OBSTETRICS

Repeated measures of urinary oxidative stress biomarkers during pregnancy and preterm birth

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OBJECTIVE: The purpose of this study was to investigate oxidative stress as a mechanism of preterm birth in human subjects; we examined associations between urinary biomarkers of oxidative stress that were measured at multiple time points during pregnancy and preterm birth.

STUDY DESIGN: This nested case-control study included 130 mothers who delivered preterm and 352 mothers who delivered term who were originally recruited as part of an ongoing prospective birth cohort at Brigham and Women's Hospital. Two biomarkers that included 8-hydroxydeoxyguanosine (8-OHdG) and 8-isoprostane were measured in urine samples that were collected at up to 4 time points (median 10, 18, 26, and 35 weeks) during gestation.

RESULTS: Urinary concentrations of 8-isoprostane and 8-OHdG decreased and increased, respectively, as pregnancy progressed. Average levels of 8-isoprostane across pregnancy were associated with increased odds of spontaneous preterm birth (adjusted odds ratio, 6.25; 95% confidence interval, 2.86—13.7), and associations were strongest with levels measured later in pregnancy. Average levels of 8-OHdG were protective against overall preterm birth (adjusted odds ratio, 0.19; 95% confidence interval, 0.10—0.34), and there were no apparent differences in the protective effect in cases of spontaneous preterm birth compared with cases of placental origin. Odds ratios for overall preterm birth were more protective in association with urinary 8-OHdG concentrations that were measured early in pregnancy.

CONCLUSION: Maternal oxidative stress may be an important contributor to preterm birth, regardless of subtype and timing of exposure during pregnancy. The 2 biomarkers that were measured in the present study had opposite associations with preterm birth; an improved understanding of what each represents may help to identify more precisely important mechanisms in the pathway to preterm birth.

Key words: epidemiology, longitudinal, oxidative stress, preterm birth

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Preterm birth (PTB) is a leading cause of neonatal death and morbidity and occurs in >1 in 10 births in the United States.[1] Despite the significance of this public health problem, mechanisms for PTB are understood poorly.[2] Many risk factors, such as maternal age, race/ethnicity, and tobacco use, have been linked to PTB; however, underlying pathways for these relationships remain unclear. This may be attributable, in part, to the difficulties in the examination of PTB in a small animal model; PTB has been induced in rodents in some studies but generally requires gene knockouts or high doses of lipopolysaccharide injection, which makes translation of results to humans difficult.[3,4] Alternatively, screening of molecular biomarkers in humans may be helpful for the identification of mechanisms that contribute to PTB.

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Inflammation and infection at the maternal-fetal interface are among the most well-established precursors to PTB; justifiably much of the research on predictive biomarkers has focused on inflammatory cytokines and other indicators of inflammation.[5] Although the predictive value of these biomarkers for use by clinicians is limited, these data provide in vivo evidence for a causative role of inflammation for some cases of PTB.[5]

Oxidative stress, defined as an imbalance between antioxidant capacity and reactive oxygen species (ROS) generation, has received less attention in the study of PTB but may play a role through multiple pathways. A number of biomarkers exist to measure oxidative stress, and each one is formed through a unique mechanism and has the potential for differing downstream physiologic effects. In the present study, we measured

2 biomarkers (8-isoprostane and 8-hydroxydeoxyguanosine [8-OHdG]) in maternal urine samples that were collected from up to 4 time points during pregnancy. 8-isoprostane is a useful biomarker of oxidative stress in humans because of its stability, sensitivity to oxidant injury, and specificity to arachidonic acid peroxidation by ROS.[6] In addition to use in studies of adverse pregnancy outcomes, levels commonly are used as markers of oxidative damage in the study of cardiovascular disease.[7] 8-OHdG, an oxidized nucleoside that is released on repair of damaged DNA, is also used commonly as a marker of oxidative stress.[8] We examined changes in these markers longitudinally across pregnancy as well as their relationship with overall prematurity and subtypes with homogenous etiologies (spontaneous PTB and PTB of placental origin).

Materials and Methods

Study population

Subjects for this nested case-control study were selected from a longitudinal birth cohort that was designed to identify predictors of preeclampsia in pregnant women who delivered at Brigham and Women's Hospital in Boston, MA, from 2006-2008.[9] Participants were recruited early in pregnancy (median, 10 weeks of gestation); each woman had completed demographic and medical history questionnaires and provided informed consent at enrollment. Gestational age was assessed by last menstrual period with verification by first-trimester ultrasound scan. Participants additionally provided urine samples from up to a total of 4 visits across gestation (median, 10, 18, 26, and 35 weeks of gestation). At delivery, detailed birth outcome and infant data were recorded. From this parent population, we selected all 130 women who delivered live singleton infants preterm or at <37 weeks completed gestation and 352 random control subjects.[9] This study was approved by institutional review boards at the University of Michigan and Brigham and Women's Hospital.

Women who delivered preterm were divided into subgroups. Women at delivery with spontaneous preterm labor or preterm premature rupture of the membranes (pPROM) were combined into a single group, because previous research has shown that women with these delivery precursors have similar patterns of placental inflammation.[10] These births (n = 56) were considered spontaneous preterm. A second category included women whose preterm deliveries were determined to be a result of preeclampsia or intrauterine growth restriction, because these groups can be combined based on a similar causes of abnormal placentation.[10] These births (n = 35) were considered placental preterm for analysis. Assignment of pregnancy outcomes was based on the criteria of the American College of Obstetricians and Gynecologists and standard clinical practice. The remaining PTBs (n = 39) did not fall into either causal-based subset (eg, followed repeated cesarean delivery or other maternal/fetal complications not listed earlier) and were not examined separately because of the lack of a hypothesized shared mechanism. Nevertheless, they were included in the primary analysis because unknown mechanisms may link oxidative stress to PTB overall, and this was an exploratory analysis.

Oxidative stress biomarker analysis

Urine samples (n = 1678 samples; n = 482 women) were stored at −80°C after collection until the time that oxidative stress biomarkers were measured. Both 8-OHdG and total 8-isoprostane were measured by Cayman Chemical (Ann Arbor, MI). For total 8-isoprostane, urine samples were hydrolyzed to deconjugate 8-isoprostane esterified to phospholipids and were passed through affinity columns for purification. Eluted samples were dried and resuspended in a buffer before measurement with enzyme immunoassay (EIA). The lower limit of detection was 3.9 pg/mL. For 8-OHdG, samples were diluted directly into buffer without purification. Concentrations were also measured with EIA, with a detection limit of 10.3 pg/mL. Levels of biomarkers below the limit of detection were replaced with the limit of detection divided by the square root of 2.[11]

To account for urine dilution, specific gravity was measured in urine samples with the use of a digital handheld refractometer (Atago Co, Ltd, Tokyo, Japan). For examining biomarker distributions and variability, concentrations were corrected for specific gravity with the following formula: $OS_c = OS([1.015 - 1]/[SG - 1])$.[12] OS_c represents the corrected biomarker concentration; *OS* is the uncorrected urinary concentration; 1.015 is the median specific gravity in all samples; and *SG* is the specific gravity of the sample. For regression analyses uncorrected concentrations were modeled, and specific gravity was included as a covariate. Extremely concentrated (specific gravity, >1.04) samples were excluded from all analyses (n = 4). Distributions of concentrations for both raw and corrected biomarkers were log-normal and ln-transformed for data analysis.

Statistical analysis

Analysis was performed using R version 3.0.2. Differences in biomarker levels by visit were tested with the use of linear mixed models with random intercepts only to adjust for intraindividual correlation, with biomarker regressed on visit of sample collection. To depict nonlinear trends in levels across gestation, generalized additive mixed models (GAMM; *mgcv* package in R)[13] were created with the biomarker regressed on a smooth term for gestational age at urine sample collection, also with random intercepts only. Predicted values from GAMM models were plotted to show average trends over time. As an additional measure of variability in biomarker concentrations, we calculated intraclass correlation coefficients (ICCs), which represent the ratio of within-to-between individual variability.[14]

Associations between oxidative stress markers and PTB were examined for overall PTB and also for the subtypes defined earlier. Odds ratios (ORs) were calculated with a geometric average biomarker concentration for each subject from levels measured at visits 1-3. Visit 4 concentrations were excluded from the average because the proportion