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.

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

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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

TABLE 1
Distributions of demographic characteristics in study population

Characteristic	Birth, n (%)			
	Term	Preterm	Spontaneous preterm	Placental preterm
Race/ethnicity (missing = 0)				
White	207 (58.8)	75 (57.7)	30 (53.6)	20 (57.1)
African American	55 (15.6)	22 (16.9)	8 (14.3)	8 (22.9)
Other	90 (25.6)	33 (25.4)	18 (32.1)	7 (20.0)
Education (missing = 11)				
High school	47 (13.7)	21 (16.3)	7 (12.5)	10 (28.6)
Technical school	52 (15.2)	25 (19.4)	12 (21.4)	5 (14.3)
Junior college/some college	101 (29.5)	38 (29.5)	16 (28.6)	12 (34.3)
College graduate	142 (41.5)	45 (34.9)	21 (37.5)	8 (22.9)
Health insurance (missing = 12)				
Private/health maintenance organization/self-pay	277 (81.0)	108 (84.4)	48 (85.7)	26 (78.8)
Medicaid/supplemental security income/MassHealth	65 (19.0)	20 (15.6)	8 (14.3)	7 (21.2)
Body mass index (missing = 4)				
<25 kg/m^2	188 (54.0)	62 (47.7)	30 (53.6)	8 (22.9)[a]
25-30 kg/m^2	94 (27.0)	32 (24.6)	15 (26.8)	9 (25.7)[a]
≥ 30 kg/m^2	66 (19.0)	36 (27.7)	11 (19.6)	18 (51.4)[a]
Tobacco use (missing =6)				
Yes	20 (5.8)	11 (8.5)	4 (7.2)	7 (20.0)[a]
No	326 (94.2)	119 (91.5)	52 (92.8)	28 (80.0)[a]
Alcohol use (missing = 10)				
Yes	19 (5.5)	1 (0.8)[a]	0	0
No	326 (94.5)	126 (99.2)[a]	54 (100)	35 (100)
Parity				
Nulliparous	160 (45.5)	55 (42.3)	24 (42.9)	20 (57.1)
Parous	192 (54.5)	75 (57.7)	32 (57.1)	15 (42.9)
Gender				
Male	158 (44.9)	56 (43.1)	27 (48.2)	16 (45.7)
Female	194 (55.1)	74 (56.9)	29 (51.8)	19 (54.3)
Use of assisted reproductive technology				
Yes	33 (9.4)	12 (9.2)	4 (7.1)	4 (11.4)
No	319 (90.6)	118 (90.8)	42 (92.9)	31 (88.6)

[a] $P < .05$ for χ^2 test that compared distributions in overall, spontaneous, or placental preterm vs term groups.

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of cases with a measure at this time point was low, because many women had already delivered (100% of cases had samples available at visit 1; 91% available at visit 2; 86% available at visit 3; 51% available at visit 4). Crude models were adjusted for specific gravity; full models included covariates that were associated with oxidative stress biomarkers in bivariate analysis that had been linked to PTB in previous studies. Covariates that were considered included maternal age, race/ethnicity, education level, health insurance provider, body mass index (BMI) at visit 1, use of tobacco or alcohol during pregnancy, parity, gender of the infant, and use of assisted reproductive technology.

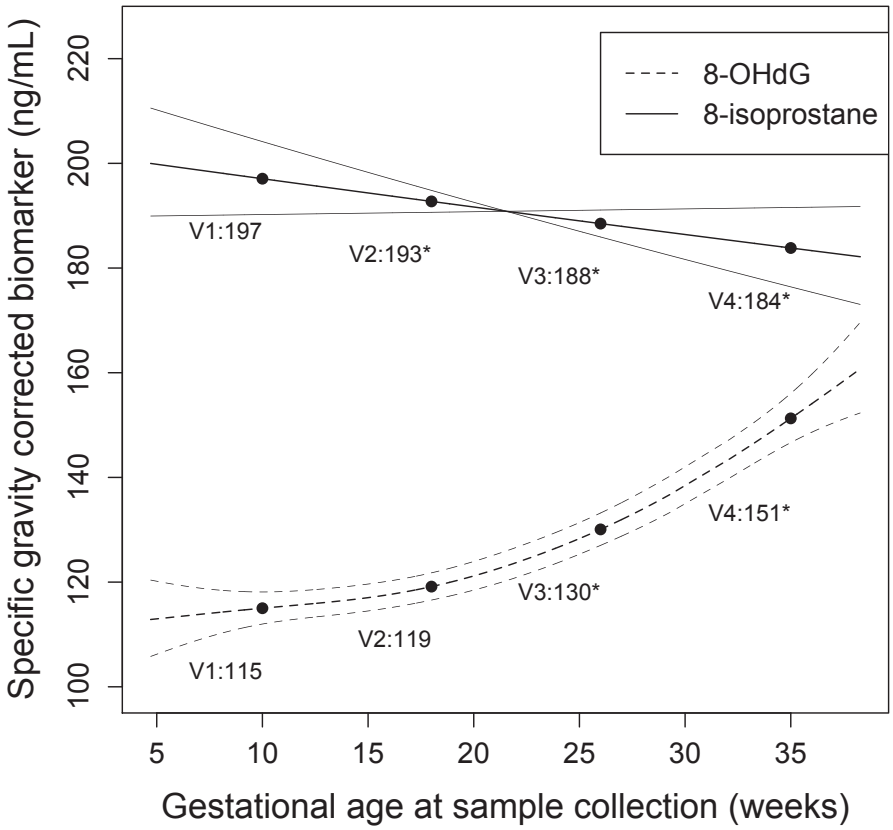
Additionally, individual logistic regression models that examined urinary oxidative stress biomarkers at each visit in relation to PTB were constructed to investigate whether oxidative stress at a particular time point was more predictive of preterm delivery. These models were created with the use of the same covariates that were included in the average models.

RESULTS

Characteristics of control subjects, cases, and spontaneous and placental cases separately are presented in Table 1. Overall, mothers were a median of 32.7 years of age at the first study visit and were predominantly white (58.5%), well-educated (38.8% with a college education or higher), and had private (79.9%) rather than public health insurance.[9] Few mothers used tobacco (6.4%) or alcohol (4.1%) during pregnancy. Most women were underweight to normal weight at visit 1 (BMI, <25 kg/m^2; 51.9%).[9] Gestational age at delivery ranged from 23.4–36.9 weeks for mothers who delivered preterm and from 37–42.7 weeks for mothers who delivered at term.

Specific gravity–corrected urinary concentrations of 8-isoprostane were higher in African American women (geometric mean, 274 pg/mL) and women of other race/ethnicity (geometric mean, 210 pg/mL) compared with white women (geometric mean, 165 pg/mL). The 8-isoprostane concentrations were also significantly higher in women with lower education levels, public health insurance and high BMI (≥ 30 kg/m^2) and in women who smoked during pregnancy. Maternal age at visit 1 was correlated inversely with 8-isoprostane average

FIGURE
Oxidative stress biomarker concentrations across pregnancy



Predicted values (95% confidence intervals) of specific gravity-corrected urinary oxidative stress biomarker concentrations are given by gestational age at sample collection from generalized additive models that were adjusted for random intercepts only. Point estimates for biomarker concentrations at visits 1 (*V1*; median, 10 weeks of gestation), 2 (*V2*; median, 15 weeks of gestation), 3 (*V3*; median, 26 weeks of gestation), and 4 (*V4*; median, 35 weeks of gestation) plotted for reference. The *asterisk* denotes significant difference ($P < .05$) in urinary biomarker concentration for the respective visit compared with visit 1 that was tested with the use of linear mixed model with random intercepts only. The *closed circles* indicate point estimates for biomarker concentrations at vists 1-4.

8-OHdG, 8-hydroxydeoxyguanosine.

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(Spearman $R = -0.23$; $P < .01$). For 8-OHdG, the only significant difference that was observed with categoric covariates was that women with private health insurance had lower levels (123 ng/mL) compared with women with public health insurance (146 ng/mL). As with 8-isoprostane, the 8-OHdG average was correlated inversely with maternal age (Spearman $R = -0.13$; $P < .01$).

Predicted values of specific gravity–corrected 8-OHdG and 8-isoprostane from GAMM are presented in the Figure. Levels of each biomarker at the median gestational age for each visit, when extracted from GAMM models, are plotted for reference. For 8-isoprostane, levels decreased slightly and linearly (indicated by crossing confidence intervals) across pregnancy; levels at visits 2, 3, and 4 were significantly lower than at visit 1. For 8-OHdG, levels increased in a quadratic form as gestation progressed, and levels at visit 3 and 4 were significantly higher than levels at visit 1. No differences in trends were observed in cases compared with control subjects.

Variability in concentrations also was captured with the use ICCs. An ICC ranges from 0–1, with 0 indicating no reproducibility in measures and 1 indicating perfect reproducibility.[14] ICCs for both urinary oxidative stress biomarkers were good; however, 8-OHdG was less reliable over time (ICC, 0.32; 95% confidence interval [CI], 0.27–0.38) compared with 8-isoprostane (ICC, 0.60, 95% CI, 0.56–0.64).[14] Correlations between biomarkers were weak but statistically significant at each study visit (Spearman $R = 0.10$-0.20; $P < .05$).

In logistic regression models of geometric average biomarker concentrations, 8-isoprostane was associated significantly with increased odds of overall PTB in crude models that were adjusted only for urinary specific gravity and in full models additionally adjusted for maternal age, race/ethnicity, education level, health insurance provider, and prepregnancy BMI. Tobacco use did not alter effect estimates. Adjusted odds ratios (aORs) of PTB in association with an interquartile range increase in urinary oxidative stress biomarker concentration are presented in Table 2. The association between 8-isoprostane and overall PTB was driven by associations with spontaneous PTB; analysis by subtypes showed large ORs for spontaneous PTB (aOR, 6.25; 95% CI, 2.86–13.7) and null associations for placental PTB (aOR, 0.94; 95% CI, 0.52–1.70). Cross-sectional logistic regression models by study visit (Table 3) showed that the odds for spontaneous PTB were lower early in pregnancy compared with subsequent visits.

Average 8-OHdG was associated strongly with reduced odds of overall PTB (aOR, 0.19; 95% CI, 0.10–0.34), and the relationship was similar in cases of spontaneous (aOR, 0.18; 95% CI, 0.09–0.40) and placental (aOR, 0.11; 95% CI, 0.04–0.32) PTB (Table 2). As with 8-isoprostane, effect estimates were similar in models that had been adjusted for specific gravity only and in models with all covariates. When ORs were calculated for each visit, we observed more protective ORs for spontaneous PTB when 8-OHdG levels were measured early in pregnancy. For placental PTB, the most protective OR was in association with levels that had

TABLE 2
Preterm birth in association with interquartile range increase in geometric average (visits 1-3) urinary oxidative stress biomarkers

	Model 1[a]			Model 2[b]		
Variable	Control cases, n	Odds ratio (95% confidence interval)	*P* value	Control cases, n	Odds ratio (95% confidence interval)	*P* value
Overall preterm birth						
8-OHdG	129,349	0.19 (0.11–0.34)	< .001	126,331	0.19 (0.10–0.34)	< .001
8-Isoprostane	129,349	2.17 (1.48–3.20)	< .001	126,331	2.22 (1.47–3.36)	< .001
Spontaneous preterm birth						
8-OHdG	56,349	0.21 (0.10–0.42)	< .001	56,331	0.18 (0.09–0.40)	< .001
8-Isoprostane	56,349	4.25 (2.21–8.15)	< .001	56,331	6.25 (2.86–13.7)	< .001
Placental preterm birth						
8-OHdG	35,349	0.17 (0.07–0.41)	< .001	33,331	0.11 (0.04–0.32)	< .001
8-Isoprostane	35,349	1.45 (0.79–2.66)	.24	33,331	0.94 (0.52–1.70)	.84

8-OHdG, 8-hydroxydeoxyguanosine.

[a] Adjusted for urinary specific gravity only; [b] Adjusted for urinary specific gravity, maternal age, race/ethnicity, education level, health insurance provider, and prepregnancy body mass index.

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been measured at visit 2 (median, 18 weeks of gestation).

COMMENT

There are plausible mechanisms for a role of oxidative stress in the pathway to PTB. ROS could (1) act as a precursor to inflammatory responses that may initiate parturition processes prematurely,[15] (2) damage collagen in the cervical stroma or fetal membranes resulting in pPROM,[16] or (3) cause apoptosis of the syncytiotrophoblast early in pregnancy by impairing spiral arteriole invasion of the myometrial wall and resulting in dysfunctional placentation.[17,18] Some previous evidence supports a relationship between oxidative stress and PTB in studies that measured maternal biomarkers.[19-22]

One prospective study (n = 508 women) examined 8-isoprostane levels in urine collected once from 5-16 weeks of gestation and observed a slight but significant trend for decreased gestational duration with increasing quintiles of 8-isoprostane.[21] Another study (n = 503 women) measured 8-isoprostane in maternal plasma that was collected once from 24-26 weeks of gestation and observed higher levels in mothers who experienced preeclampsia or had small-for-gestational-age infants.[19] Neither group observed an association with PTB, although the number of cases in each study was small (n = 48 and 37 women, respectively). Other studies have examined 8-isoprostane levels in amniotic fluid and found higher levels in women with term PROM[23] and pPROM.[20] Finally, 2 studies examined differences in maternal serum levels of malondialdehyde, which is another marker of lipid peroxidation, in mothers who delivered preterm compared with term. One case-control study observed higher levels of malondialdehyde in mothers who delivered preterm (n = 30 women) vs term (n = 30 women); however, in another cohort study (n = 200 women), no differences were observed.[24,25] Importantly, in both studies, blood samples were taken immediately before or after delivery.

Our results provide strong evidence for an association between urinary 8-isoprostane levels and spontaneous PTB in a large nested case-control study that examined urinary concentrations at multiple time points during pregnancy. We also examined urinary excretion patterns during pregnancy that have not been explored previously. Urinary concentrations decreased slightly across pregnancy but generally showed good intraindividual reproducibility, which suggests that future studies that will examine this marker may need to do so only at one time point. However, by measuring levels at multiple time points, we were able to illustrate that associations with spontaneous PTB were strongest when 8-isoprostane was measured at visits 2-4, compared with visit 1. This supports the hypothesis that oxidative stress levels later in pregnancy may cause PTB by weakening tissue integrity or initiating events that lead to spontaneous preterm labor.

The aforementioned study in which 8-isoprostane was measured in urine also examined 8-OHdG in urine samples and found slight but significant associations with decreased gestational length and birthweight, but not with PTB.[22] A previous smaller study by the same group (n = 18 cases of low birthweight, growth restriction, or preterm; n = 34 control subjects) reported significantly higher 8-OHdG concentrations measured in urine from the third trimester in cases compared with control subjects[22];

TABLE 3
Preterm birth in association with interquartile range increase in urinary oxidative stress biomarkers by study visit

	8-hydroxydeoxyguanosine			8-isoprostane		
Variable	Control cases, n	Odds ratio (95% confidence interval)	*P* value	Control cases, n	Odds ratio (95% confidence interval)	*P* value
Overall preterm birth						
Visit 1	123,326	0.25 (0.14–0.46)	< .01	123,326	1.72 (1.18–2.51)	.01
Visit 2	114,289	0.21 (0.10–0.42)	< .01	114,289	2.33 (1.44–3.77)	< .01
Visit 3	107,282	0.44 (0.24–0.81)	< .01	107,282	2.05 (1.31–3.19)	< .01
Visit 4	59,294	0.45 (0.23–0.90)	.02	59,294	1.76 (1.08–2.88)	.02
Spontaneous preterm birth						
Visit 1	54,326	0.26 (0.12–0.56)	< .01	54,326	2.72 (1.46–5.06)	< .01
Visit 2	52,289	0.30 (0.13–0.71)	< .01	52,289	7.10 (2.80–18.0)	< .01
Visit 3	47,282	0.33 (0.14–0.74)	< .01	47,282	4.45 (1.96–10.1)	< .01
Visit 4	24,294	0.75 (0.28–2.01)	.57	24,294	5.27 (1.82–15.3)	< .01
Placental preterm birth						
Visit 1	33,326	0.21 (0.07–0.58)	< .01	33,326	1.02 (0.62–1.69)	.93
Visit 2	29,289	0.12 (0.04–0.40)	< .01	29,289	1.19 (0.60–2.34)	.62
Visit 3	30,282	0.40 (0.17–0.94)	.04	30,282	0.79 (0.44–1.43)	.44
Visit 4	12,294	0.19 (0.04–0.85)	.03	12,294	0.59 (0.29–1.19)	.14

Models were adjusted for urinary specific gravity, maternal age, race/ethnicity, education level, health insurance provider, and prepregnancy body mass index.

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however, those results were not specific to PTB. In another study, where 8-OHdG was measured in urine collected once from 24-26 weeks of gestation, concentrations were higher in women who went on to deliver a low birthweight infant, but no association was observed with PTB.[20] Finally, one study that measured 8-OHdG in umbilical cord serum found elevated levels in samples from babies who were born both preterm and with low birthweight.[26]

Unexpectedly, we observed that women with higher urinary concentrations of 8-OHdG had reduced odds of PTB. This was true for both spontaneous and placental PTB; ORs were most protective for spontaneous PTB at visit 1 (median, 10 weeks of gestation) and for placental PTB at visit 2 (median, 18 weeks of gestation). We hypothesized that 8-OHdG, like 8-isoprostane, would be associated with increased odds of PTB because 8-OHdG is also used commonly as a marker of oxidative stress generated on repair of oxidative DNA damage.[8] Despite the common understanding that these markers both represent oxidative stress, levels of the 2 markers were correlated lowly with one another in this and a previous study,[21] which indicates that they are truly representative of different physiologic processes.

One explanation for the protective effect of 8-OHdG is that it is associated with unmeasured confounders in this population that strongly reduce the risk of PTB. For example, 8-OHdG levels increase with exercise[27] and ferritin levels.[21] Current literature shows that associations between these factors and PTB are generally null;[28,29] however, previous studies are not definitive, and these or other prenatal factors could be confounders that reduce the risk of PTB. An alternative hypothesis hinges on the fact that 8-OHdG tracks not only oxidative DNA damage but also the successful execution of the excision repair process.[8] It is possible that, in mothers in whom this process is impaired, there is increased risk of PTB. Under this scenario, 8-OHdG is not a marker of oxidative stress but instead the mother's ability to repair oxidative damage. This hypothesis has been suggested in another study in which women who delivered infants with birth defects had lower urinary 8-OHdG levels compared with mothers with normal infants.[21] This possibility deserves exploration in future research.

Consistent with previous studies, we observed that 8-OHdG levels were moderately variable within-women across gestation and that levels were higher in later, compared with early, pregnancy. Another study that examined urinary 8-OHdG concentrations at 6-8

weeks, 15-20 weeks, and 26-30 weeks of gestation also observed that levels were elevated later in pregnancy.[30] A third study that measured levels at 20 and 30 weeks of gestation and also at delivery showed that levels were higher in the urine that was collected at delivery compared with earlier in pregnancy, although these results could have been complicated by the laboring processes.[31]

Our study had several limitations. The measurement of 8-isoprostane in EIA liquid chromatography-mass spectrometry (LC/MS) may be preferable because of its improved specificity for the isomer. However, the correlation between levels measured with Cayman Chemical EIA and LC/MS is strong; specificity is improved in EIA after affinity purification, which was used in this study; and other isoprostane isomers may also be produced by ROS.[32] Also, LC/MS methods would have been cost-prohibitive; we would have been unable to analyze samples from as many subjects and time points, which was a major strength of our study. Urinary 8-OHdG analysis with EIA is preferred to LC/MS, and correlation between results from the 2 methods is generally strong.[8] Also, the study population was drawn from a prospective cohort of pregnant women who delivered at a major tertiary care center in the Boston area. When the results are extended to other populations, subject characteristics should be taken into account; these associations may not be consistent in women who are at high risk of having a PTB.

There were many advantages to our study design as well. We measured 8-isoprostane and 8-OHdG in maternal urine samples from repeated time points, which allowed us to create stable averages of maternal oxidative stress levels across pregnancy to investigate associations with PTB. With these repeated measures, we were also able to examine variability in levels across pregnancy and associations with PTB at individual exposure windows when vulnerability could differ. Additionally, we measured urinary rather than plasma concentrations of 8-isoprostane, which are preferred because plasma may be susceptible to autoxidation during storage, although urine samples, with low lipid concentrations, are not.[33] Last, this represents one of the largest studies to date to examine oxidative stress markers in relation to PTB, and it included by far the largest number of preterm cases.

In conclusion, we observed that increased 8-isoprostane levels were associated with increased odds of spontaneous, but not placental, PTB; ORs were highest in association with levels that were measured later in pregnancy. Conversely, we found that 8-OHdG levels were associated significantly with decreased odds of both spontaneous and placental preterm subtypes, particularly for measures that were taken early in pregnancy. These results were unexpected but could provide valuable information about heretofore unidentified factors that are preventative of PTB. Although the present study was not designed to identify predictive markers of PTB for the purposes of screening or prevention, these results may further the understanding of the role of oxidative stress in PTB. Better characterization of this relationship could lead to future treatment and prevention strategies. ■

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