Secretory Phospholipase A2 Is Associated with the Odds of Acute Coronary Syndromes through Elevation of Serum Amyloid-A Protein

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Abstract

Keywords

- acute coronary syndromes
- secretory phospholipase A2
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- serum amyloid-A
- coronary heart disease
- case-control study
- odds ratio

In coronary heart disease (CHD), levels of secretory phospholipase A2 (sPLA2) are commonly increased. Serum amyloid-A (SAA) is increased in acute coronary syndromes (ACS) as well. It is needed to verify the hypotheses that sPLA2 is associated with the odds of ACS through elevation of SAA. We conducted a case-control study with 57 male patients with ACS and 30 controls matched by gender category. Levels of sPLA2, SAA, and myeloperoxidase (MPO) were measured by immunoreactive assay on the basis of a double-antibody sandwich technique. Levels of sPLA2, MPO, and SAA were significantly higher in patients than those in controls ($11,359.0 \pm 10,372.4$ pg/mL vs. $1,320.5 \pm 654.5$ pg/mL, $p = 0.00$; 438.6 ± 310.7 ng/mL vs. 271.1 ± 176.8 ng/mL, $p = 0.01$; $10,995.2 \pm 2,842.6$ ng/mL vs. $3,861.7 \pm 3,173.5$ ng/mL, $p = 0.00$). There were significant correlations between age, visceral obesity, MPO, sPLA2, and SAA ($r = 0.43$; $p = 0.00$; $r = 0.30$; $p = 0.00$; $r = 0.28$; $p = 0.00$ and $r = 0.53$; $p = 0.00$). On multivariate logistic regression analyses, there were significant and independent associations between sPLA2 and SAA with odds of ACS [OR (95% CI) = 14.2 (2.1 to 98.6), $p = 0.00$; OR (95% CI) = 44.9 (6.9 to 328.4), $p = 0.00$]. Our findings suggest that sPLA2 may be associated with the odds of ACS compared with controls through increased inflammation, represented by elevated SAA.

In patients with stable coronary artery disease (CAD), secretory phospholipase A2 (sPLA2) is commonly increased and acts as a good prognosticator for the future cardiovascular events.^{1,2} Even a single test of plasma sPLA2 activity in those with acute coronary syndromes (ACS) would indicate an important scientific information regarding prediction for the next cardiovascular events.³ Phospholipase A2 enzymes hydrolyze phospholipids at the *sn*-2 position to produce lysophospholipids and fatty acids.⁴ This enzyme produces

isoprostanes, a prostaglandin-like compound formed in vivo from the free radical-catalyzed peroxidation of essential fatty acids, which has strong mitogenic activity. It induces thrombocyte aggregation and vasoconstriction⁵ and possibly stimulates acute inflammatory response. In the future, a promising inhibitor of this enzyme activity would be an evolving new therapy in atherosclerosis.

Serum amyloid-A (SAA) is a family of proteins that form a major component of the acute-phase inflammatory

response.⁶ In response to inflammation, injury, infection, or stress, SAA is produced in the liver, likewise C-reactive protein (CRP). Thereby, it is regarded as a sensitive biomarker of an acute inflammatory state.

Although the recent progress of management of ACS had better been developed and implemented as stated in the current guidelines, the mortality of this syndrome cannot be disregarded. Possibly this is because of the athero-mechanism of sPLA2, which is not yet elucidated. This enzyme would hypothetically be having an important role in atherosclerosis via increased levels of SAA and myeloperoxidase (MPO). So, the purpose of the study is to verify the associations between sPLA2 and MPO and the odds of ACS via increased elevation of SAA.

Methods

Patients and Study Design

This was a case-control study conducted from October 28, 2008, to June 3, 2009. Initially, 95 male patients with ACS were enrolled; finally, only 52 male patients aged 30 to 70 years were recruited as case. Thirty male patients aged 30 to 70 years participated and were considered as a control group. The control group was matched in terms of gender (only male gender recruited). Inclusion criteria were as follows: (1) male patients who suffered from ACS, fulfilling criteria of ESC Committee on Practice Guidelines on non-ST elevation of ACS⁷ and ST-segment elevation of acute myocardial infarction (MI),⁸ and (2) male patients who agreed to participate in the study. Exclusion criteria were patients who had diabetes mellitus, chronic kidney diseases, liver dysfunction, and systemic infection. Participants had signed informed consent to participate into the study. And all data in terms of height, weight, smoking status, blood pressure, waist circumference, alcohol, and medications were collected. The study was conducted in Sanglah Hospital of Bali as a referral hospital in Bali Island and surrounding area; the hospitals had been accredited as a teaching hospital and affiliated to Faculty of Medicine-Udayana University of Bali. The Health and Medical Research Ethics Committee of Faculty of Medicine of Udayana, in Denpasar, Bali, Indonesia, had approved the study's proposal.

Biochemical Markers Measurement

Serum was separated from whole blood after centrifugation and immediately kept at -20° until assay was done. All assays were conducted based on manufacturer's instruction. Levels of immunoreactive sPLA2 in plasma were measured using Cayman's sPLA2 (human type IIA) EIA Kit (Cayman Chemical Co., Ann Arbor, MI) immunometric assay that can be used for quantification of sPLA2 in plasma, synovial fluid, and other sample matrices. The EIA has a minimum detectable concentration of 15.6 pg/mL. Precision is represented by the intra-assay and interassay variation with coefficient of variation (CV) successively of 4.8 to 12.4% and of 4.8 to 16.8%.

Activity of myeloperoxidase was determined using enzyme immunoassay technique, and presented in ng/mL. The minimum detectable concentration of the MPO ELISA assay as

measured by 2 SD from the mean of a zero standard is estimated to be 0.25 ng/mL. Intra-assay precision and inter-assay precision are shown by CV of 1.7 to 4.9% and 3.4 to 11.7%.

Concentration of SAA protein was measured using enzyme-linked immunoassay (Anogen, Ontario, Canada) and expressed as ng/mL, the minimum detectable dose of human SAA using standard calibrator diluent is 1.1 ng/mL. The SAA concentration of serum/plasma ranges from 1,000 to 5,000 ng/mL in apparently healthy individuals. The intra-assay precision represented by CV ranged from 2.8 to 10.3%. Levels of homocysteine were measured using fluorescence polarization immunoassay (Abbott, Wiesbaden, Germany) and unit was expressed in $\mu\text{mol/L}$. Lipid profiles, blood glucose, serum creatinine, and blood urea nitrogen were determined using the standard techniques. All assays were performed blinded to clinical data. All specimens available were shipped to Prodia Clinical Laboratory, Jakarta, Indonesia, for the sake of standardization and quality assurance.

Statistical Analyses

Statistical analyses were performed using the SPSS for Windows version 20.0 software (SPSS Inc. Chicago, IL). The Shapiro-Wilk test was done to assess normality of continuous variables, and Leven's test was assessed to test the homogeneity of variance. The continuous variable, which was not normally distributed, then was logarithmically transformed to meet the assumption of parametric analyses. Correlations between age, waist circumference, sPLA2, MPO, and SAA as dependent variable and other related variables were analyzed using Pearson correlation statistics. ROC (receiver operating characteristics) curve was done to determine the cut-off point of independent variables and intermediate variables with regard to the best sensitivity, specificity, and accuracy; next, the results were used as cut-off points in the logistic regression model. Univariate and multivariate logistic regression analyses were conducted to assess the associations between sPLA2, MPO, SAA, age, and serum creatinine with ACS. The adjusted odds ratio (OR) with (95% confidence interval) was calculated to prove the hypotheses. It represented the importance of significant and independent predictors of ACS. The analyses results were narrated and explained by tables and graphs. Significance tests were two-tailed, and p -values less than 0.05 were considered as statistically significant.

Results

We recruited 57 male patients with ACS and 30 male controls. Matching was aimed to gender category only. Characteristics of patients and controls are given in Table 1. Age and systolic blood pressure are higher in patients than in controls. Waist circumference, fasting blood sugar, and serum creatinine are significantly greater in patients than in controls group. But, total-cholesterol, LDL-cholesterol, and apolipoprotein AI (apoAI) are interestingly lower in patients than those in controls. The studied variables such as sPLA2, MPO, and SAA are significantly higher in patients than in controls. As expected, patients are more likely older, to have low

Table 1 Baseline characteristics of patients

	Control (30)	Case (57)	p
Age (year)	41.5 ± 15.3	56.3 ± 11.0	0.00
Body mass index (kg/m ²)	23.7 ± 2.9	24.2 ± 2.9	0.48
Waist circumference (cm)	85.9 ± 7.8	89.6 ± 8.1	0.05
Blood pressure (mm Hg)			
Systolic	113.6 ± 10.6	122.9 ± 20.9	0.02
Diastolic	77.8 ± 8.7	77.0 ± 10.7	0.72
Fasting blood sugar (mg/dL)	85.8 ± 8.1	101.1 ± 30.2	0.01
Serum creatinine (mg/dL)	0.9 ± 0.1	1.3 ± 0.8	0.01
Total cholesterol (mg/dL)	212.4 ± 29.5	177.9 ± 40.3	0.00
HDL-cholesterol (mg/dL)	46.7 ± 8.1	35.9 ± 8.5	0.00
LDL-cholesterol (mg/dL)	141.5 ± 25.7	120.8 ± 33.5	0.00
Triglyceride (mg/dL)	127.0 ± 43.9	138.3 ± 55.4	0.34
sPLA2 (pg/mL)	1,320.5 ± 654.5	11,359.0 ± 10,372.4	0.00
MPO (ng/mL)	271.1 ± 176.8	438.6 ± 310.7	0.00
SAA (ng/mL)	3,861.7 ± 3,173.5	10,995.2 ± 2,842.6	0.00
Homocysteine (μmol/L)	13.9 ± 2.2	14.3 ± 4.6	0.65

Abbreviations: sPLA2, secretory phospholipase A2; MPO, myeloperoxidase; SAA, serum amyloid-A.

Note: Data are presented as mean ± SD, unless noted otherwise.

HDL-cholesterol, and have higher systolic blood pressure, fasting blood sugar levels, and more obese. Oxidative stress and inflammation as represented by MPO and SAA are found more elevated in patients than in controls. There are no significant differences between groups in terms of homocysteine concentrations (►Table 1).

Interestingly in ►Fig. 1 and ►Table 2, there are significant bivariate correlations between age, visceral obesity (measured by waist circumference), and oxidative stress (represented by MPO and sPLA2) with concentrations of SAA, representing inflammation. On multivariate linear regression analysis, there are still significant correlations of age, waist circumference, and sPLA2 with SAA as dependent variables.

Fig. 1 Positive correlation between concentration of sPLA2 and SAA in logarithmic.

On univariate logistic regression analysis (►Table 3), there are significant associations between age, kidney function (serum creatinine), sPLA2, MPO, SAA with the odds of ACS as dependent variables. And after being adjusted, there are only sPLA2 and SAA that act as significant and independent predictors of the odds of ACS (►Table 3).

Discussion

In this study, we have revealed the significant and independent associations between sPLA2, SAA, and ACS. The result is obtained after being adjusted with other significant variables between the two groups. The association between sPLA2 concentrations and the odds of ACS was independent of the other known predictors and other comorbidities of ACS, including the presence or absence of a history of MI and the presence or absence of clinical signs of heart failure at hospital admission. sPLA2 is a member of the phospholipase A2 family and is widely studied. It is broadly expressed in hepatocytes, macrophages, platelets, and vascular smooth muscle cells.⁵ Production of sPLA2 type IIA is upregulated in response to proinflammatory substances such as interleukin-1 β , interleukin-6, tumor necrosis factor- α , interferon- γ , and oxidized LDL-cholesterol.⁹ The hydrolysis of the sn-2 acyl group of glycerophospholipids with additional production of fatty acids and lysophospholipids is considered as an important catalytic function of this enzyme. We measured, in the study, the immunoreactive levels of sPLA2, and interestingly Mallat et al³ demonstrated that “a single determination of sPLA2 activity, obtained within the 2 days after the onset of ischemic symptoms, provides powerful prognostic information in those with ACS.” This suggests that activation of sPLA2

Table 2 Correlations between age, waist circumference, MPO, and sPLA2 as independent variables and SAA as dependent variable in acute coronary syndromes and control patients

Predictors	Bivariate coefficient correlations (<i>r</i>)	<i>p</i>	Multivariate coefficient correlations (<i>r</i>)	<i>p</i>
Age	0.43	0.00	0.23	0.03
Waist circumference	0.30	0.00	0.21	0.03
sPLA2	0.53	0.00	0.34	0.00
MPO	0.28	0.00	0.15	0.30

Abbreviations: SAA, serum amyloid-A; sPLA2, secretory phospholipase A2; MPO, myeloperoxidase.

Note: sPLA2 was logarithmically transformed.

might be a significant and common mechanism among patients at risk for death or MI after an ACS. A higher level of sPLA2 was related to producing future cardiovascular events, namely, coronary revascularization procedures and hospital readmission, as demonstrated in a previous study during a follow-up of 2 years.¹⁰ Our study recruited all types of ACS, not only unstable angina, to represent a broader spectrum of ACS in our hospital. And the objective of our study is not to measure the prognostics power of sPLA2 as demonstrated by previous studies, but rather to elucidate the mechanism of association between the enzymes with ACS via increased elevations of SAA. On multivariate logistic regression analysis, this was confirmed that sPLA2 and SAA being significant and independent predictors of ACS with adjusted OR (95% CI) successively of 14.2 (2.1 to 98.6; *p* = 0.00) and 44.9 (6.1 to 328.4; *p* = 0.00) (►Table 3).

SAA consists of a group of three functionally related apolipoproteins. During the acute-phase response, this reactant

protein dislocates apoAI and apoAII from high-density lipoprotein to form larger, denser high-density lipoprotein particles that have diminished ability to catalyze cholesterol esterification and efflux. The above pathomechanism of SAA on apoAI was shown in our study by a significant and negative correlation between SAA and apoAI (*r* = 0.52; *p* = 0.00) (►Fig. 2). These particles may subsequently promote foam cell formation.¹¹ Within 24 hours of acute MI, SAA levels will increase and reach peak in the next 3 days after chest pain onset. ATIMI 11A substudy found that “elevated levels of SAA predicted risk of 14-day mortality in patients with unstable angina or NSTEMI but that SAA and hs-CRP provided essentially the same prognostic information.”¹² Johnson et al¹³ showed “a strong independent relationship between SAA and future cardiovascular events in female patients, similar to that found for hs-CRP. This is consistent with the hypotheses that systemic inflammation may promote atherosclerotic plaque destabilization, in addition to exerting a possible direct

Table 3 Association between age, serum creatinine, MPO, SAA, and sPLA2 with the odds of acute coronary syndromes represented by odds ratio

Predictors	Bivariate OR (95% CI)	<i>p</i>	Multivariate OR (95% CI)	<i>p</i>
Age, year ^a				
< 49.5 years	1.0 ^b 98.8 (3.4–28.2)	0.00	1.0 ^b 0.8 (0.1–6.3)	0.8
> 49.5 years				
Serum creatinine, mg/dL ^a				
< 1.05 mg/dL	1.0 ^b 6.2 (2.2–17.2)	0.00	1.0 ^b 6.9 (0.9–54.6)	0.06
> 1.05 mg/dL				
sPLA2, pg/mL ^a				
< 1,567.0 pg/mL	1.0 ^b 29.3 (8.5–100.4)	0.00	1.0 ^b 14.2 (2.1–98.6)	0.00
> 1,567.0 pg/mL				
MPO, ng/mL ^a				
< 247.0 ng/mL	1.0 ^b 3.3 (1.3–8.6)	0.01	1.0 ^b 0.5 (0.1–3.1)	0.47
> 247.0 ng/mL				
SAA, ng/mL ^a				
< 6,056.0 ng/mL	1.0 ^b 47.0 (12.4–177.9)	0.00	1.0 ^b 44.9 (6.1–328.4)	0.00
> 6,056.0 ng/mL				

Abbreviations: SAA, serum amyloid A; sPLA2, secretory phospholipase A2; MPO, myeloperoxidase; OR, odds ratio.

^aValue was determined using ROC (receiver operating characteristic) curve.

^bReference value.

Fig. 2 Negative correlation between concentration of SAA in logarithmic and apoA1.

effect on atherogenesis.” Our study showed that concentration of SAA is significantly higher in patients with ACS compared with controls ($10,995.2 \pm 2,842.6$ vs. $3,861.7 \pm 3,173.5$; $p = 0.00$) (see ►Table 1); this verifies the hypotheses of inflammatory response in ACS patients.

Several circulating inflammatory markers, including hs-CRP, interleukin-6, vascular cell adhesion molecule, and more recently, MPO,¹⁴ have been shown to be elevated in patients with ACS and associated with adverse clinical outcomes (MI/death) at follow-up. On bivariate logistic regression analysis, initially MPO was significantly associated with the odds of ACS, but after being adjusted with sPLA2 and SAA, there is no evidence that this substance behave as a significant predictor (see ►Table 3). This could possibly be explained by some assumptions such as (1) this enzyme is not acting as a significant and independent role in pathophysiology of ACS and (2) determination of plasma MPO is not representing the real oxidative process in vivo. Ideally, the impact of MPO on either apoA1 or HDL-cholesterol should be based on assaying apoA1-chlorotyrosine¹⁵ because tyrosine molecule is considered to be sensitive to the effect of MPO.

Limitation of the Study

This study has several limitations, namely, this is observational and retrospective in nature; consequently, the causation is not fully established using such study design. In addition, there are some imbalances in variables between patients and controls as a consequence of observational study, so we should consider cautiously in inferring the study results.

In conclusion, our study has suggested that sPLA2 may be associated with the odds of ACS through activation of inflammation, represented by elevated SAA in patients with ACS compared with sex-matched controls.

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