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Association of asymmetric dimethylarginine levels with treadmill-stress-test-derived prognosticators

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ABSTRACT

Background: Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide production. The purpose of this study was to assess the correlation between ADMA and treadmill stress test outcome parameters with known prognostic value, in patients with intermediate risk for coronary artery disease (CAD).

Methods: Study participants were referred for treadmill exercise stress test (EST) due to symptoms of suspected CAD. Participants with prior history of CAD, cerebrovascular events, peripheral artery disease, systemic inflammatory disease or use of anti-inflammatory agents were excluded. ADMA levels were measured before EST.

Results: The study prospectively enrolled 209 individuals (165 males, aged 58.1 ± 10.9). A significant negative correlation was detected between ADMA and maximal exercise time ($r = -0.556$, $p < 0.001$), metabolic equivalents (METs) ($r = -0.555$, $p < 0.001$) and Duke treadmill score (DTS) ($r = -0.347$, $p < 0.001$). Subjects who exercised to ≥10 METs ($n = 114$) had lower ADMA levels than those who achieved <7 METs ($n = 30$) (0.58 ± 0.06 vs 0.87 ± 0.08 μmol/L, $p < 0.001$), and those with DTS < 5 ($n = 63$) had higher ADMA (0.75 ± 0.19 vs 0.64 ± 0.15 μmol/L, $p < 0.001$) compared to those with DTS ≥5 ($n = 146$). In multivariable analysis, ADMA remained an independent predictor of DTS ($R^2 = 0.210$; beta = −10.5; 95% confidence interval −14.9 to −6.2; multivariate $p < 0.001$) and METs ($R^2 = 0.500$; beta −8.5; 95% confidence interval −9.7 to −6.0; multivariate $p < 0.001$) after adjustment for age, BMI, gender, diabetes, smoking status, dyslipidemia, hypertension and family history of premature CAD.

Conclusion: ADMA is correlated to EST parameters with proven prognostic value. This implies that ADMA itself might be a useful prognosticator in patients with suspected CAD.

Introduction

Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide (NO) production, has been demonstrated to have a pathophysiologic role in cardiovascular disease (CVD), serving at the same time as a potential biomarker of cardiovascular risk [1–3]. Since the pioneer study of Valconen et al. [1], showing that middle-aged smoking men in the highest quartile of ADMA levels were at an almost 4-fold risk for acute coronary events, several other studies followed showing a strong association between ADMA and CAD [2–4].

Electrocardiographic exercise stress test (EST) is the most commonly used method to evaluate intermediate-risk individuals with symptoms of suspected CAD and normal baseline ECG [5,6]. Although its use as a diagnostic tool has been partly substituted by imaging stress methods, its prognostic value is the most well-established among CAD evaluation tests. Exercise capacity, Duke treadmill score (DTS) and blood pressure (BP) response are established EST parameters, with diagnostic and prognostic value [7–9].

The goal of this study was to assess correlations between ADMA and EST outcome parameters with known clinical prognostic value, namely

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exercise capacity, expressed in metabolic equivalents (METs), DTS and BP response, in patients with intermediate risk for CAD.

Methods

Population

The study participants were individuals without a history of atherosclerotic disease, referred to our hospital with symptoms of suspected CAD. All patients were evaluated for pre-test likelihood for CAD by sex, age and type of pain according to the Diamond and Forrester model [10]. Individuals with prior history of CAD, cerebrovascular events, peripheral artery disease and systemic inflammatory disease were excluded. Use of anti-inflammatory agents, including aspirin and corticosteroids, anticoagulants and statins was also an exclusion criterion.

Major cardiovascular risk factors, including hypertension, dyslipidemia, diabetes mellitus, obesity, smoking and family history of CAD were assessed. Hypertension was defined as current use of any antihypertensive medication or resting BP of ≥140/90 mm Hg taken on 3 separate visits [11]. Dyslipidemia was defined as the presence of fasting total cholesterol levels >220 mg/dL (5.7 mmol/L) or LDL concentrations >160 mg/dL (4.1 mmol/L), diabetes mellitus as fasting plasma glucose ≥126 mg/dL (7 mmol/L) or currently using anti-diabetic medication [12], and obesity was determined as body-mass index (BMI, weight/height^2) ≥30 kg/m^2. Family history of premature CAD was defined as male or female first-degree relative diagnosed with CAD under the age of 55 and 65 years, respectively. Subjects also reported their physical activity status on a 3-level scale ranging from sedentary (no or irregular physical activity), active (2.5–5 h a week on physical activity or aerobic exercise training of at least moderate intensity, or 1–2.5 h a week on vigorous intense exercise) and fit (more than 2.5–5 h a week on physical activity or aerobic exercise training of at least moderate intensity, or more than 1–2.5 h a week on vigorous intense exercise) [13].

All participants provided informed consent to participate in the study, which was approved by the institutional review board.

Exercise stress test

EST was performed on a treadmill with ECG and BP monitoring. The standard Bruce protocol was used and participants were encouraged to continue their exercise until they had achieved at least 90% of their age-predicted maximal heart rate. During each 3-min stage of exercise, at peak exercise and every 2 min of the recovery period, data on symptoms, heart rhythm, heart rate (HR), BP, estimated workload in metabolic equivalents (METs) and ST-segment deviations were recorded [14]. BP response was classified either as normal or exaggerated BP response (EBPR, if SBP or DBP exceeded 200 mm Hg or 100 mm Hg at peak exercise) [15]. Chronotropic response was expressed by the chronotropic response index, which is a measure of maximal HR in relation to chronotropic reserve and is assessed by the following formula: $[(HR_{peak} - HR_{rest}) / (220 - \text{age in years}) - HR_{rest}] \times 100$ and normal values were considered ≥0.8 [16]. Duke treadmill score (DTS) was assessed based on the duration in minutes, maximal ST-segment deviation during or after exercise and angina symptoms using the following formula: duration of exercise in minutes − (5 × the maximal ST-segment deviation during or after exercise, in millimeters) − (4 × the treadmill angina index), where angina index was 0 for no angina, 1 for non-limiting angina, and 2 for exercise-limiting angina. Subjects with DTS ≥5 are considered low risk, those with score <5 and >−10 are intermediate risk whereas those with DTS ≤−10 are high risk [17].

Biochemical measurements

Blood was drawn using the standard venipuncture technique in the morning, in a dark quiet room, under controlled temperature (22–24 °C). All subjects abstained from food, tobacco, alcohol, and caffeine-containing drinks for at least 12 h before venipuncture. Blood samples were obtained before EST. After centrifugation at 2000 ×*g* at 4 °C for 15 min, plasma or serum was collected and stored at −80 °C until assayed. Routine chemical methods were used to determine serum lipid concentrations, glucose, and serum creatinine (Architect 1600 clinical chemistry analyzer, Abbott Diagnostics, Abbott Park, IL, USA). ADMA concentrations were measured by an enzyme-linked immunosorbent assay (DLD Diagnostika GmbH, Hamburg, Germany). The sensitivity of this particular ADMA kit is 0.05 μmol/L, and its specificity is 100% for ADMA, <0.02% for arginine, 1.0% for NMMA, and 1.2% for symmetrical DMA. High-sensitivity C-reactive protein (hsCRP) was measured using an immunonephelometric assay (CardioPhase hs CRP, Dade Behring, Marburg, Germany). The detection limit for this assay was 0.175 mg/L, with expected values 0–3 mg/L, while the coefficient of variation (CV) was 3.1%. Fibrinogen was measured using the Dade Fibrinogen Determination Reagents (Dade Behring, Marburg, Germany), with expected values 0.18–3.50 g/L and CV 1.6%. Creatinine clearance as an estimate of the glomerular filtration rate (calculated by the Cockroft–Gault formula) was calculated in all subjects.

Statistical analysis

All continuous variables are presented as mean ± standard deviation (SD) (or as median and interquartile range for non-normally distributed variables), whereas nominal variables are summarized as counts and percentages. The distribution of variables was analyzed with the Kolmogorov–Smirnov test. Differences between two groups were evaluated with Student's *t*-test for normally distributed continuous variables, while non-parametric tests (Mann–Whitney *U* test) were used for non-normally distributed ones. X^2 test with Yates correction for continuity was used to determine the relationship between categorical variables. Spearman's correlation test was used to assess correlations between continuous variables and corresponding plots were created. Multivariable linear regression analyses were applied to test for independent associations between ADMA levels and EST-derived parameters (namely DTS and achieved METs). Because ADMA distribution deviated significantly from the Gaussian, a model with ADMA entered as a binary variable was run to confirm that, despite the violation of the normality assumption for ADMA, the results of the multivariable analysis are sound. Two-sided p values less than 0.05 were considered statistically significant. Statistical analysis was performed using SPSS version 21.

Results

Table 1 summarizes the characteristics of the study population and their EST parameters. The study population consisted of 209 individuals (165 male, aged 58.1 ± 10.9 years). Over a quarter (26.8%) of the participants were obese (BMI ≥ 30 kg/m^2) and most of them (70%) reported a sedentary lifestyle, while 33% were active smokers.

The mean peak HR in EST was 152.5 ± 19.9 bpm and most of the participants had a satisfactory chronotropic response (~70% achieved chronotropic index ≥ 0.8). Their maximal systolic and diastolic BP at peak exercise was 173.2 ± 18.5 and 78.1 ± 8.4 mm Hg, respectively, and 20 individuals (9.5%) had an EBPR. Mean DTS was 5.97 ± 5.55 while mean METs and total exercise time achieved were 9.93 ± 2.99 and 8.32 ± 2.51 min, respectively.

A significant negative correlation was detected between ADMA levels and maximal exercise time (Spearman's rho = −0.511, p < 0.001), achieved METs (Spearman's rho = −0.510, p < 0.001) (Fig. 1A) and DTS (Spearman's rho = −0.373, p < 0.001) (Fig. 1B).

Moreover, subjects were subdivided into three groups according to achieved METs (<7 METs [n = 30], 7 to 9 METs [n = 65], and ≥10 METs [n = 114]). Subjects who exercised to ≥10 METs (n = 114) had lower ADMA levels than those who achieved <7 METs (n = 30)

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