43 (36,50)	44 (36, 51)	46 (38,55)	<0.0001
Male Sex	38%	45%	41%	48%	0.0039
Black	58%	48%	44%	46%	<0.0001
Hypertension	26%	27%	29%	42%	<0.0001
Diabetes	7%	8%	9%	16%	<0.0001
Current Smoker	26%	24%	26%	30%	0.1248
Body Mass Index	27.8 (24.4, 31.8)	28.2 (24.7, 33.6)	28.4 (24.5, 32.8)	29.2 (25.3, 34.2)	0.0005
Cholesterol (mg/dL)	169 (148,194)	178 (153, 201)	179 (157, 205)	183 (161, 213)	<0.0001
HDL (mg/dL)	49 (41, 59)	48 (40, 57)	48 (40, 59)	46 (38, 54)	<0.0001
LDL (mg/dL)	99 (78, 121)	105 (82, 126)	106 (86, 127)	112 (86, 135)	<0.0001
Triglycerides (mg/dL)	81 (61, 120)	92 (67, 144)	98 (68, 144)	109 (77, 168)	<0.0001
Statin Use	3%	6%	6%	5%	0.1786
Systolic BP (mmHg)	119 (109, 130)	120 (111, 132)	119 (111, 130)	126 (115, 139)	<0.0001
Diastolic BP (mmHg)	76 (70, 83)	77 (71, 83)	76 (71, 83)	79 (73, 86)	<0.0001
eGFR [*]	102 (91, 118)	98 (87, 112)	95 (83, 107)	92 (81, 108)	<0.0001
CAC > 10 AU [†]	13%	19%	17%	28%	<0.0001
sICAM-1 [‡] (ng/mL)	553 (408, 752)	563 (403, 749)	603 (461, 839)	686 (505, 949)	<0.0001
sVCAM [§] (ng/mL)	843 (651, 1125)	897 (680, 1240)	1011 (771, 1430)	1181 (851, 1733)	<0.0001
10 yr ASCVD risk %	1.3 (0.3, 4.2)	1.9 (0.6, 4.4)	2 (0.6, 4.7)	3.2 (1.1, 8.6)	<0.0001

Values are medians (interquartile range) for continuous variables and percentages for categorical variables.

^{*} eGFR: estimated glomerular filtration rate

[†] CAC: coronary artery calcium

[‡] sICAM-1: soluble intercellular adhesion molecule-1

[§] sVCAM: vascular cell adhesion molecule

^{||} ASCVD: atherosclerotic cardiovascular disease.

Table 2

Correlations Between sESAM and Inflammatory Markers

	Overall	Men	Women	Black	White	Hispanic
D-Dimer	0.07 (< 0.01)	0.14 (< 0.01)	0.06 (0.03)	0.09 (< 0.01)	0.12 (< 0.01)	0.07 (0.19)
LP-PLA2 [*]	0.21 (< 0.01)	0.18 (< 0.01)	0.21 (< 0.01)	0.19 (< 0.01)	0.16 (< 0.01)	0.21 (< 0.01)
Mass						
LP-PLA2 [*]	0.14 (< 0.01)	0.12 (< 0.01)	0.14 (< 0.01)	0.11 (< 0.01)	0.12 (< 0.01)	0.10 (0.05)
Activity						
hsCRP [‡]	0.16 (< 0.01)	0.19 (< 0.01)	0.18 (< 0.01)	0.17 (< 0.01)	0.20 (< 0.01)	0.14 (< 0.01)
IL-18 [‡]	0.20 (< 0.01)	0.18 (< 0.01)	0.20 (> 0.01)	0.23 (< 0.01)	0.16 (< 0.01)	0.15 (0.03)
MCP-1 [§]	0.29 (< 0.01)	0.29 (< 0.01)	0.28 (< 0.01)	0.31 (< 0.01)	0.23 (< 0.01)	0.25 (< 0.01)
sVCAM ^{//}	0.27 (< 0.01)	0.25 (< 0.01)	0.29 (> 0.01)	0.24 (< 0.01)	0.29 (< 0.01)	0.33 (< 0.01)
sICAM [#]	0.18 (< 0.01)	0.17 (< 0.01)	0.19 (> 0.01)	0.18 (< 0.01)	0.20 (< 0.01)	0.19 (< 0.01)

Spearman correlation coefficients for sESAM (p-values).

^{*} LP-PLA2: lipoprotein associated phospholipase 2 mass[‡] hsCRP: high sensitivity C-reactive protein[‡] IL-18: Interleukin-18[§] MCP-1: monocyte chemoattractant protein-1^{//} sVCAM: soluble vascular cell adhesion molecule[#] sICAM: soluble intercellular adhesion molecule.

Table 3

Association of Continuous sESAM with ASCVD and Total CVD

Variable	Hazard Ratio ^{//}	95% CI	P Value
ASCVD (n = 162) [#]			
No adjustment	1.52	1.40–1.66	<0.0001
Model 1 [*]	1.31	1.19–1.44	<0.0001
Model 2 [†]	1.30	1.17–1.44	<0.0001
Model 3 [‡]	1.29	1.16–1.44	<0.0001
Model 4 [§]	1.30	1.16–1.45	<0.0001
CVD (n = 201) ^{**}			
No adjustment	1.49	1.37–1.61	<0.0001
Model 1 [*]	1.28	1.18–1.40	<0.0001
Model 2 [†]	1.26	1.14–1.39	<0.0001
Model 3 [‡]	1.25	1.13–1.39	<0.0001
Model 4 [§]	1.29	1.16–1.43	<0.0001

^{*} Model 1: adjusted for age, sex, race, hypertension, diabetes, current smoking, BMI, hypercholesterolemia, low HDL, triglycerides, statin use

[†] Model 2: Model 1 plus eGFR

[‡] Model 3: Model 2 plus sICAM and sVCAM

[§] Model 4: Model 2 plus CAC

^{//} Hazard Ratios reflect a 1-SD increase (sESAM = 15.8)

[#] ASCVD is defined by first nonfatal and fatal myocardial infarction, stroke, coronary revascularization, or CV death.

^{**} CVD is defined by ASCVD in addition to hospitalization for congestive heart failure or atrial fibrillation