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Increased asymmetric dimethylarginine and enhanced inflammation are associated with impaired vascular reactivity in women with endometriosis

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ABSTRACT

Objective: Enhanced inflammatory responses which may inhibit vascular reactivity, are associated with endometriosis development. Asymmetric dimethylarginine (ADMA), an inhibitor of endogenous nitric oxide synthase, is also implicated in endothelial dysfunction. We aimed to determine whether plasma ADMA and systemic inflammation are associated with endothelial function in women with endometriosis.

Methods: We evaluated 41 women with and 28 women without endometriosis. Plasma levels of lipids and inflammatory markers such as high sensitive-C reactive protein (hs-CRP), serum amyloid protein A (SAA), and interleukin-6 (IL-6) were measured in the two groups. We also measured levels of ADMA and symmetric dimethylarginine (SDMA). High-resolution ultrasonography measured flow-mediated vasodilation (FMD) to assess vasodilatory responses.

Results: FMD was significantly lower in women with endometriosis compared to those without endometriosis ($8.39 \pm 0.43\%$ vs $10.79 \pm 0.54\%$, $P = 0.001$). While plasma lipid levels did not differ significantly between groups, levels of AMDA, but not SDMA, were significantly higher in women with endometriosis (409.7 ± 10.1 pmol/L vs 383.0 ± 48.3 pmol/L, $P = 0.04$). Inflammatory markers were also significantly higher in these women (hs-CRP: 1053.3 ± 252.0 ng/mL vs 272.0 ± 83.3 ng/mL, $P = 0.02$; SAA: 8.00 ± 1.53 μg/mL vs 3.82 ± 0.42 μg/mL, $P = 0.04$; IL-6: 2.73 ± 0.75 pg/mL vs 1.05 ± 0.60 pg/mL, $P = 0.04$). FMD was negatively correlated with plasma levels of ADMA ($r = -0.37$, $P = 0.01$) and log hs-CRP ($r = -0.34$, $P = 0.01$).

Conclusion: Increased plasma ADMA levels and enhanced inflammation are associated with inhibited endothelial function in women with endometriosis.

1. Introduction

Endometriosis, a common gynecological disorder characterized by growth of the endometrial gland and stroma outside the uterus, causes several symptoms such as dysmenorrhea, hypermenorrhea, and chronic abdominal pain. Endometriosis has been noted in 20–50% of patients undergoing gynecological laparotomies [1]. Endometriosis is diagnosed in women of reproductive age and is often accompanied by infertility in 5–10% of cases.

Endometriosis is accompanied by inflammation, and endometrial tissue can activate macrophages that express scavenger receptors and produce pro-inflammatory cytokines such as interleukin (IL)-6, IL-1, and tumor necrosis factor-alpha. Oxygen free radicals may promote the development of endometriosis and infertility by stimulating the growth and adhesion of endometrial cells in the peritoneal cavity [2].

Endothelial dysfunction is one of the earliest events during the course of atherosclerosis [3]. Nitric oxide (NO), which is produced during arginine oxidation by NO synthase in endothelial cells, has anti-atherosclerotic effects. Endothelium-dependent vasodilation, which is mediated through the release of vasodilators such as NO [4], may be affected by several factors. Vascular inflammation may decrease the production and activity of endothelium-derived NO [5]. Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO synthesis, also suppresses vascular NO production, while symmetric dimethylarginine (SDMA), a stereoisomer of ADMA, lacks NO synthesis inhibitory activity. Reduced NO activity induces leukocyte adhesion, thrombosis, and vasoconstriction, and accelerates the progression of atherosclerosis [6]. Moreover, plasma ADMA levels are associated with higher levels of C-reactive protein (CRP), an indicator of systemic inflammation [7], and are increased in subjects with atherosclerotic diseases [8].

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In this study, we measured endothelium-dependent vasodilation in women with and without endometriosis to determine whether vascular reactivity is inhibited in women with endometriosis. We also measured levels of ADMA and SDMA and markers of inflammation and analyzed their associations with endothelial function.

2. Methods

2.1. Subjects

We evaluated 41 Japanese women with American Fertility Society (AFS) stage III to IV ovarian endometriomas, and 28 Japanese women without endometriosis between April 1, 2009 and March 31, 2010. Surgeons completed operative records, which noted the presence or absence of endometriosis and the stage of endometriosis according to the revised AFS criteria. Exclusion criteria included the presence of diseases such as diabetes mellitus, hypertension, cardiovascular disease, dyslipidemia, systemic lupus erythematosus, and any other infection. Women who were smokers and/or used any medication were also excluded.

Endometriosis was diagnosed by laparoscopy, and control subjects underwent the same surgery for uterine myomas. Endometriosis was confirmed by histopathologic examination. Written informed consent was obtained from each subject before admission to the study. The study design was approved by the Ethics Committee of Aichi Medical University.

2.2. Laboratory analysis

Venous blood samples were obtained between 8:00 and 10:00 AM following a 12-h fasting period. Basal body temperature was used to determine menstrual cycle phase, with all women showing a biphasic basal body temperature pattern. Blood samples were drawn at the mid-follicular phase (day 7–10) of the menstrual cycle.

Levels of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL)-cholesterol were measured using enzymatic methods. Levels of high-density lipoprotein (HDL)-cholesterol were determined using similar methods after precipitation of apolipoprotein B-containing lipoproteins with sodium phosphotungstate in the presence of magnesium chloride. Levels of estradiol (E2) and follicle-stimulating hormone (FSH) were measured using radioimmunoassays. Plasma levels of CA-125, one of the markers for evaluating the severity of endometriosis, were measured by enzyme immunoassay. High sensitive (hs)-CRP levels were measured using the Behring Latex-Enhanced CRP assay on the Behring Nephelometer Analyzer System (Dade, Behring). Serum amyloid A protein (SAA) levels were determined by a latex agglutination turbidimetric immunoassay. Interleukin-6 (IL-6) levels were measured by chemiluminescent enzyme immunoassay.

2.3. ADMA and SDMA

Plasma ADMA and SDMA levels were measured by high-performance liquid chromatography (HPLC), using precolumn derivatization with o-phthalaldehyde (OPA). Plasma samples and internal standards were extracted and incubated with the OPA reagent (5.4 mg/mL OPA in borate buffer, pH 8.5 containing 0.4% mercaptoethanol). OPA derivatives of ADMA and SDMA were separated on a C6H5 column (Macherey and Nagel) with the fluorescence monitor set at an excitation wavelength of 340 nm and an emission wavelength of 455 nm [9].

Table 1
Subjects characteristics and concentrations of plasma lipids.

	Endometriosis	Control	*P*
Age (years)	40.1 ± 1.1	38.7 ± 1.0	0.2
BMI (kg/m^2)	19.8 ± 0.4	21.6 ± 0.3	0.2
Estradiol (pg/mL)	87.5 ± 12.1	76.4 ± 9.0	0.4
FSH (mIU/mL)	11.4 ± 1.6	18.1 ± 3.6	0.8
CA125 (U/mL)	91.5 ± 12.1	28.2 ± 4.4	0.04
Total cholesterol (mg/dL)	184.1 ± 4.7	188.9 ± 4.2	0.9
Triglyceride (mg/dL)	83.5 ± 5.5	83.9 ± 8.7	0.4
HDL cholesterol (mg/dL)	66.6 ± 1.8	62.1 ± 1.3	0.2
LDL cholesterol (mg/dL)	103.4 ± 4.1	113.4 ± 3.3	0.9

Data are expressed as mean ± SE; BMI, body mass index; FSH, follicle-stimulating hormone; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

2.4. Endothelial function

Patients rested in the supine position for 10 min before initiating the examinations. High-resolution Doppler ultrasonography equipment (Sonovista-Color model MEU-1582, Mochida) with a 10-MHz transducer was used to image the right brachial artery, and vasodilatory responses were measured. A nontortuous segment of the brachial artery was scanned longitudinally 4–5 cm above the elbow, where the clearest image could be obtained. When an adequate transducer position was determined, the skin was marked and the arm was kept in a constant position throughout the study. After baseline images of the brachial artery were obtained and arterial flow velocity was determined, a blood pressure cuff encircling the proximal portion of the arm was inflated to 250 mmHg for 5 min, and then rapidly deflated. Increased blood flow after cuff deflation, termed reactive hyperemia, results in flow-mediated vasodilation (FMD) [10]. Flow velocity in the artery was determined again, and 1 min after cuff deflation, the brachial artery was imaged. Blood pressure and heart rate were recorded during the investigation. The diameter of the brachial artery was measured from the anterior to posterior interface between the media and adventitia ("m" line) at the end of diastole, incident with the R wave on a continuously recorded electrocardiogram. Diameters for four cardiac cycles were determined from the images, and these measurements were averaged. All scans were recorded for later analysis. FMD was calculated as the percent increase in arterial diameter during hyperemia and was used as an index of endothelium-dependent vasodilation [10]. Blood flow was calculated by multiplying the time velocity integral of the angle-corrected Doppler flow signals by the heart rate and the mean cross-sectional vessel area. Intraobserver and interobserver variability for repeated measurements were 0.03 ± 0.02 and 0.05 ± 0.03 mm, respectively. Variability for FMD performed on 2 separate days was 2.1 ± 0.9%.

2.5. Statistical analysis

Data are expressed as mean ± standard error (SE). Differences in subject characteristics, plasma levels of E2, FSH, CA-125, lipids, inflammatory markers, ADMA and SDMA and FMD were analyzed by Student's unpaired *t*-test when there was normal distribution or by Mann–Whitney test when the parameters did not exhibit normal distribution. Regression lines were determined by the least squares method. $P < 0.05$ was considered significant.

3. Results

No significant differences between the endometriosis and control groups were found in age, body mass index (BMI), plasma E2 and FSH levels, and levels of TC, TG, LDL-C, and HDL-C. Plasma CA-125 was significantly elevated in the endometriosis group (Table 1).