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Asymmetric dimethylarginine is related to the predicted stroke risk in middle-aged Japanese men

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ABSTRACT

Background: Asymmetric dimethylarginine (ADMA) has recently been investigated as a risk marker for cardio- and cerebrovascular diseases. However, whether ADMA levels are related to the risk of stroke in the Japanese general population remains unclear.
Methods: We examined 769 Japanese men (mean age, 47 ± 5 years) undergoing health examinations. Each subject's ADMA level and various vascular risk factors were assessed; the predicted 10-year stroke risk was calculated using the point-based prediction model from the Japan Public Health Center Study.
Results: In a multiple linear regression analysis, age, body mass index, estimated glomerular filtration rate, and current smoking status were significant independent determinants of ADMA levels. A significant odds ratio (OR) for high predicted stroke risk (10-year risk ≥ 5%)was noted in the highest quartile of ADMA levels (OR, 2.47; 95% CI, 1.002–6.07), compared with the lowest quartile, after adjusting for potential confounding factors. A significant OR for high predicted stroke risk was also found for each standard deviation increment in ADMA level (adjusted OR, 1.46; 95% CI, 1.10–1.92).
Conclusion: Elevated ADMA levels were significantly associated with an increased predicted stroke risk, suggesting that measuring ADMA levels may be useful for identifying middle-aged Japanese men with a high risk of stroke.

1. Introduction

Stroke and its resulting complications are currently major causes of death and disability [1]. The incidence and mortality rates for stroke in Japan have been very high for most of the past century and have exceeded those for heart disease [2]. Moreover, the number of stroke patients in Japan is expected to continue to increase as the number of elderly individuals in the population increases. Therefore, in Japan, precise information is needed regarding the causes of stroke and the identification of subjects at high risk for stroke.

Researchers have focused on asymmetric dimethylarginine (ADMA) as a risk marker for vascular diseases because it contributes directly to endothelial dysfunction and atherosclerotic burden by inhibiting nitric oxide synthesis [3,4]. Previous studies have also reported that increased ADMA levels are predictive of cardiovascular outcomes in specific patient populations, such as in patients with end-stage renal disease [5] or in those with coronary artery disease [6,7]. Community-based cohort studies have also shown that increased ADMA levels are significantly associated with all-cause mortality [8], total cardiovascular deaths [9], and total cardiovascular events [9]. However, no significant relationship has been found between ADMA levels and incident stroke in any previous studies. Moreover, whether the ADMA level is related to the risk of first-time stroke in the Japanese general population remains unknown.

We previously showed that the ADMA levels in stroke patients were significantly higher than those in control subjects [10]. In the present study, we aimed to identify the independent determinants of ADMA levels among various stroke risk factors and to examine whether elevated ADMA levels are related to the predicted risk of stroke, as estimated by the risk prediction model from the Japan Public Health Center (JPHC) study [11], in middle-aged Japanese men without known cardio-cerebrovascular disease (CCVD).

2. Methods

2.1. Study population

This study was conducted during an annual medical checkup at a company that develops precision equipment in Kanagawa, Japan, in 2005. The overall potential study population included 1084 male, daytime employees involved in desk work who were 24–63 years of age and underwent health examinations. Of these individuals, 315 (29.0%)

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were excluded: 302 (27.8%) were under the age of 40 years (only subjects aged 40–69 years were included in the JPHC study), 9 (0.8%) had a history of CCVD, and 4 (0.4%) had atrial fibrillation. Ultimately, 769 subjects participated in the present study. The study protocol was approved by the ethics committee of Nippon Medical School, Tokyo, Japan, and all participants provided written informed consent.

2.2. Data collection

A self-reported questionnaire was used to collect data regarding the subjects' smoking habits, exercise habits, alcohol intake, family history of CCVD, and medical history, including information on prescription drug use. Regular exercise was defined as continuous exercise for at least 15 min, ≥3 days/week, for at least 1 year. Weekly alcohol intake was calculated by combining daily alcohol consumption and the frequency per week. Excess alcohol intake was defined as an alcohol intake of ≥300 g/week, based on the results of the JPHC study [12]. Anthropometric measurements and blood samplings were performed in a room maintained at 22 ± 2 °C. The systolic and diastolic blood pressures (BPs) were measured by well-trained staff members using the right arm of a seated subject, after at least 5 min of rest, with a mercury sphygmomanometer and the optimal cuff size for each subject's arm circumference. The first and fifth Korotkoff sounds were used to determine the systolic and diastolic BP, respectively. Hypertension was defined as a systolic BP ≥ 140 mm Hg, a diastolic BP ≥ 90 mm Hg, or the use of antihypertensive medications.

2.3. Biochemical measurements

Blood samples were obtained from the antecubital vein after overnight fasting. Diabetes was defined as a fasting plasma glucose level ≥126 mg/dL or the use of glucose-lowering medications. Standard enzymatic methods were used to measure the levels of total serum cholesterol, triglycerides, creatinine, and plasma glucose. Serum high-density lipoprotein (HDL) cholesterol levels were measured using direct methods and serum low-density lipoprotein (LDL) cholesterol levels were calculated using the Friedewald's formula in the 761 subjects with serum triglyceride levels <400 mg/dL [13]. Dyslipidemia was defined as total cholesterol levels ≥220 mg/dL, an HDL cholesterol level <40 mg/dL, a triglyceride level ≥150 mg/dL, or the use of lipid-lowering medications. After the aforementioned measurements were obtained, the residual sera were stored at −30 °C for 5 years and were used to measure the ADMA levels in the present study. ADMA levels were measured by high-performance liquid chromatography (HPLC) using orthophthalaldehyde for fluorescence determination (SRL, Tokyo, Japan) in accordance with a modified version of a previously described method [14]. In brief, HPLC was performed on a Hitachi L-7100 system (Hitachi, Tokyo, Japan) equipped with a FP-2025 fluorescence detector (Jasco, Tokyo, Japan) (excitation and emission wavelengths of 248 and 450 nm, respectively) and a PEGASIL octyldecyl silane column (diameter, 4.6 mm; length, 250 mm; Chemical Inspection and Testing Institute, Tokyo, Japan). Samples were eluted with 75 mmol/L aqueous sodium acetate buffer. The estimated glomerular filtration rate (eGFR) was calculated according to the following equation for Japanese men, as recommended by the Japanese Society of Nephrology [15]: eGFR (mL/min/1.73 m^2) = 193 × serum creatinine$^{-1.094}$ × age$^{-0.287}$. In the present study, chronic kidney disease (CKD) was categorized into 3 groups: G1 for eGFR ≥ 90, G2 for 60 ≤ eGFR ≤ 89, and G3–5 for eGFR ≤ 59 mL/min/1.73 m^2 [16].

2.4. Assessment of individual stroke risk level

An individual's risk level for future stroke onset was estimated by the point-based prediction model for total incident strokes for Japanese adults, which was recently developed on the basis of findings from the JPHC study [11]. The details of the algorithm for the risk prediction model are described in the original report. Briefly, 6 risk factors (age, sex, smoking status, body mass index [BMI], BP, and diabetes status) were used to calculate the predicted risk of stroke in the JPHC study. Age, BP, and BMI were categorized according to their values, and smoking status was classified as current smoker or non-smoker. The subjects were identified as presently taking antihypertensive medications or not. In addition, the subjects were identified as presently having diabetes or not. Finally, a corresponding point value was given to each risk factor, and the total score was used to estimate the individual's 10-year risk of stroke. In the present study, the predicted 10-year stroke risk was categorized as low (<1%), intermediate (1 to <5%), or high (≥5%) risk.

2.5. Statistical analysis

All statistical tests were performed using the SPSS software program (version 19.0.0, IBM, Armonk, NY, USA). Continuous variables, other than triglyceride levels, are expressed as means ± standard deviation (SD). Triglyceride levels were transformed to the common logarithm for statistical analysis and expressed as the geometric mean (95% confidence interval [CI]) because of their skewed distribution. Categorical data are expressed as the number of subjects (percent of total). The clinical characteristics for each ADMA level quartile were compared by an analysis of variance for continuous variables and the χ^2 test for categorical variables. The ADMA levels were compared by Student's *t*-test between 2 groups or by an analysis of variance followed by multiple comparisons with the Bonferroni correction for the 3 groups. Correlations between ADMA levels and other studied variables were evaluated by the Pearson's moment correlation coefficient. Factors with a P value < 0.20, as determined by the Pearson's correlation analysis, were included in a multiple linear regression analysis in order to identify independent determinants of the serum ADMA level. A logistic regression analysis was performed to obtain the odds ratio (OR) for a high predicted stroke risk in the second, third, and fourth ADMA level quartiles, relative to the lowest quartile, as well as in each quartile and each 1-SD increase in the ADMA level. All statistical tests were two-sided, and a P value < 0.05 was considered significant.

3. Results

The study subjects were divided into quartiles according to their ADMA levels (0.33–0.41 μmol/L, 0.42–0.44 μmol/L, 0.45–0.48 μmol/L, and 0.49–0.79 μmol/L, from the lowest to the highest quartile). The subjects' clinical characteristics are summarized in Table 1. The mean ADMA level for the entire subject population was 0.45 ± 0.05 μmol/L, and the mean age was 47 ± 5 years. Age, BMI, diastolic BP, HDL cholesterol level, triglyceride level, eGFR, and current smoking status were significantly different among the groups.

ADMA levels were significantly higher in the subjects with hypertension, dyslipidemia, a current smoking habit, or CKD than in subjects without these comorbidities (Table 2). A simple correlation analysis showed that ADMA levels were significantly correlated with age, BMI, diastolic BP, total cholesterol level, HDL cholesterol level, LDL cholesterol level, triglyceride level, serum creatinine level, eGFR, and current smoking status (Table 3). A multiple linear regression analysis indicated that age ($\beta = 0.10$, $P = 0.009$), BMI ($\beta = 0.10$, $P = 0.015$), eGFR ($\beta = -0.11$, $P = 0.002$), and current smoking status ($\beta = 0.18$, $P < 0.001$) were significant independent determinants of the ADMA level.

When the subjects were divided into groups based on low, intermediate, and high predicted stroke risk, according to the JPHC study risk prediction algorithm, ADMA levels were significantly higher in the high-predicted-risk group than in either the immediate- or low-predicted-risk groups (Fig. 1). The ADMA levels in the intermediate-