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Title: Inflammatory and oxidative stress markers associated with decreased cervical length in pregnancy

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ABSTRACT

Problem: We assess whether inflammatory and oxidative stress markers early in pregnancy are associated with decreasing cervical length in the second trimester.

Methods of study: **This is a secondary analysis of a nested case-control study of preterm birth conducted at a tertiary care center from 2006-2008. Plasma inflammatory markers included: interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), and c-reactive protein (CRP); and urine oxidative stress markers included: 8-hydroxydeoxyguanosine (8-OHdG) and 8-Isoprostane, measured at two study visits (median 10 and 18 weeks of gestation). The primary outcome was cervical length<10th percentile measured between 16 to 24 weeks of gestation. Logistic regression models were used, adjusting for body mass index, age, race, parity, tobacco use, education, and gestational age at cervical length measurement.**

Results: Among 384 observed women, the10th percentile cervical length was 3.0 cm. **IL-10 levels were significantly higher among women with a cervical length<10th percentile compared to women with a longer cervix (mean IL-10: 95.5 vs. 25.8 pg/mL, p<0.01). Similarly, IL-6 levels were significantly higher among women with a cervical length<10th percentile (mean IL-6: 25.2 vs. 4.3 pg/mL, p<0.01).** After controlling for potential confounders, an increase in IL-10 was significantly associated with a cervical length<10th percentile at both 10 and 18 weeks (AOR: 1.74; 95% CI: 1.18-2.58); p=0.005). At 18 weeks only, IL-6 was also significantly associated with a cervical length<10th percentile (AOR: 1.54; 95% CI: 1.11-2.13; p=0.009). Other inflammatory biomarkers, including CRP, IL-1β, TNF-α, and oxidative stress biomarkers, 8-OHdG and 8-isoprostane, were not associated with cervical length.

Conclusions: There was a significant association between the cytokines IL-6 and IL-10 early in pregnancy and decreased cervical length, suggesting an **imbalance of immune regulation** could impact cervical length.

INTRODUCTION

Preterm birth is a significant public health problem, impacting over 10% of births worldwide[1]. The underlying mechanisms leading to preterm birth remain poorly understood, and in many instances, preterm birth is preceded by asymptomatic shortening of the uterine cervix[2]. Women with a short cervix in the second trimester are at a substantially increased risk of subsequent preterm birth[3]. Inflammation and infection are among the most well-established

precursors of preterm birth[4,5]. Prior studies have focused on whether inflammatory markers are associated with preterm birth, but data remain limited with regards to these biomarkers in relation to mid-trimester cervical length[6-9].

A number of biomarkers with known **physiologic effects exist to measure both oxidative stress and inflammation[10,11]. Pro-inflammatory cytokines are some of the most well-studied markers associated with preterm birth, most notably interleukin-6 (IL-6)[7,12]. Another protein, interleukin-10 (IL-10), has been shown to be both a marker of inflammation as well as of anti-inflammation[13]. C-reactive protein (CRP) is a strong marker of overall inflammation that has been shown to be associated with preterm birth. Oxidative stress, defined as an imbalance between antioxidant capacity and reactive oxygen species (ROS) generation, has received less attention, but has been implicated as playing a role in preterm birth[14]. 8-isoprostane is a useful biomarker of oxidative stress due to its stability, sensitivity to oxidant injury, and specificity to arachidonic acid peroxidation by ROS[15]. 8-hydroxydeoxyguanosine (8-OHdG), an oxidized nucleoside that is released in repair of damaged DNA, is also a commonly used marker of oxidative stress[16].** In prior analyses, we have analyzed oxidative stress and inflammatory cytokine biomarkers longitudinally during pregnancy in relation to preterm birth[14,17]; however, to date, there is limited data measuring these biomarkers at multiple time points in association with cervical length among a large population of pregnant women[6-9,18,1917]; and no studies have assessed oxidative stress markers in conjunction with cervical length.

In the current study, we assessed whether five biomarkers of inflammation, including interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), and c-reactive protein (CRP), and two biomarkers of oxidative stress, 8-hydroxydeoxyguanosine (8-OHdG) and 8-Isoprostane, collected at two time points early in pregnancy (median 10 and 18 weeks gestation, respectively) were associated with decreasing cervical length between 16-24 weeks of gestation. We hypothesized that inflammatory pathways early in pregnancy implicated in preterm labor could also be a precursor to decreasing cervical length.

MATERIALS AND METHODS

Study population: The current study is a secondary analysis of a case and control study of preterm birth conducted among women enrolled in the LIFECODES birth cohort at Brigham and Women's Hospital (Boston, MA) from 2006 to 2008[20]. Briefly, in that original analysis, all singleton cases of preterm delivery (N=130) in the birth cohort were randomly matched 1:3 to singleton term controls (N=352)[20]. Similar to prior analyses from this dataset investigating secondary research questions, our goal was to characterize the association between biomarkers of inflammation and oxidative stress and cervical length in a population that would be generalizable to the overall cohort. We utilized inverse probability weightings created from the probability of selection from the parent study population for cases (90.1%) and controls (33.9%)[21]. This adjustment negates the effect of oversampling preterm births and makes these results more generalizable to pregnant women in the base cohort population[22]. The study was approved by the Institutional Review Board at Brigham and Women's Hospital.

During the first trimester (median of 10 weeks gestation), enrolled women completed a demographic questionnaire, as well as supplied urine and blood samples for biomarker analysis. Participants provided plasma and urine samples at two time points generally before assessment of second trimester cervical length (median 10 and 18 weeks of gestation, respectively). All women had samples at 10 weeks and 333 (87%) of women had samples at 18 weeks either before or within 1 week of cervical length assessment. Samples were also taken at 2 study visits later in pregnancy (median 26 and 35 weeks), which were not used as a goal of the current analysis was to assess for a temporal relationship between biomarkers early in pregnancy and mid-trimester cervical length. Results of inflammation and oxidative stress biomarkers at all four time points in relation to preterm birth have been presented elsewhere[14,17].

Study variables: The following demographic variables were assessed at enrollment, including: age, race/ethnicity, education, parity, body mass index (BMI) (calculated by dividing a subject's weight by the square of her height, kg/m2), any tobacco use, alcohol use, and use of assisted-reproductive technology.

The primary exposures were measures of inflammation, namely IL-6, IL-10, CRP, IL-1β, and TNF-α; and of oxidative stress, 8-OHdG and 8-Isoprostane. For the primary analysis, each biomarker was defined as an interquartile range increase in the geometric average of levels measured at 10 and 18 weeks gestation (or of one measure if two were not available); and then

biomarkers were assessed separately at 10 and 18 weeks gestation. Distributions for both inflammation and oxidative stress biomarkers were natural log-transformed.

The primary outcome was cervical length, categorized into <10th percentile vs. ≥10th percentile. The distribution of cervical length in the current study was consistent with prior large observational studies assessing the relationship between cervical length and the risk of spontaneous preterm birth[23,24]. Cervical length in centimeters was assessed by ultrasound between 16 to 24 weeks at the time of fetal survey at a mean gestational age of 18.4 weeks (SD 1.43). Initial cervical length assessment was performed transabdominally with more detailed evaluation done transvaginally if clinically indicated or for either a cervical length <3.0 cm or an inability to adequately visualize both the internal and external os. For cases of transvaginal measurement, once the probe was introduced and the cervical canal visualized, it was then slightly withdrawn to avoid compressing the cervical stroma. Regardless of the probe type used, measurements were then made by tracing the distance between the visualized internal and external cervical os. This procedure was repeated with the shortest measurement being recorded. In the current study, a single trained sonographer retrospectively reviewed measurements on existing ultrasound images to ensure uniformity of sonographic measurements.

Oxidative stress and inflammatory biomarker analysis: Details about how **oxidative stress and inflammatory biomarkers** were measured and analyzed are described in detail in prior analyses[14,17]. Briefly, plasma samples were analyzed for inflammatory biomarkers at the University of Michigan Cancer Center Immunological Monitoring Core (Ann Arbor, MI). Cytokines were analyzed using Milliplex MAP High Sensitivity Human Cytokine Magnetic Bead Panel (EMD Millipore Corp., St. Charles, MO), **and for individual measures below the limit of detection (LOD) (0.128 pg/mL for all cytokines), values reported numerically were kept as is.** CRP was measured using a DuoSet enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN) **and the lower LOD was 10 pg/mL and upper LOD was 100 pg/mL.**

For oxidative stress markers, urine samples were stored at -80C after collection until measurement. Both 8-OHdG and 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 then dried and resuspended in a buffer before measurement with enzyme

immunoassay (EIA)[14]. **The LOD was 3.9 pg/mL.** For 8-OHdG, samples were diluted directly into buffer without purification, and concentrations were measured by EIA. **The LOD was 10.3 pg/mL.** Consistent with prior analyses, to account for urine dilution, specific gravity was measured in urine samples with a digital handheld refractometer (Atago Co, Ltd, Tokyo, Japan). Then to assess biomarker distributions and variability, concentrations of oxidative stress markers were corrected for specific gravity with the following formula: $OS_C = OS([1.015 – 1]/[SG – 1])$[25]. 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.

Statistical analysis: Distributions for both inflammation and oxidative stress biomarkers were ln-transformed (i.e. natural log transformed) for data analyses. The distribution of cervical lengths was tested for normality. As described above, we utilized inverse probability weightings created from the probability of selection from the parent study population in all unadjusted and adjusted regression models. Odds ratios for being <10th percentile for cervical length were calculated for an association with a geometric average biomarker concentration calculated from the two study time points at 10 and 18 weeks gestation. Univariate models were adjusted for specific gravity for oxidative stress markers as they were drawn from urine samples. Multivariable models adjusted for *a priori* covariates that have been linked to short cervical length in previous studies[14,17,26], which included: age, parity, race/ethnicity, education, tobacco use, BMI, and gestational age at assessment of cervical length. Additionally, separate multivariable logistic regression models at 10 and 18 weeks were constructed to investigate whether the association between biomarkers and cervical length differed in magnitude at a specific time point, adjusting for the same covariates above. We also conducted a sensitivity analysis at 18 weeks excluding 51 women (13%) who had biomarkers measured >1 week after cervical length to determine whether the above results held for a temporal association. All analyses were performed using STATA (STATACORP, version 10.0, College Station, TX).

RESULTS

Among the 482 women included in the original case-control study of preterm birth, 402 (83%) women had a cervical length assessed on second trimester ultrasound. Of those, 11 women had a cervical length assessed either before 16 weeks or after 24 weeks and were hence

excluded from the current analysis as this was outside the clinically proscribed window. An additional 7 women did not have biomarker measures from either study visit and were hence excluded. The current analysis is limited to the remaining 384 women (80%), and there were no statistically significant differences ($p<0.05$) between women in the current analysis compared to excluded women by maternal age, race, parity, BMI, tobacco use, or gestational age at delivery (data not shown).

Among the 384 pregnant women observed, the median age was 32 years (interquartile range, IQR 28.7-35.8), over half (56%) were white, over two thirds (67%) had completed a college education, and most women were multiparous (56%) (Table 1). Consistent with the original case-control study, 116 women (30%) delivered preterm (<37 weeks gestation). The median cervical length was 3.6 cm (IQR 3.2-4.1) and the 10^{th} percentile was 3.0 cm. There were no statistically significant differences between women with a cervical length $<10^{th}$ percentile compared to women with a longer cervical length.

IL-6 levels were markedly higher among women with a cervical length$<10^{th}$ percentile compared to women with a longer cervix (geometric mean IL-6: 25.2 vs. 4.3 pg/mL, $p<0.001$) (Table 2). Similarly, IL-10 levels were significantly higher among women with a cervical length$<10^{th}$ percentile (geometric mean IL-10: 95.5 vs. 25.8 pg/mL, $p=0.002$). Other biomarkers of inflammation and oxidative stress did not significantly differ by cervical length.

We present unadjusted and adjusted analyses of the association between cervical length $<10^{th}$ percentile in association with an interquartile range increase in the geometric average of inflammatory and oxidative stress biomarkers in Table 3. In unadjusted analysis, IL-10 was associated with an increased odds of cervical length $<10^{th}$ percentile (odds ratio, OR: 1.61; 95% CI: 1.12-2.30; $p=0.009$). In multivariable analyses of geometric average biomarker concentrations, after adjusting for maternal race, age, parity, education, BMI, tobacco use, and gestational age at cervical length assessment, IL-10 was significantly associated with an increased odds of cervical length $<10^{th}$ percentile (adjusted odd ratio, AOR: 1.74; 95% CI: 1.18-2.58); $p=0.005$). No significant associations were observed for the inflammatory markers, IL-6, IL-1β, TNF-α, and CRP, and the oxidative stress markers, 8-OHdG and 8-isoprostane.

When associations between biomarkers and cervical length $<10^{th}$ percentile were examined separately at 10 and 18 weeks gestation, IL-6 was also significantly associated with an increased odds of cervical length $<10^{th}$ percentile at 18 weeks (AOR: 1.54; 95% CI: 1.11-2.13;

p=0.009), but not at 10 weeks. Similar to the overall analysis, the association between IL-10 and cervical length <10th percentile persisted at 10 weeks (AOR: 1.42; 95% CI: 1.01-1.99; p=0.04) and 18 weeks (AOR: 1.78; 95% CI: 1.20-2.65); p=0.004).

We also conducted a sensitivity analysis at 18 weeks excluding 51 women (13%) who had biomarkers measured >1 week after cervical length to determine a temporal association, and the adjusted odds ratios between IL-6 (AOR: 1.67; 95% CI: 1.19-2.33; p=0.003) and IL-10 (AOR: 1.99; 95% CI: 1.32-2.98; p=0.001) and cervical length <10th percentile were even stronger. **Given the possibility for over correction in adjusted models between interrelated biomarkers and other variables in the final model, namely tobacco use and BMI, multivariable models were re-run adjusting for race, gestational age, and maternal age only, and these results were consistent with the overall findings.**

COMMENT

The current study measured inflammatory and oxidative stress biomarkers at two time points early in pregnancy before assessment of a shortened cervix among 384 pregnant women. We found a significant association between the cytokines IL-10 and IL-6 and a cervical length <10th percentile, after adjusting for maternal race, age, parity, education, BMI, tobacco use, and gestational age at cervical length assessment. Notably, other inflammatory biomarkers, including CRP, IL-1β, and TNF-α, and oxidative stress biomarkers, 8-OHdG and 8-Isoprostane, were not associated with cervical length. These results provide further evidence that **an imbalance of immune regulation** could impact cervical length, highlighting a critical gap in knowledge regarding cervical insufficiency.

We found that IL-6 was associated with a cervical length <10th percentile at only 18 weeks, while IL-10 was associated with a shortened cervix at both 10 and 18 weeks. This could be because IL-6 increased later in pregnancy, while IL-10 appeared unchanged throughout pregnancy[13,26,27]. Prior studies conducted among smaller populations of pregnant women have noted an inconsistent association between inflammatory markers and cervical length. Among 104 women at high risk for preterm birth, inflammatory markers, including elafin, surfactant protein-D, and IL-6, were not associated with cervical length, both of which were measured twice between 20-27 weeks[18]. In another study among 94 women, the inflammatory markers IL-1β, IL-8, TNF-α, and matrix metalloproteinase-8 measured in mid-trimester cervical fluid were

also not associated with cervical length or shortening between 20-24 weeks[28]. However, among 41 women with a previous preterm birth, shorter mid-trimester cervical length was associated with pro-inflammatory cytokines (IL-6, IL-10, IL-4, IL-13, and IL-1β)[8]. Another study conducted among 112 women with a previous preterm delivery found that only granulocyte-macrophage colony-stimulating factor and monocyte chemotactic protein-1 were increased among those women with a cervical length<25 mm, however the majority of inflammatory markers (including IL-8, IL-8, and TNF- α) were not[9]. Additionally, shortening of the cervix has been associated with the rate of rise of CRP among women with a short cervix from 20 to 33 weeks[19]. Differences in results across studies may be due to the relatively small sample sizes (on average 50-100 women), heterogeneity in study design, serum assay techniques, collection of varying inflammatory markers, gestational age at collection, and patient populations studied.

These findings suggest that cervical shortening may not simply be anatomic in origin, but a shift in the normal balance of cervical inflammation may trigger biochemical and immunologic changes that precede mid-trimester cervical shortening[8,29]. In a prior analysis of this dataset, IL-6, IL-10, as well as TNF- α were associated with an increased odds of preterm birth[4,17]. In some studies, lower levels of IL-10 have been associated with an increased risk of preterm birth, which may be because IL-10 is anti-inflammatory, while other studies have found no association[11,30,31]. However, higher levels of IL-10 associated with shortening of cervical length and pre-term birth could be a normal physiologic response to an anti-inflammatory state[27]. Both IL-6 and IL-10 have been shown to be highly correlated with each other in the current dataset[17], suggesting they may represent closely related physiologic processes. Future cellular and epidemiologic studies are needed to understand possible mechanisms that could explain these associations. Additionally, whether anti-inflammatory compounds that target anti-IL-6 and IL-10 activity upstream could be possible therapeutic interventions to prevent shortening of cervical length in pregnancy need to be studied[10].

In the current study, we did not find an association between two measures of oxidative stress, 8-isoprostance and 8-OHdG, with shortening of cervical length. Of note, in a prior analysis, we found that 8-isoprostane and 8-OHdG were associated with preterm birth, and these two oxidative stress markers were highest and associations with preterm birth were strongest later in pregnancy, compared to the current study which utilized these measures early in

pregnancy prior to shortening of cervical length[14,32,33]. However, not all studies have demonstrated an association between 8-isoprostane and 8-OHdG with preterm birth[34-36].

This study must be interpreted within the context of its design. An important limitation is that a transvaginal ultrasound was done only on those women in whom a cervical view could not be accurately obtained from transabdominal imaging at the time of the second trimester fetal survey. Of note, the cervical length at the 75th and 25th percentile (4.1 cm and 3.3 cm) in the current study were very similar to prior large observational studies assessing the relationship between cervical length and the risk of spontaneous preterm birth (4 cm and 3 cm)[23,24]. Though prior research has demonstrated conflicting results regarding the usefulness of transbadominal assessment of cervical length, recent studies comparing transvaginal to transabdominal sonography of the cervix suggest that transabdominal cervical length may be shorter than or similar to mean transvaginal cervical length[37,38]. These results will need to be replicated in women undergoing universal transvaginal screening for cervical length. **In the current analysis, biomarker values below the limit of detection (LOD) reported numerically were kept as is, and hence calculated effect estimates may be attenuated compared to either excluding these values or substituting with the LOD. However, it is unlikely these values below the LOD had a significant impact on the effect estimates given that biomarkers with values below the LOD were infrequent (<5%).** The current study utilized plasma and urinary biomarkers, and it is possible that markers closer to the maternal-fetal interface, such as amniotic or cervicovaginal fluid, would be more predictive of changes in cervical length[4]. Additionally, cervical length was assessed at a single time point during fetal anatomic survey, and hence we cannot comment on the contribution of these biomarkers to cervical length shortening over time relative to having a shorter cervix. This is a secondary analysis of nested case-control study of preterm birth, and these results may not be generalizable to all women. We utilized inverse probability weighting to adjust for the effect of oversampling preterm births so that these results would be more generalizable to pregnant women in the base cohort population.

A strength of the current study is we utilized biomarker measurements at two time points early in pregnancy before assessment of cervical length among a relatively large cohort of women, while many studies have relied on concurrent measurement of biomarkers and cervical length at one time point in smaller cohorts with <100 participants. To our knowledge, following a MEDLINE search, the current study is the first study to investigate whether oxidative stress

biomarkers are associated with cervical length and is the largest sample size to assess inflammatory markers at two time points in relation to cervical length.

In conclusion, we observed that higher levels of IL-6 and IL-10 early in pregnancy were associated with an increased risk of having a shorter cervix in the second trimester. A better understanding and characterization of the role of inflammatory and oxidative stress biomarkers in the shortening of cervical length could lead to future targeted prevention and treatment strategies earlier in the pathologic process before the onset of preterm labor.

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TABLES

Table 1. Characteristics of participants overall and by cervical length (N=384)*			
Characteristic	**Overall N (%) N=384**	**By cervical length****	
		<10th %tile N=41	**≥ 10th %tile N=343**
Age			
<25 years	40 (10.4)	6 (14.6)	34 (9.9)
25-≤35 years	227 (59.1)	25 (61.0)	202 (58.9)
>35 years	117 (30.5)	10 (24.4)	107 (31.2)
Race/ethnicity			
White	215 (56.0)	24 (58.5)	191 (55.7)
Black	67 (17.5)	5 (12.2)	62 (18.1)
Latina	55 (14.3)	8 (19.5)	47 (13.7)
Other or unknown	47 (12.2)	4 (9.8)	43 (12.5)
Education#			
High school or less	13 (3.4)	1 (2.6)	12 (3.6)
Junior college/some college	44 (11.7)	7 (18.0)	37 (11.0)
College graduate or greater	319 (84.8)	31 (79.5)	288 (85.5)
Parity			
0	171 (44.5)	20 (49.8)	151 (44.0)
1+	213 (55.5)	21 (51.2)	192 (56.0)
Tobacco use during pregnancy#	24 (6.3)	3 (7.5)	21 (6.2)
Alcohol use during pregnancy#	17 (4.5)	2 (5.0)	15 (4.4)
Use of assisted reproductive technology	35 (9.1)	5 (12.2)	30 (8.8)
Body mass index at enrollment			
<25 kg/m^2	198 (51.6)	26 (63.4)	172 (50.2)
25-30 kg/m^2	104 (27.1)	7 (17.1)	97 (28.3)
>30 kg/m^2	82 (21.3)	8 (19.5)	74 (21.6)
Gestational age at delivery			

<37 weeks	116 (30.2)	18 (43.9)	98 (28.6)
37-≤39 weeks	112 (29.1)	11 (26.8)	101 (29.5)
>39 weeks	156 (40.6)	12 (29.2)	144 (42.0)

Table 2. Distribution of inflammatory and oxidative stress biomarkers overall and by cervical length

*The above frequencies are of non-weighted results.

**There were no statistically significant differences ($p<0.05$) by cervical length for the above variables using chi-square statistic.

#Missing data for 2.1% of participants for education, 0.8% for tobacco use, and 1.6% for alcohol use.

Biomarkers	**Geometric Mean (SD)**	**By cervical length**		**p-value***
		length <10th %tile	**≥ 10th %tile**	
Inflammatory markers				
IL-6 (pg/mL)	6.69 (33.83)	25.18	4.33	<0.001
IL-10 (pg/mL)	33.62 (140.88)	95.47	25.79	0.002
CRP (ug/mL)	10.06 (12.28)	11.21	9.92	0.52
1L-IB (pg/mL)	1.13 (4.49)	0.68	1.19	0.49
TNFa (pg/mL)	3.62 (5.30)	3.18	3.67	0.57
Oxidative stress markers				
8 OHDG	135.24 (127.86)	135.22	135.87	0.97
8-isoprostane	272.59 (181.82)	263.27	273.82	0.74
*Student's t-test were used to compare geometric means between cervical length <10th %tile vs. ≥ 10th %tile.				

Table 3. Cervical length <10th percentile in association with an interquartile range increase in the geometric average of inflammatory and oxidative stress biomarkers		
Biomarkers	**Unadjusted Analysis OR (95% CI); p-value**	**Adjusted Analysis AOR (95% CI); p-value***
Inflammatory markers		
IL6	1.18 (0.78-1.79); 0.42	1.32 (0.91-1.91); 0.13
IL10	**1.61 (1.12-2.30); 0.009**	**1.74 (1.18-2.58); 0.005**
1L-IB	0.90 (0.60-1.35); 0.63	1.08 (0.65-1.79) 0.75
TNFa	0.61 (0.35-1.07); 0.08	0.58 (0.29-1.15); 0.12

CRP	0.98 (0.66-1.45); 0.94	1.09 (0.66-1.79); 0.72
Exposure		
Oxidative stress markers		
8 OHDG#	1.04 (0.49-2.19); 0.91	1.09 (0.47-2.50); 0.82
8-isoprostane#	1.01 (0.64-1.58); 0.96	0.95 (0.57-1.57); 0.84

Bolded results reflect statistically significant findings ($p<0.05$).

#Adjusted for urinary specific gravity

*Adjusted for the following variables in multivariate analysis: maternal race, age, parity, education, body mass index, tobacco use, and gestational age at cervical length assessment. Models adjusted for inverse probability weightings.

Table 4. Cervical length <10th percentile in association with a natural log unit increase in inflammatory and oxidative stress biomarkers at visit 1 and visit 2		
Biomarkers	**Visit 1**	**Visit 2**
	Adjusted Analysis AOR (95% CI); p-value*	**Adjusted Analysis AOR (95% CI); p-value***
Inflammatory markers		
IL-6	1.06 (0.75-1.51); 0.70	**1.54 (1.11-2.13); 0.009**

IL1-0	**1.42 (1.01-1.99); 0.04**	**1.78 (1.20-2.65); 0.004**
IL-1B	0.83 (0.61-1.14); 0.26	0.84 (0.55-1.27); 0.41
TNFa	0.81 (0.46-1.43); 0.47	0.65 (0.36-1.18); 0.16
CRP	1.01 (0.70-1.46); 0.92	1.12 (0.72-1.73); 0.61
Oxidative stress markers		
8 OHDG#	0.90 (0.57-1.44); 0.68	1.30 (0.51-3.31); 0.57
8-isoprostane#	1.04 (0.70-1.55); 0.81	0.89 (0.60-1.33); 0.57

Bolded results reflect statistically significant findings ($p<0.05$).

#Adjusted for urinary specific gravity

*Adjusted for the following variables in multivariate analysis: maternal race, age, parity, education, body mass index, tobacco use, and gestational age at cervical length assessment. Models adjusted for inverse probability weightings.