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Asymmetric dimethylarginine is not a good predictor of ischemia using myocardial perfusion scintigraphy

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Background/aim: Asymmetric dimethylarginine (ADMA) plays role in the pathogenesis of coronary artery disease and related mortality and morbidity through a number of mechanisms. We hypothesized that plasma ADMA levels would be increased in the presence of reversible ischemia as measured by GATED single photon emission computed tomography (SPECT) myocardial perfusion scintigraphy (MPS).

Materials and methods: Fasting i.v. blood samples were drawn before testing. All patients underwent 99mTc-sestamibi GATED SPECT MPS with a one-day stress-rest protocol; the images were visually analyzed. Post-stress GATED parameters, including ejection fraction, end systolic and end diastolic volumes, and automatic stress defect scores, were recorded.

Results: The plasma ADMA levels were higher in the ischemic group than in the non-ischemic group (0.46 ± 0.19 vs. 0.40 ± 0.15; P = 0.016). Plasma ADMA levels (odds ratio [OR] = 13.5; 95% confidence interval [CI] = 1.7-109.01; P = 0.015) and sex (OR = 2.49, 95% CI = 1.18-5.26; P = 0.017) were independent predictors of ischemia. There was no linear correlation between plasma ADMA levels and both the GATED SPECT and stress test parameters.

Conclusion: Our data support the hypothesis that increased baseline ADMA levels are independently related with the presence of reversible ischemia.

Key words: Asymmetric dimethylarginine, single photon emission computed tomography, ischemia

1. Introduction

Asymmetric dimethylarginine (ADMA) is an analog of L-arginine that acts as a nitric oxide (NO) synthesis inhibitor and contributes to the pathogenesis and related mortality and morbidity of coronary artery disease (CAD) (1–3). High levels of ADMA cause atherosclerosis by triggering endothelial dysfunction through the inhibition of NO synthesis (4). ADMA also promotes apoptosis, which inhibits the mobilization and differentiation of endothelial progenitor cells (5).

Gated single photon emission computed tomography (SPECT) myocardial perfusion scintigraphy (MPS) is a highly reliable method for determining the presence and severity of myocardial ischemia in CAD and can independently predict the extent of future cardiac events (6–8). To date, there has been only one report comparing reversible ischemia during stress echocardiography using the wall motion score with plasma ADMA levels (9). In this study, we hypothesized that plasma ADMA levels would be increased in the presence of reversible ischemia compared to normal subjects using gated SPECT MPS.

2. Materials and methods

2.1. Study population

This study included gated myocardial SPECT images from 170 consecutive patients [69 (40.6%) males and 101 (59.4%) females; mean age 57 ± 9 years] with ischemia (n = 87) or no ischemia (n = 83). None of the patients had cardiac valve disease, cardiomyopathy, malignant arrhythmias, acute or chronic liver disease, renal failure,

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or suspected pregnancy, and none were breast-feeding at the time of enrollment. Patients who had a reversible fixed defect were excluded. Gated SPECT MPS was performed for the diagnosis of CAD. A detailed history, including risk factors, current medications, body mass index (BMI), waist circumference, blood pressure before and after exercise test, metabolic equivalents, and baseline heart rate, was collected for all patients. Fasting intravenous (i.v.) blood samples were drawn before the stress test. All patients were examined by 99mTc-sestamibi gated SPECT MPS with a 1-day stress-rest protocol. All subjects gave informed consent prior to their enrollment in the study, which was approved by our local ethics committee.

2.2. Imaging protocol

Blood pressure, heart rate, and electrocardiogram findings were monitored during the test. The test endpoints included physical exhaustion, anginal complaints, dyspnea, a significant decrease in blood pressure greater than 10 mmHg, and achievement of the maximal age-related heart rate. In patients that could not perform the treadmill exercise test, a pharmacological stress test consisting of i.v. adenosine (0.14 mg kg^{-1} min^{-1} for 6 min) or dobutamine (up to a maximum dose of 40 μg kg^{-1} min^{-1} in 15 min) was administered until the required age-related heart rate was reached. Beta-blockers and calcium antagonists were discontinued for 48 h and long-acting nitrates were discontinued for 24 h before gated myocardial SPECT. Caffeine- and theophylline-containing foods, medications, and tobacco were discontinued 1 day before the study. At peak exercise, 296–370 MBq (8–10 mCi) of 99mTc-sestamibi was injected intravenously and continued for another 1–2 min after the tracer injection. Resting images were obtained 3–4 h after stress imaging with an injection that was 3 times the initial dose. Images were acquired 45 min after the stress-injection and 60 min after the rest-injection with an E.Cam single-headed gamma camera (Siemens, Germany) equipped with all-purpose high-resolution collimators. The studies were prefiltered with a Butterworth filter and back-projected with a ramp filter. After reconstruction, the data were displayed as tomographic slices and bull's-eye maps (polar plots). The images were also visually analyzed. Poststress gated parameters, including the ejection fraction, end systolic and end diastolic volumes, and automatic stress defect scores, were recorded.

2.3. ADMA measurement

Serum specimens were separated from whole blood samples by centrifugation at 5000 rpm for 10 min and stored at –20 °C until analysis. Hemolytic and lipemic serum samples were excluded from the study. All assays were performed using enzyme-linked immunosorbent assay kits (BioVendor Research and Diagnostic Products, Candler, NC, USA) read on a BioTek Epoch (Winooski, VT, USA) microplate reader. Before the assay, all samples were brought to room temperature and mixed carefully to avoid foam development. Standards, controls, and samples (50 μL) were added to the wells of coated microtiter strips. Antiserum (50 μL) was added to all wells and mixed on an orbital shaker. The plate was then covered with adhesive foil and incubated at 2–8 °C for 20 h. Next, all wells were washed 4 times with 250 μL of washing buffer. After removing the residual liquid, 100 μL of enzyme conjugate was added and the washing process was repeated. The plates were then incubated for 30 min on an orbital shaker at room temperature (25 °C). The reaction was stopped by adding 100 μL of stopping solution. The plates were read at 450 nm with a reference wavelength of 570 nm. The standard curve was as predicted and the control results were in the appropriate ranges.

2.4. Statistical analysis

Statistical analyses were performed using PASW 18 statistical software (SPSS Inc., Chicago, IL, USA). Normality analyses of continuous variables were performed with histogram curves. Continuous variables are presented as mean ± standard deviation. When two groups of data were compared, we performed an independent sample t-test. If there were more than two groups, we used one-way analysis of variance. The Spearman test was performed to determine the linear correlation between two continuous variables. Categorical variables were compared between two groups using a chi-square test and are presented as frequency and percentage. Receiver operating curve (ROC) analyses were performed to determine the cut-off value of ADMA levels for estimating reversible ischemia. Multivariate logistic regression analyses were performed to estimate independent predictors of ischemia. Univariate analyses of variables with $P < 0.1$ were selected as covariates. Statistical significance was set at $P < 0.05$.

3. Results

The characteristics of the patients are presented in Table 1. The plasma ADMA levels were higher in the ischemic group than in the nonischemic group (0.46 ± 0.19 vs. 0.40 ± 0.15; P = 0.016). Male patients had a 2.5-fold higher frequency of ischemia. The frequencies of beta-blocker, antiaggregant, and nitrate use were higher in the ischemic group. In addition, a prior history of revascularization was more common in the ischemic group. There was no linear correlation between the plasma ADMA levels and the gated and stress test parameters (Table 2). We obtained a sensitivity value of 0.90 and a specificity value of 0.08 for a cut-off value of 0.19 in ROC analyses (area under the curve = 0.618; P = 0.008). We found that plasma ADMA level (odds ratio [OR] =13.5; 95% confidence interval [CI] = 1.7–109.01; P = 0.015) and sex (OR = 2.49; 95% CI = 1.18–5.26; P = 0.017) were related to ischemia by gated SPECT MPS when age, sex, plasma ADMA level, and BMI were used as covariates (Table 3).

Table 1. Characteristics of the patients.

	Nonischemic patients (n = 87)	Ischemic patients (n = 83)	P
Age (years)	57 ± 9	60 ± 9	0.069
Sex (male; n, %)	22 (27)	47 (54)	<0.001
BMI (kg/m^2)	30.1 ± 4.9	29.5 ± 5.2	0.415
Hypertension (n, %)	54 (65.1)	62 (71.3)	0.385
Diabetes (n, %)	19 (22.9)	21 (24.1)	0.602
Hyperlipidemia (n, %)	31 (37.3)	32 (36.8)	0.939
ADMA (μmol/L)	0.40 ± 0.15	0.46 ± 0.19	0.016
Currently smoking (n, %)	19 (22.9)	27 (31.0)	0.232
Medication (n, %)			
Beta blocker	22 (27.5)	41 (47.7)	0.007
Calcium channel blocker	18 (22.5)	13 (15.1)	0.223
Antiaggregant	25 (31.3)	43 (50.0)	0.014
ACE inhibitor	28 (35.0)	25 (29.1)	0.413
Digital	0 (0)	1 (1.2)	0.333
Nitrate	0 (0)	9 (10.5)	0.003
Oral antidiabetic or insulin	12 (14.6)	15 (17.4)	0.620
Antihyperlipidemic	17 (20.7)	25 (29.1)	0.212
Prior revascularization (n, %)	9 (30)	21 (70)	0.023
Baseline heart rate (min^{-1})	80.7 ± 13.0	78.7 ± 12.2	0.327
Systolic blood pressure (mmHg)	122.9 ± 16.4	124.5 ± 14.9	0.510
Diastolic blood pressure (mmHg)	73.3 ± 8.7	74.5 ± 8.0	0.343
Waist circumference (cm)	100.9 ± 10.9	99.7 ± 13.1	0.534

Table 2. Correlations of ADMA levels between both gated and stress parameters.

	Plasma ADMA levels	
	R	P
Baseline heart rate	–0.088	0.258
Diastolic blood pressure before stress test	0.047	0.547
Systolic blood pressure before stress test	0.036	0.645
Duration of stress test	–0.067	0.400
Metabolic equivalent tasks	–0.188	0.018
Systolic blood pressure after stress test	–0.001	0.993
Diastolic blood pressure after stress test	0.065	0.405
Stress defect score	0.054	0.488
Summed difference score	–0.033	0.673
Ejection fraction	0.033	0.681
End systolic volume	–0.023	0.778
End diastolic volume	–0.008	0.921

Table 3. Results of multivariate logistic regression analyses where ischemia is the dependent variable.

	OR	95% CI for OR	P
Age (years)	0.985	0.941–1.031	0.513
Sex*	2.493	1.180–5.260	0.017
BMI (kg/m^2)	0.975	0.899–1.056	0.530
Plasma ADMA (μmol/L)	13.5	1.7–109.01	0.015
Metabolic equivalent tasks	0.878	0.748–1.030	0.110
History of bypass	2.841	0.279–28.972	0.378
Prior revascularization	1.186	0.383–3.671	0.767
Use of beta blocker	2.138	0.907–5.040	0.083
Use of antiaggregant	1.297	0.571–2.942	0.534
Use of nitrate	0.464	0.001– ...	0.999

*Male sex increased 2.5-fold in the ischemic group.

4. Discussion

Our study suggests that plasma ADMA levels are increased during ischemia and that an increased ADMA level is a predictor of reversible ischemia. Elevated plasma concentrations of ADMA have been found in nonmyocardial infarction heart disease, and clinical and laboratory studies have reported elevated ADMA levels in patients diagnosed with ischemic heart disease. Djordjević et al. (10) reported increased ADMA levels in ischemic heart disease with median ADMA values of 0.75 (0.31–2.73) μmol/L in stable angina pectoris (SAP) patients and 0.94 (0.34–3.13) μmol/L in unstable angina pectoris (USAP) patients, whereas in healthy subjects the ADMA level was 0.31 (0.17–0.87) μmol/L. The USAP group displayed 95.65% sensitivity and 96.30% specificity for ADMA. Cao et al. (11) also showed that ADMA levels were significantly elevated in acute coronary syndrome patients compared with controls or SAP patients. Similarly, Krempl et al. (12) reported that baseline ADMA concentrations were significantly lower in controls than in patients with CAD (0.59 ± 0.23 vs. 0.76 ± 0.17 μmol/L), and that patients with unstable angina had significantly higher baseline ADMA levels than patients with stable angina (0.82 ± 0.18 vs. 0.73 ± 0.15 μmol/L). Valkonen et al. (13) reported that ADMA levels of >0.62 μmol/L resulted in a 3.9-fold increased risk of acute coronary events in males. The ADMA level tended to increase in the SAP group compared to the noncoronary heart disease group, although the difference was not significant. There are also studies confirming CAD by percutaneous coronary angiography. A prospective study (14) showed that the plasma ADMA level in patients with significant CAD was notably higher than in the controls (0.66 ± 0.17 and 0.44 ± 0.09 μM, respectively). The same authors used a larger sample size (n = 997) to confirm these results, showing that plasma ADMA levels were significantly higher in patients with significant CAD (0.10 μmol/L) than in those with insignificant CAD (0.47 ± 0.10 μmol/L) and normal coronary arteries (0.42 ± 0.08 μmol/L) (15). In this study, we found that the ADMA level was higher in ischemic patients than in nonischemic patients and was independently related to ischemia by multivariate regression analysis. However, it appears that ADMA is not a suitable agent for clinical use because of poor diagnostic accuracy: we calculated 90% sensitivity for a cut-off value of 0.19 in ROC analyses with a specificity of 0.08.

Significant positive correlations have been reported between the coronary atherosclerotic score, which represents the extent and severity of coronary atherosclerosis, and plasma ADMA levels (r = 0.518) (14). Song et al. (16) used a multivariate stepwise logistic regression analysis to show that plasma ADMA levels were positively correlated with the severity of coronary atherosclerosis (r = 0.684). Furthermore, single (1.52 ± 0.61 μmol/L), double (1.67 ± 0.80 μmol/L), and multibranched (2.60 ± 0.62 μmol/L) CAD groups had significantly higher levels of ADMA than controls. However, we did not detect a correlation between the baseline plasma ADMA level and the summed stress and summed difference scores, which indicate the extent and severity of ischemic heart disease, respectively.

To date, studies have verified CAD anatomically by percutaneous coronary intervention, while nuclear medicine and echocardiographic techniques can evaluate myocardial function. There is only one study describing the relationship between ADMA levels and functional status

of ischemic myocardial tissue (9). This study reported a significant increase in ADMA in patients with stable angina and increased wall motion scores after exercise stress echocardiography. However, this was not related to stress-induced ischemia. Ours is only the second study to compare plasma ADMA levels with the functional status of myocardial tissue, and it is the first to do so using gated SPECT MPS.

In conclusion, our data support the hypothesis that increased baseline ADMA levels are independently related to the presence of ischemia; however, these values are not suitable for the clinical prediction of ischemia and are not related to the extent or severity of ischemia, or with other functional parameters, including poststress ejection fraction, end systolic volume, and end diastolic volume, in scintigraphy.

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