BASIC SCIENCE: OBSTETRICS

Elevated asymmetric dimethylarginine concentrations precede clinical preeclampsia, but not pregnancies with small-for-gestational-age infants

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OBJECTIVE: The purpose of this study was to investigate maternal plasma concentrations of asymmetric dimethylarginine (ADMA) in mid pregnancy and at the time of disease in women who experience preeclampsia, compared with women with uncomplicated pregnancies and women with small-for-gestational-age infants.

STUDY DESIGN: Plasma samples were collected at mid-pregnancy and at the time of delivery from 31 women with uncomplicated pregnancies, from 12 women with small-for-gestational-age infants, and from 15 women with preeclampsia. ADMA and L-arginine concentrations were measured using high-pressure liquid chromatography.

RESULTS: Maternal ADMA concentrations were elevated at mid pregnancy and remained elevated at delivery in women who later experienced preeclampsia (0.45 ± 0.09 μmol/L) compared with women with uncomplicated pregnancies (0.34 ± 0.08 μmol/L; $P < .01$) and with women with small-for-gestational-age infants (0.33 ± 0.06 μmol/L; $P < .01$).

CONCLUSION: Maternal ADMA concentrations are higher in mid pregnancy in women who experience preeclampsia, compared with women with uncomplicated pregnancies and small-for-gestational-age infants. Elevated ADMA concentration before clinical onset of preeclampsia suggests a role of this nitric oxide synthase inhibitor in the pathophysiologic condition of preeclampsia.

Key words: arginine, asymmetric dimethylarginine, preeclampsia, pregnancy, small-for-gestational-age

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Pregnancy syndrome preeclampsia complicates approximately 3-7% of all pregnancies and continues to be a major contributor to maternal and neonatal morbidity and death.[1] The pathogenesis of preeclampsia is unclear; reduced placental perfusion and endothelial dysfunction appear to be central to the pathophysiologic condition of the syndrome.[2,3]

Nitric oxide, which is produced by endothelial cells, is proposed to contribute to vasodilation and the resulting decrease in vascular resistance that is observed during normal pregnancy.[4] Reduced nitric oxide production/availability has been postulated to contribute to the increased blood pressure, systemic vascular resistance, and sensitivity to pressors that are noted in preeclampsia.[4,5] Naturally occurring methylated arginine analogues are endogenous inhibitors of the 3 isoforms of nitric oxide synthase (NOS).[6,7] Asymmetric dimethylarginine (ADMA) and N-mono-methyl L-arginine (L-NMMA) compete with L-arginine, the substrate for NOS. However, plasma ADMA concentrations are more than 10-fold higher than NMMA and are therefore considered more relevant biologically. In contrast, the methylated arginine analogue symmetric dimethylarginine (SDMA) does not compete with L-arginine.

Maternal plasma ADMA concentration has been reported to be higher in women with preeclampsia.[8-10] In addition, Savvidou et al[11] reported that elevated ADMA concentrations preceded preeclampsia. ADMA concentration was higher at 23-25 weeks' gestation in women who later experienced preeclampsia; interestingly, ADMA concentration was also elevated in other women with abnormal uterine artery Doppler measurements, which included women who later had a pregnancy with a growth-restricted infant in the absence of preeclampsia. These data suggest that elevated plasma ADMA levels may not be limited to preeclampsia and may contribute to the pathophysiologic condi-

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tion of other pregnancy disorders with reduced placental perfusion. The objective of this study was to investigate maternal plasma ADMA concentrations at both mid pregnancy and at delivery in women who later experienced preeclampsia and in women with small-for-gestational-age (SGA) infants but without preeclampsia, compared with uncomplicated pregnancies.

MATERIALS AND METHODS

Study population

This was a nested case-control study from an ongoing investigation of preeclampsia at the University of Pittsburgh, Magee-Womens Hospital, and Magee-Womens Research Institute. The study was approved by the institutional review board, and informed consent was obtained from all subjects.

Subjects were recruited to the study at the time of admission to Magee-Womens Hospital for prenatal care. All subjects, both cases and control subjects, were nulliparous healthy women without known medical complications. Subjects were matched for parity and maternal age. The medical records of subjects were juried regarding pregnancy outcome by a group of physician and research investigators and consisted of uncomplicated control pregnancies (n = 31), subjects with SGA infants and without preeclampsia (n = 12), and subjects with preeclampsia (n = 15). Preeclampsia was diagnosed by the presence of gestational hypertension, proteinuria, and hyperuricemia beginning after week 20 of pregnancy with resolution of blood pressure and proteinuria postpartum. Gestational hypertension was defined as an absolute blood pressure of ≥140 mm Hg systolic and/or ≥90 mm Hg diastolic after 20 weeks' gestation. Proteinuria was defined as ≥300 mg per 24-hour urine collection, ≥2+ protein on voided urine sample, ≥1+ protein on catheterized urine specimen, or a protein-creatinine ratio of ≥0.3. Hyperuricemia was defined as plasma uric acid concentration ≥1 SD above reference values at the gestational age the sample was obtained (eg, term, >5.5 mg/dL).

SGA infants were defined by infant birthweight ≤10th percentile, after adjustment for gestational age, race, and gender, in an otherwise uncomplicated pregnancy. SGA infants with clinical or pathologic evidence of chronic intrauterine infection or chromosomal abnormalities were excluded from the study. Because birthweight percentile alone cannot distinguish constitutional smallness from failure to reach genetic growth potential (definition of intrauterine growth restriction [IUGR]), we attempted to identify IUGR infants as a subset of SGA infants with additional clinical evidence of fetal growth restriction that included birthweight percentile <5, asymmetric fetal growth profile, abnormal umbilical artery Doppler waveform where available, oligohydramnios, and/or elevated cord blood erythropoietin. The diagnosis of SGA and the subset of IUGR were determined at delivery. The umbilical artery systolic/diastolic ratio was considered abnormal if it was elevated above the 95th percentile for gestational age or if diastolic blood flow was either absent or reversed. Erythropoietin was measured in venous cord blood EDTA plasma samples by enzyme-linked immunosorbent assay in duplicate (catalog no. DEP00; R&D Systems, Minneapolis, MN); range, 2.5-200 mIU/mL; the interassay coefficient of variation was between 3.5% and 4.6%. Cord blood erythropoietin concentrations were considered to be elevated if they were ≥95th percentile for gestational age. Babies were considered to have asymmetric growth when the growth percentiles for baby weight were significantly less than for baby length and/or head circumference. SGA infants were considered IUGR if at least 2 of these 5 criteria were met. As shown in Table 1, 8 of the 12 SGA infants had clinical characteristics that were consistent with IUGR.

Blood samples

Maternal venous EDTA plasma samples were collected between 9-21 weeks' gestation during a routine prenatal visit and again at the time of admission for delivery. Samples were stored at -70°C for later analysis. These samples are part of a collection that were accumulated longitudinally over the last 10 years. With a frequency of preeclampsia of 3% by strict criteria, there are approximately 50 subjects with preeclampsia and 150 subjects with SGA. Power analysis based on previously published data[11] indicated that we would require at least 10 subjects with preeclampsia or SGA and 20 uncomplicated control pregnancies to observe a 35% increase in ADMA concentration with an alpha of 0.05 and 80% power. Samples were chosen for analysis on the basis of availability. Samples were stored on average 5.2 ± 1.1 years before ADMA quantitation; this duration was not different in the different diagnostic groups. Storage time has not been found to significantly affect the measurement of ADMA concentration in properly stored samples.

L-ADMA concentration and L-arginine quantitation

Maternal plasma ADMA and L-arginine concentrations were determined by high-pressure liquid chromatography as described by Teerlink et al.[12] In brief, samples were processed by solid phase extraction with Oasis MCX cation exchange SPE columns (30 mg, 1 mL; Waters Corp, Milford, MA). L-NMMA was used as an internal standard; 50 μL of plasma from patients was spiked with 50 μL of 1 μmol/L L-NMMA and 900 μL of phosphate-buffered saline solution, pH 7.4. Extracted plasma samples were injected onto a prepared high-pressure liquid chromatography system, and the amino acids were derivatized online with a Waters 717 plus autosampler. Separation of the amino acids was performed with a Symmetry C18 column (3.9 x 150 mm, 5 μm particle size; Waters Corp) with a Sentry Guard column (3.9 x 20 mm, 5 μm particle size; Waters Corp). Standard curves were generated with known quantities of ADMA, SDMA, L-arginine, and L-NMMA that were spiked into pooled control plasma. All samples were measured in duplicate, and the average was used for statistical analysis. The lower limit of detection is 0.01 μmol/L, with an interassay variability of <5%.