Malondialdehyde-Modified Low Density Lipoprotein as Oxidative-Stress Marker in Vasospastic Angina Patients

Based on a Guideline of the Japanese Circulation Society

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Summary

Vasospastic angina (VSA) is caused by endothelial dysfunction and hypercontraction of vascular smooth muscle cells. Although oxidative-stress can induce endothelial dysfunction, the relationship of VSA and the oxidative-stress marker malondialdehyde-modified low density lipoprotein (MDA-LDL) remains unclear. Purpose: Serum MDA-LDL was evaluated in candidate VSA patients.

The subjects were 84 patients admitted to our hospital because of chest pain at rest. We stratified the patients into 3 groups; definite VSA, suspected VSA, and unlikely VSA according to a Japanese Circulation Society (JCS) guideline. The patients classified as definite VSA or suspected VSA were considered as "clinical VSA".

Forty cases were classified as definite VSA, 35 as suspected VSA, and 9 as unlikely VSA. Thus, clinical VSA was the diagnosis in 75 cases. The patient characteristics showed that the average age of the patients was 60.2 years old (men, 61%). The serum MDA-LDL level of the clinical VSA group (126.3 ± 38.0 U/L) was significantly higher than the unlikely VSA group (98.7 ± 31.1 U/L). Serum MDA-LDL was positively correlated with total cholesterol (T-Chol), low-density lipoprotein cholesterol (LDL-C), triglycerides, and fasting blood glucose. Multivariate analysis showed that serum MDA-LDL was the most predictive marker for making a diagnosis of clinical VSA (Odds ratio 1.064, 95% confidence interval 1.014-1.145, $P = 0.008$). In a population with positive or borderline ECG change, the positive rate in the acetylcholine provocation test was significantly higher in the MDA-LDL higher group compared to the MDA-LDL lower group (81% versus 37%, $P = 0.032$).

: Serum MDA-LDL might be a useful biomarker of VSA and have additional value for the diagnosis of clinical VSA. (Int Heart J 2017; 58: 335-343)

Key words: Diagnostic biomarker, Acetylcholine provocation test

Vasospastic angina (VSA) is caused by focal or diffuse spasm of coronary arteries, and is characterized by ischemic chest pain at rest as distinct from exertion. If a chest pain attack is accompanied by ST segment elevation in several leads of an electrocardiogram, this syndrome is known as variant angina which was first described by Prinzmetal and colleagues in 1959.[1)] People in Europe and the United States develop angina pectoris due to severe coronary atherosclerosis to a greater extent than Japanese people, while variant angina is more prevalent in Japan than in Europe and the United States.[2)] However, recent studies have reported that coronary artery spasm could be associated with the onset of acute coronary syndrome, and it has been gradually realized that vasospastic angina is not rare even in western countries.[3,4)] This is because there are local and racial differences in the morbidity of VSA, and the genetic background of the patient may be involved in a part of the mechanism of VSA.

Since there was no systematic diagnostic method for VSA, the Japan Circulation Society (JCS) issued the first guideline for VSA in 2010[5)] and then updated it in 2013.[6)] It has subsequently been referred to in many countries around the world.[7)] This guideline shows a flow chart using symptoms such as chest pain, 12-lead electrocardiogram (ECG) change, and ambulatory electrocardiogram monitoring. It is very useful for making a diagnosis in Japan. Cardiac catheterization with a provocative test using acetylcholine or ergonovine has also been shown to be a useful diagnostic tool.[8)] It is known that the etiology of VSA involves multiple environmental factors such as smoking,[9,10)] alcohol consumption,[11)] lipid disorder,[12)] abnormal glucose metabolism,[13)] psychological stress (abnormal autonomic nervous system function),[14)] and genetic factors.[15-17)] The pathology of VSA is associated with endothelial cell dys-

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function and hypercontraction of vascular smooth muscle cells (VSMC). Multiple environmental factors are considered to be triggers of coronary spasm.

The prognosis of VSA is usually good due to improvements in lifestyle and proper medical treatment. Medical treatment with calcium channel blockers (CCB), nitrate, and nicorandil can prevent cardiovascular events via inhibition of the mechanisms of coronary spasticity. Recently, statins such as fluvastatin have been reported to be effective in the management of VSA.[18] Some VSA patients have a poor prognosis due to sudden death, acute myocardial infarction (AMI), or ventricular fibrillation (VF)[19,20] if they do not receive proper medical treatment.

MDA-LDL,[21] which is a type of oxidative LDL, is known as a marker of oxidative stress[22] and increases to high levels in serum due to smoking[23] and diabetes mellitus (DM).[24] Oxidative stress may induce endothelial dysfunction[25,26] and subsequent vascular spasms, and MDA-LDL might also be involved in the mechanism of VSA. However, it has not yet been determined whether MDA-LDL is associated with the clinical status of VSA. In this study, we evaluated serum MDA-LDL in consecutive candidate patients for a diagnosis of VSA without significant coronary stenosis using invasive coronary angiography in reference to the JCS guideline.

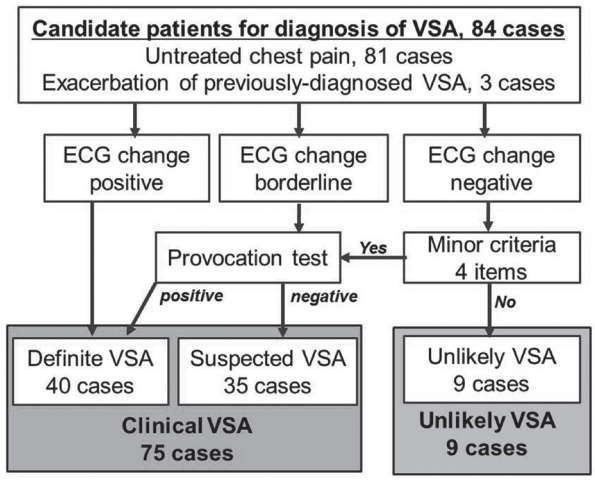

Figure 1. Flow-chart of this study according to JCS guideline. Candidate patients for diagnosis of VSA were divided into 3 groups (definite VSA, suspected VSA, or unlikely VSA) using ischemic ECG change, minor criteria, and acetylcholine provocation test.

Methods

Study population and flow chart according to JCS guideline: This study was retrospectively analyzed using a prospectively accumulated database for coronary artery diseases which was constructed using an electronic medical record system and a reporting system for cardiac catheter examinations (Cardio Agent™ Pro, Toshiba Medical Systems Corporation, Tochigiken, Japan). The study population consisted of 84 consecutive candidate patients for a diagnosis of VSA or previously-diagnosed VSA with exacerbation of chest pain admitted to the National Hospital Organization Disaster Medical Center between January 2008 and October 2014. These patients came to our hospital due to symptoms of chest pain at rest and/or exertion that appeared particularly during the night and early morning and underwent examinations consisting of blood tests, 12-lead electrocardiograms, ambulatory electrocardiogram monitoring, and elective or emergent invasive coronary angiography. The judgment concerning a diagnosis of VSA was made based on the Japanese Circulation Society guideline[5] as shown Figure 1. Subjects were divided into 3 groups; definite VSA, suspected VSA, or unlikely VSA. Definite VSA was defined as positive ischemic ECG changes at the time of a heart attack or the presence of a positive result in the acetylcholine provocation test even if there was a borderline ischemic ECG change at the time of the heart attack or no ischemic ECG change but with satisfying minor criteria. The minor criteria included the improvement of angina attacks using nitrates and at least one of 4 items; 1) the chest pain appeared at rest particularly between midnight and early morning; 2) the chest pain exhibited marked diurnal variation in exercise tolerance (in particular, reduction of exercise capacity in the early morning); 3) angina attacks induced by hyperventilation; and 4) angina attacks suppressed by calcium channel blockers but not beta-blockers. When the acetylcholine provocation test had a negative result in these patients, they were classified as suspected VSA. If a patient without ischemic ECG change did not meet the minor criteria, they were considered to be unlikely VSA. A final diagnosis of "clinical VSA" was made by classifying it as definite VSA or suspected VSA. These patients underwent medical treatment to suppress coronary artery spasms (ie, calcium channel blockers, the vasodilatory drug nicorandil, nitrates, or statins) according to the JCS guideline. We excluded patients who had angina pectoris requiring PCI due to atherosclerotic stenosis (75% or more), post-implantation of a drug-eluting stent, fatal arrhythmogenic heart diseases such as Brugada syndrome and long-QT syndrome, aortic dissection, cerebrovascular disease, respiratory disease, or endocrine disease (Basedow disease). Non-DM patients with a normal coronary artery who underwent invasive coronary angiography due to chronic heart failure or valvular disease served as the control cohort. This study was approved by the ethics committees of our institutions.

Electrocardiograms and ambulatory electrocardiograms: Ischemic ECG change was defined according to the JCS guideline as follows. An ischemic change was defined as a transient ST elevation of 0.1 mV or more, an ST depression of 0.1 mV or more, or the new appearance of negative U waves, recorded in at least two contiguous leads on 12-lead ECG during an angina attack. If the ischemic ECG and angina attack were prolonged, patients should be treated using fast-acting nitrates as directed in the guidelines for the management of acute coronary syndrome. In general, it is estimated that patients with VSA have chest pain in only 20-30% of the episodes of ischemic ST change, and many events of coronary spasm are asymptomatic. Because attacks are prevalent between midnight and early morning at rest, ischemic ECG changes are not often recorded in 12-lead ECG at an outpatient department. In these cases, an ambulatory electrocardiogram (Holter recording) is the best practical tool. If ischemic ECG changes were not detected in a 12-lead ECG in non-emergent cases, an ambulatory electrocardiogram was considered.

Biochemical analysis: The serum concentration of MDA-LDL

was determined by enzyme-linked immunosorbent assay (ELISA) using anti-MDA-modified LDL antibody (SRL Inc., Tokyo). The normal ranges of MDL level were 46-82 U/L in males < 45 years and females < 55 years and 61-105 U/L in males ≥ 45 years and females ≥ 55 years. The concentrations of T-Chol, LDL-C, high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were determined by enzymatic methods (T-Chol and TG, Kyowa Medex Co. Ltd., Tokyo; LDL-C and HDL-C, Sekisui Medical Co. Ltd., Tokyo; SRL Inc., Tokyo). Fasting blood glucose was determined by hexogenase/glucose-6-phosphate dehydrogenase assay (SHINOTEST Corporation, Kanagawa, Japan). HbA1c was measured by high-performance liquid chromatography (Sekisui Medical Co. Ltd., Tokyo). Serum cystatin C level was measured by a gold colloid aggregation method (SRL Inc., Tokyo). C-reactive protein (CRP) was determined by measuring the antigen-antibody reactions of supernatants from blood samples and a liquid chemical reagent including a latex binding anti-human CRP mouse monoclonal antibody (Nittobo Medical Co. Ltd., Fukushima, Japan) using a spectrophotometer. BNP was also determined by measuring the antigen-antibody reactions using alkaline phosphatase-labeled anti-human BNP mouse monoclonal antibody with a chemical reagent (FUJIREBIO Inc., Tokyo).

Invasive coronary angiography: All cases in this study underwent invasive coronary angiography to check for the narrowing of coronary arteries. Experienced interventional cardiologists performed invasive coronary angiography with 4F catheters and analyzed all angiographic data quantitatively with validated automated edge-detection software (CAAS II; Pie Medical, Maastricht, The Netherlands). The outer diameter of the catheter tip, unfilled with contrast agent, was used as the calibration standard. Minimal lumen diameter, reference diameter, and percentage diameter stenosis of the lesion were measured from multiple projections in diastole. Coronary arteries without ≥ 50% luminal narrowing and apparent atherosclerosis in the initial coronary angiography were eligible for inclusion in this study. Coronary lesions with ≥ 50% luminal narrowing with atherosclerosis, which subsequently were not dilated by intracoronary nitrate administration, were considered as organic stenosis and thus excluded. If more than 50% coronary stenosis existed initially and was completely relieved by intracoronary nitrate administration, we judged this susceptible to a coronary artery spasm. We classified more than 90% stenosis with a response to nitrates as significant coronary artery spasm. The acetylcholine provocation test was performed in elective cases at the discretion of the operator. However, if serum troponin levels were elevated in emergency admissions (acute coronary syndrome), the test was not conducted in order to avoid worsening of the myocardial damage.

The acetylcholine provocation test protocol has been described previously.[8] Briefly, first an initial diagnostic coronary angiography is performed without intracoronary administration of isosorbide dinitrate. Next is the injection of acetylcholine chloride (OVISOT®, Daiichi Sankyo Co. Ltd., Tokyo) into the coronary arteries for 10 seconds, followed by angiography at 30, 60, 90, and/or 120 seconds. The predetermined doses of intracoronary acetylcholine were 20-25 μg, 50 μg, and/or 100 μg. Stenosis exceeding 90% was defined as significant coronary spasm. After checking for coronary artery spasm, the final angiogram was obtained with intracoronary administration of isosorbide dinitrate up to maximal dilation. Importantly, temporary pacing is necessary to prevent bradycardia during the acetylcholine provocation test.

Medical therapy: Medical therapy to dilate the coronary arteries (ie, a calcium channel antagonist, nicorandil, and nitrates) was discontinued before elective coronary angiography. Prescribed statin therapy and other medications were continued during the hospital stay and coronary angiography. In emergent cases, invasive coronary angiography was performed regardless of any kind of medication. After diagnosing clinical VSA using the JCS guideline, vasodilatory drugs were administered at the discretion of the operator.

Statistical analysis: Continuous variables are presented as the mean ± SD or as median ± interquartile range. Statistical differences between the groups were determined using the chi-square test and Student's *t*-test. Values of $P < 0.05$ were considered to be statistically significant. Multivariate logistic regression analysis was performed to determine independent correlates using clinical variables with a P value < 0.20 in univariate analysis, conventional risk factors according to the JCS guideline, and variables of clinical significance. Regression analysis to examine the linear association between continuous variables was estimated using the Spearman correlation test. Receiver-operating characteristic (ROC) analysis was used to determine the optimal cut-off values of the various biomarkers including serum MDA-LDL for diagnosis of clinical VSA. The ROC curve represents the relationships between sensitivity and specificity by plotting true-positive rates against false positive rates as the cut-off level of the model varies. The area under curve (AUC) provides a measure of overall accuracy that is the independent decision criterion. The best cut-off value was defined as the point of the highest sum of sensitivity and specificity. Statistical analysis was performed using JMP9 (SAS Campus Drive, Cary, NC, USA).

RESULTS

Patient characteristics and lesion characteristics: A flow chart of this study is shown in Figure 1. The patients were classified into 3 groups depending on the diagnosis according to the JCS guideline as follows; 40 patients as definite VSA, 35 as suspected VSA, and 9 as unlikely VSA. A final diagnosis of "clinical VSA", defined as definite VSA or suspected VSA, was made in 75 patients. Analysis of the patient characteristics revealed significant differences in gender, chest pain at rest, and serum MDA-LDL (Table I). Current smoking was more prevalent among patients classified as clinical VSA. The clinical VSA group had more significant ischemic ECG change because making a diagnosis of VSA based on the flow-chart of the JCS guideline depended essentially on this ECG change. There were no differences in medicines, ie, statins, angiotensin-converting enzyme inhibitors (ACE-I), and/or angiotensin II receptor blockers (ARB) at baseline.

Figure 2 shows the serum MDA-LDL levels in every diagnostic group according to the flow chart of the JCS guideline. In Figure 2A, the serum MDA-LDL levels of definite VSA ($n = 40$, 126.8 ± 41.1 U/L) were significantly higher than unlikely VSA ($n = 9$, 98.7 ± 31.1 U/L). The serum MDA-LDL level of suspected VSA ($n = 35$, 125.8 ± 34.7 U/L) was slightly higher than that of unlikely VSA, but the difference was not

Table I. Patient Characteristics

	Clinical VSA, $n = 75$	Unlikely VSA, $n = 9$	P
Age (years)	63.9 ± 10.8	61.7 ± 14.1	0.573
Male sex	38 (51%)	1 (11%)	0.025
Height (cm)	159.8 ± 10.6	156.3 ± 6.2	0.337
Weight (kg)	60.2 ± 12.6	54.7 ± 9.0	0.209
Body mass index	22.4 ± 3.5	22.4 ± 3.6	0.408
Symptom			
Chest pain at rest	70 (83%)	5 (55%)	0.0005
Chest pain on exertion	21 (28%)	4 (44%)	0.308
Syncope	6 (8%)	0 (0%)	0.379
Hypertension	46 (61%)	5 (55%)	0.737
Dyslipidemia	38 (51%)	4 (44%)	0.724
Diabetes mellitus	10 (13%)	2 (22%)	0.472
Current smoker	25 (33%)	1 (11%)	0.173
Ex-smoker	14 (19%)	1 (11%)	0.576
Alcohol consumption	42 (56%)	4 (44%)	0.511
ECG change			0.067
Positive	22 (29%)	0 (0%)	
Borderline	16 (21%)	1 (11%)	
Negative	37 (49%)	8 (89%)	
Laboratory data			
T-Chol (mg/dL)	199.5 ± 29.8	197.8 ± 48.6	0.883
HDL-C (mg/dL)	56.5 ± 14.2	60.2 ± 18.8	0.472
LDL-C (mg/dL)	112.1 ± 22.6	108.9 ± 34.8	0.734
TG (mg/dL)	143.4 ± 84.3	102.6 ± 56.2	0.161
MDA-LDL (U/L)	126.3 ± 38.0	98.7 ± 31.0	0.039
Fasting blood glucose (mg/dL)	101.8 ± 21.9	103.3 ± 29.9	0.849
Hemoglobin A1c (%)	5.9 ± 0.7	6.3 ± 1.2	0.139
1,5AG (μg/mL)	18.1 ± 7.6	16.3 ± 7.4	0.489
Creatinine (mg/dL)	0.72 [0.62-0.86]	0.65 [0.60-0.77]	0.579
Cystatin C	0.95 ± 0.70	0.84 ± 0.13	0.644
C-reactive protein(mg/dL)	0.07 [0.03-0.25]	0.05 [0.03-0.12]	0.378
BNP (pg/mL)	26.0 [14.2-61.4]	50.1 [11.9-135.1]	0.985
Medication at baseline			
ACE-I and/or ARB	26 (35%)	3 (33%)	0.936
Statin	24 (32%)	1 (11%)	0.195

ACE-I indicates angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blockers; and BNP, brain natriuretic peptide.

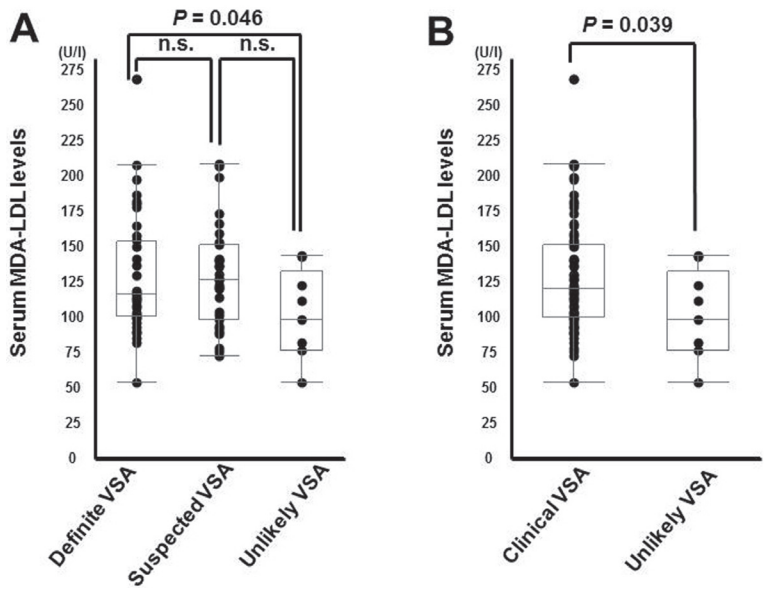

Figure 2. Serum MDA-LDL levels in candidate patients for diagnosis of VSA. Serum MDA-LDL levels of clinical VSA ($n = 75$, 126.3 ± 38.0 U/L) were significantly higher than unlikely VSA ($n = 9$, 98.7 ± 31.0 U/L, $P = 0.039$).

significant. Definite VSA and suspected VSA had approximately equal results, and the difference was not significant. In Figure 2B, the serum MDA-LDL level of clinical VSA (n = 75) was significantly higher than that of unlikely VSA (n = 9) (126.3 ± 38.0 versus 98.7 ± 31.1 U/L, P = 0.039).

Relationship between serum MDA-LDL and other biomarkers: Figure 3 shows the relationship between serum MDA-LDL level and other biomarkers. Serum MDA-LDL level was positively correlated with T-Chol level (Y = -8.560 + 0.663 X, r^2 = 0.300, P < 0.0001) and LDL-C level (Y = 35.869 + 0.783 X, r^2 = 0.295, P < 0.0001), but there was no relationship with HDL-C level (Y = 146.791 – 0.411 X, r^2 = 0.025, P = 0.150). Interestingly, it was also positively related with TG (Y = 98.839 + 0.176 X, r^2 = 0.145, P = 0.0003) and fasting blood glucose (Y = 85.597 + 0.370 X, r^2 = 0.048, P = 0.044).

Invasive coronary angiography and acetylcholine provocation test findings: Table II presents the results of invasive coronary angiography and the acetylcholine provocation test. The acetylcholine provocation test was conducted in 60 patients (54 patients in clinical VSA group and 6 patients in unlikely VSA group). The acetylcholine provocation test was positive in 60% (36 of 60 cases). In the clinical VSA group, the acetylcholine provocation test was positive in 67% (36 of 54 cases). All cases in the unlikely VSA group were negative.

Multivariate analysis for diagnosis of clinical VSA: To determine the factors for diagnosis of clinical VSA, multivariate analysis was performed using the results of univariate analysis with consideration of the confounding factors and conventional risk factors according to the JCS guideline (Table III). Serum MDA-LDL level was the most independent factor among the other clinical factors (odds ratio 1.064, 95% confidence interval 1.014-1.145, P = 0.008).

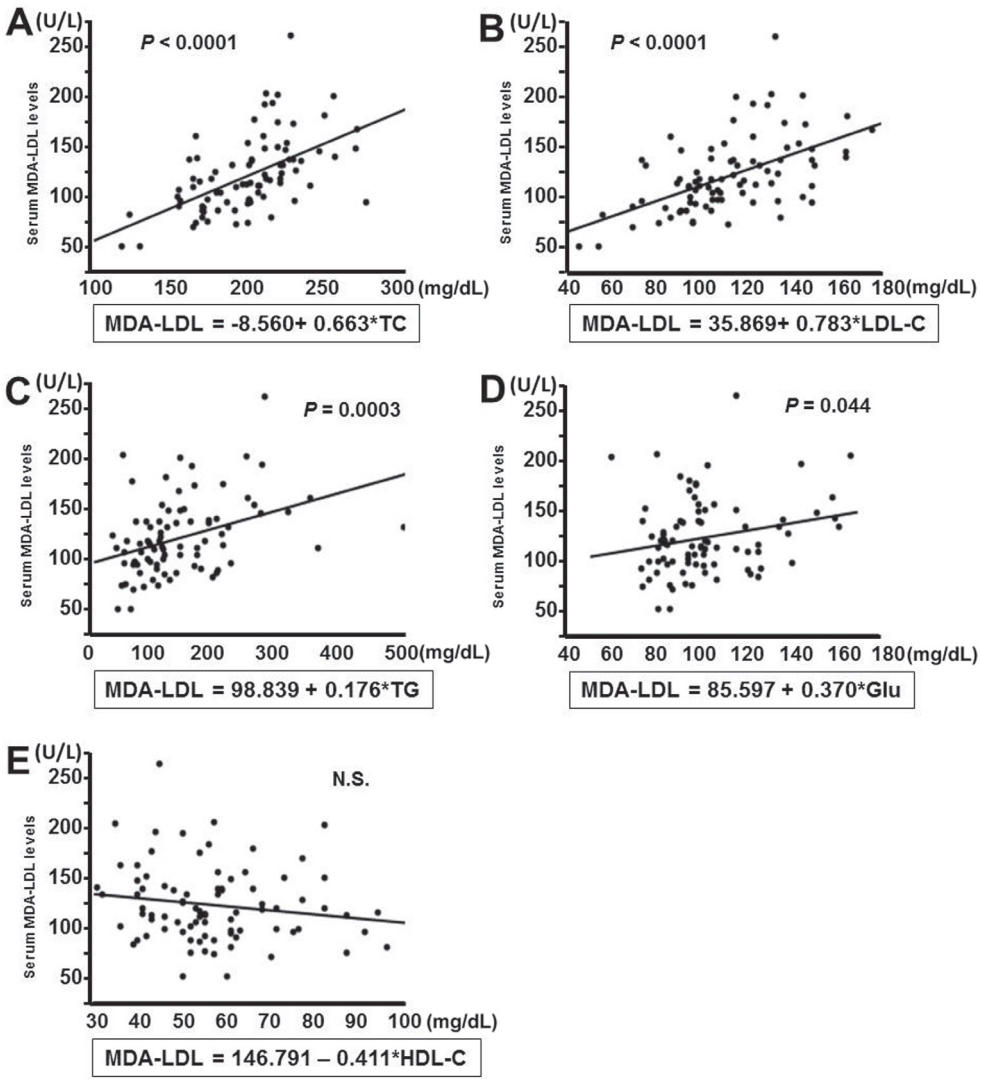

Figure 3. Correlation of serum MDA-LDL level and biomarkers. Serum MDA-LDL level was positively correlated with T-Chol level (**A:** Y = -8.560 + 0.663 X, r^2 = 0.300, P < 0.0001), LDL-C level (**B:** Y = 35.869 + 0.783 X, r^2 = 0.295, P < 0.0001), TG (**C:** Y = 98.839 + 0.176 X, r^2 = 0.145, P = 0.0003), and fasting blood glucose (**D:** Y = 85.597 + 0.370 X, r^2 = 0.048, P = 0.044). However, serum MDA-LDL level had no relationship with HDL-C level (**E:** Y = 146.791 – 0.411 X, r^2 = 0.025, P= 0.150).

Table II. Invasive Coronary Angiography and Acetylcholine Provocation Test

Acetylcholine provocation test	Clinical VSA, $n = 75$	Unlikely VSA, $n = 9$
Positive	36 (48%)	0 (0%)
Negative	18 (24%)	6 (67%)
Significant spasm in the first shot	1 (1%)	0 (0%)
Positive, formerly	2 (3%)	0 (0%)
Not done	18 (24%)	3 (33%)

Table III. Multivariate Analysis for the Factors to Diagnose Clinical VSA

	Odds ratio	95% Confidence interval	*P*
Male sex	13.610	0.895-934.179	0.023
Chest pain at rest	6.286	0.403-121.930	0.185
ECG change, positive or borderline	7.433	0.722-248.480	0.097
Current smoker	0.320	0.007-12.826	0.520
Alcohol consumption	1.253	0.143-12.401	0.838
Triglycerides	0.999	0.982-1.022	0.969
MDA-LDL	1.064	1.014-1.145	0.008
Hemoglobin A1c	0.394	0.091-1.362	0.143

Predictive value of serum MDA-LDL for VSA and relationship between coronary spasm induced by acetylcholine provocation test and serum MDA-LDL levels: To determine the ability of the predictive value for the diagnosis of clinical VSA, ROC analyses were performed for various biomarkers. Serum MDA-LDL level had the largest AUC (AUC 0.695) and a sensitivity of 93% and specificity of 45% as well as the best cutoff value (110.8 U/L) (Figure 4). Among the patients that underwent coronary angiography with the acetylcholine provocation test, the MDL-LDL higher group (> 110.8 U/L, $n = 37$) had a slightly positive result compared to the MDL-LDL lower group (< 110.8 U/L, $n = 23$) (68% versus 48%, $P = 0.12$). Furthermore, in a population with a positive or borderline ECG change, the positive rate of the acetylcholine provocation test was significantly higher in the MDA-LDL higher group ($n = 16$) compared to the MDA-LDL lower group ($n = 8$) (81% versus 37%, $P = 0.032$) (Figure 5). There was no significant difference among a population with a negative ECG change.

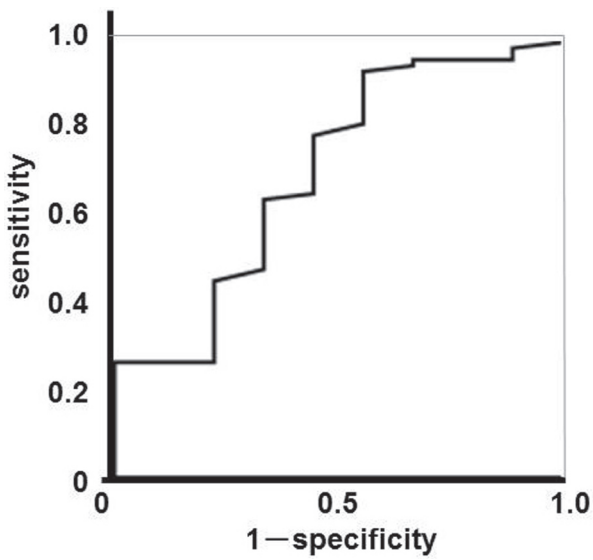

Figure 4. ROC analysis to determine proper value of serum MDA-LDL level for making a diagnosis of clinical VSA. Serum MDA-LDL level had the largest AUC (AUC 0.695) and sensitivity of 93%, specificity of 45%, and the best cutoff value of 110.8 U/L.

Discussion

The important findings of the present study are as follows. First, the serum MDA-LDL level was elevated in the clinical VSA group which was diagnosed according to the JCS guideline among the candidate patients for VSA. Second, the serum MDA-LDL level was positively correlated with T-Chol and LDL-C, which were associated with the formation of atherosclerotic plaque, and also with triglycerides and fasting blood glucose which are known markers of endothelial dysfunction. Third, serum MDA-LDL was the most valuable marker for diagnosing clinical VSA among conventional markers according to the multivariable analysis, and had good sensitivity and specificity as exhibited by the best cutoff value (110.8 U/L) by ROC analysis. Fourth, the patients with high MDA-LDL levels had a high frequency of positive response to the acetylcholine provocation test. Therefore, the results of the present study suggest that serum MDA-LDL might be a useful biomarker for the diagnosis of clinical VSA.

Pathogenesis of VSA: VSA patients usually have an angina attack at rest between around midnight and the early morning due to a coronary artery spasm.[6] In most cases, there is no significant stenosis or only mild stenosis by invasive coronary angiography in these patients. The etiology of VSA is thought to be due to vascular endothelial dysfunction and the hypercontraction of vascular smooth muscle cells.[27] From the perspective of endothelial dysfunction, the endothelium plays an important role in the modulation of vascular tone via endothelium-derived relaxing factors (EDRF) such as nitric oxide (NO) and prostaglandins and endothelium-derived hyperpolarizing factor (EDHF). NO is expressed by the eNOS gene of vascular endothelial cells and the released NO has a relaxant effect on VSMCs. The biological circumstantial factors which induce coronary spasm are known to be multiple agents such as smoking, alcohol consumption, lipid disorder,

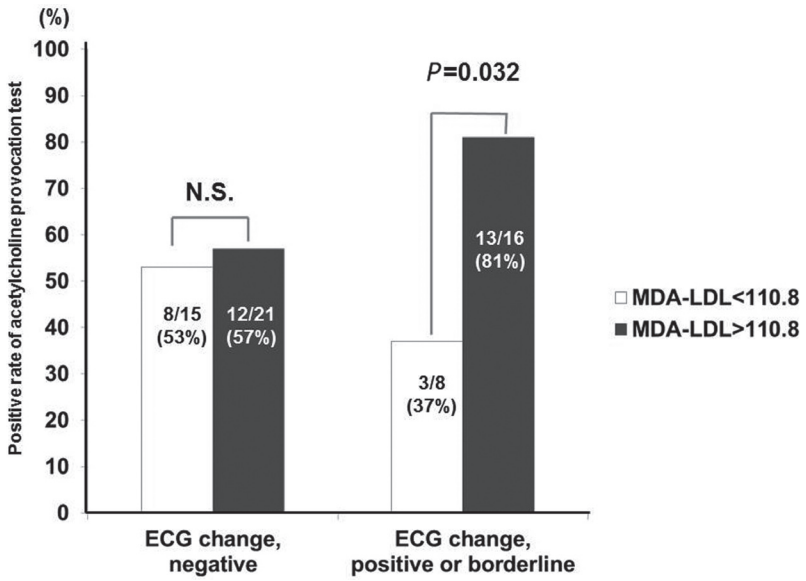

Figure 5. Positive rate of acetylcholine provocation test according to ischemic ECG change and serum MDA-LDL levels. In a population with positive or borderline ECG change, inducibility of coronary spasm was significantly higher in the MDA-LDL higher group (n = 16) compared to the MDA-LDL lower group (n = 8) (81% versus 37%, P = 0.032). There was no significant difference among a population with negative ECG change.

psychological stress (autonomic disorder), and oxidative stress. For these reasons, a VSA attack is believed to be induced by the overlapping of multiple factors and multiple pathways.

Serum MDL-LDL as a biomarker of VSA: It is reported that VSA patients in general have a good prognosis, however, some cases deteriorate into AMI, fatal arrhythmia, and CPA.[28-30] To avoid serious cardiovascular events, the first step is to obtain a precise diagnosis of VSA for patients with chest pain. The next step is to prevent a coronary spasm attack with appropriate medical treatment. Chest pain at rest between midnight and the early morning that is accompanied by ST-T change suggests suspected VSA, but a change in the ECG does not always occur and cannot be detected completely using ambulatory electrocardiogram monitoring. Although coronary angiography with the acetylcholine or ergonovine provocation test has been shown to be useful as a diagnostic aid, coronary angiography and the acetylcholine/ergonovine provocation test[8] are invasive and therefore challenging. An adjuvant noninvasive examination before performing coronary angiography is desperately needed in clinical practice.

Yoneyama, *et al* reported that metabolic planar imaging using 123I-β-methyl-iodophenyl pentadecanoic acid might be useful in the diagnosis of coronary artery spasm. While this method is non-invasive, it is expensive from a medical economics perspective.[31]

MDA-LDL,[21] which is modified LDL-C, is a known oxidative stress marker,[22] and the serum MDA-LDL level increases in patients complicated by smoking[23] or DM.[24] It is conceivable that the oxidative marker MDA-LDL may be associated with endothelial dysfunction in the mechanism of VSA because oxidative stress can generally induce endothelial dysfunction.[26] Actually, oxidized LDL is reported to be associated with endothelial dysfunction in previous myocardial infarctions.[32] In our study, the serum MDA-LDL level was high in definite VSA patients or clinical VSA, and had a positive correlation with not only T-Chol and LDL-C, but also triglycerides and fasting blood glucose. Furthermore, ROC analysis and multivariate analysis were found to be useful for predicting clinical VSA. These results suggest that serum MDA-LDL might be superior as a marker of VSA, and may be involved in the mechanism of coronary spasm.

Recently, Ito, *et al* reported that patients with a positive status with respect to the ergonovine provocation test showed higher levels of serum MDA-LDL than negative patients.[33] In the present study, we have shown that patients with clinical VSA categorized according to the JCS guideline had higher levels of serum MDA-LDL than unlikely VSA patients. Patients who are positive to the acetylcholine provocation test also had slightly higher levels compared to negative patients, but the difference was not significant. The reason for this discrepancy in these studies was that selection bias to cardiac catheter examination with provocation testing affected the results of both studies. Sueda, *et al* reported the positive rate in the acetylcholine provocation test was 66.9% in rest angina and 9.1% in non-ischemic heart disease.[34] Furthermore, they showed the positive rate in the ergonovine provocation test was 55.5% in rest angina, but was 3.7% in non-ischemic heart disease.[35] Our data showed the positive rate in the acetylcholine provocation test was 60%, and this frequency was similar to previous studies. On the other hand, the data from Ito, *et al* showed that the positive rate was 27% in the ergonovine provocation test, leading to the conclusion their population included relatively quite a few patients without spastic response. Because the JCS guideline is enable to make a diagnosis of clinical VSA using diagnostic tools (ie, symptoms, ECG changes, provocation test, and clinical course), it should be easy to acquire a high proportion as clinical VSA within the candidate patients of VSA. Additional studies are needed with a larger population using the JCS guideline. In addition, Ito, *et al* suggested that MDA-LDL was a predictor of cardiac events

after implantation of drug-eluting stents,[36] and MDA-LDL might have an effect on the clinical course in chronic stage VSA patients.

Serum MDA-LDL as the target of medical treatment: MDA-LDL may be important to medical treatment after making the diagnosis of VSA. Lifestyle improvements such as smoking cessation, reducing alcohol consumption, and mental health management is essential as the first-line treatment. Medical treatment using nitrates, calcium channel blockers, or an ATP sensitive potassium channel opener like nicorandil is useful for dilating a spastic coronary artery. However, a different approach is required because a refractory angina attack is sometimes experienced due to conventional medical treatment. Management of the triggers of VSA, which is in the upstream of the mechanism of coronary spasm, is essential as the target of an alternative approach.

It is known that patients with VSA have high degrees of coexistence with lipid disorder,[37] impaired glucose tolerance,[13,38,40] and renal dysfunction.[39] Hypertriglycemia, impaired glucose tolerance, and oxidative stress are known important factors for endothelial dysfunction and coronary spasm. Lee, *et al* reported that a high level of cystatin-C was independently associated with the prevalence of VSA patients in a Korean VSA registry.[39] Medical treatment for these factors should be conducted in addition to medical therapy that dilates the coronary artery. It is noted that endothelial dysfunction at mild plaque sites induced coronary spasm around the plaque itself, and also subsequently plaque rupture and, in part, acute coronary syndrome. Nobuyoshi, *et al* presented data using multivariable analysis in 239 patients that indicated the coronary spasm was associated with the onset of acute coronary syndrome.[41] Therefore, we believe MDA-LDL is an important target for treating VSA. Recently, it has been reported that the statin fluvastatin is an effective treatment for VSA,[18] and this result is thought to be due to its pleiotropic effect against oxidative stress. Statins reduce T-Chol and LDL-C, and also lead to a lowering of serum MDA-LDL level.[42,43] If serum MDA-LDL is involved in the mechanism of coronary spasm, it is easy to understand the effect of statin on VSA. Smoking cessation is highly recommended because active smoking can induce serum MDA-LDL elevation. These findings suggest that serum MDA-LDL could be a target of upstream therapy for coronary spasm.

VSA is always accompanied by the possibility of ACS,[44] AMI, fatal arrhythmia, and CPA.[19,20] It is not rare that a first time attack causes a critical situation in the emergency room. To prevent sudden cardiac death, an early diagnosis, early medical intervention, and early prevention of a VSA attack are needed. In our results, the measurement of serum MDA-LDL may support the early diagnosis and medical intervention at an early stage for VSA.

Limitations: A coronary artery spasm is a multi-factor problem. However, in familial VSA, abnormal genes such as eNOS genetic polymorphism and 1 phospholipase C - δ protein missense mutation are a dominant problem in VSA attacks compared to acquired factors. In this regard, MDA-LDL is not necessarily high in this situation. We were not able to conduct genetic screening in the present study because genetic backgrounds are not routinely determined in Japan. Going forward, the collection of data in familial VSA cases is needed to resolve this difficult situation.

In conclusion, serum MDA-LDL may be a useful biomarker of VSA and have additional value for the diagnosis of clinical VSA. In the future, the risk stratification and/or prognostic value of using serum MDA-LDL should be investigated.

Disclosures

The authors declare no conflicts of interest.

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