Phthalate metabolites and bisphenol-A in association with circulating angiogenic biomarkers across pregnancy

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ABSTRACT

Introduction: Phthalates and bisphenol-a (BPA) are endocrine disrupting compounds with widespread exposure that have been linked to adverse birth outcomes and developmental effects. We hypothesized that these associations may be mediated in part through altered placental development and function consequent to exposure. To investigate this question, we examined associations between plasma bio-markers of angiogenesis and urinary biomarkers of exposure to phthalates and bisphenol-a (BPA) measured at repeated time points across pregnancy.
Methods: We utilized a nested case-control population of 130 mothers who delivered preterm and 352 who delivered term from a prospective birth cohort. Placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) were measured in plasma samples collected from up to four visits during pregnancy (median 10, 18, 26, and 35 weeks). Phthalate metabolites and BPA were measured in urine samples collected at the same visits as indices of exposure.
Results: In linear mixed effects models adjusted for urine dilution and gestational age at sample collection, oxidized di-2-ethylhexyl phthalate (DEHP) metabolites were associated with decreases in PlGF as well as increases in the sFlt-1 to PlGF ratio. These results were slightly attenuated in fully adjusted models. Other phthalate metabolites did not show consistent relationships with either sFlt-1 or PlGF. BPA, however, was associated with increased sFlt-1 as well as the sFlt-1 to PlGF ratio in both crude and adjusted models.
Discussion: We observed associations between urinary DEHP metabolites and BPA and biomarkers of angiogenesis during pregnancy that may be indicative of disrupted placental development and/or function during gestation.

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1. Introduction

Phthalate diesters and bisphenol-A (BPA) are chemicals used in a wide variety of consumer products that humans worldwide come into contact with daily, making exposure a common occurrence. Both are classified as endocrine disrupting compounds because of their anti-androgenic and estrogenic properties, respectively. Much research on the human health effects of exposure to these com-pounds has focused on birth outcomes and fetal development, as they have been shown to cross the placental barrier [1]. Particularly, maternal exposures to phthalates and BPA during pregnancy have been linked to fetal growth parameters [2], preterm birth [3], and altered neurodevelopment in infants [4] and children [5].

Growth of the fetus during pregnancy is strongly tied to the successful implantation, development and functioning of the

placenta. Poor implantation in the first trimester can lead to inadequate villous perfusion and has been associated with conditions such as preeclampsia, intrauterine growth restriction, miscarriage, and stillbirth. On a more subtle level, function of the placenta and adequate substrate supply to the fetus may be important for optimal development, particularly of the brain [6]. While a number of these outcome measures have been studied in association with environmental exposures, few have examined biomarkers of the more subtle but nevertheless consequential changes.

Angiogenic markers have been explored in obstetric research as a means to identify and predict preeclampsia [7,8]. Placental growth factor (PlGF), a member of the vascular endothelial growth factor (VEGF) family, is a protein that plays a role in vascularization of the placenta early in pregnancy and is secreted in increasing quantities as pregnancy progresses [9]. Lower than average levels may indicate poor placental development and/or function. Soluble fms-like tyrosine kinase-1 (sFlt-1, also known as sVEGFR-1), binds to VEGF with consequent anti-angiogenic activity [10]. Thus sFlt-1 levels higher than average signal problematic placentation. In addition to observed associations with preeclampsia, these biomarkers may provide further evidence for more subtle complications in placental development or function. In the present study we examine the relationships between urinary phthalate metabolites or BPA and each of these plasma angiogenic biomarkers utilizing repeated measures across pregnancy.

2. Methods

2.1. Study population

The pregnant women in this nested case-control study were selected from an ongoing prospective cohort of women recruited early in gestation at Brigham and Women's Hospital in Boston, MA, between 2006 and 2008. As part of the parent study mothers provided demographic and anthropometric information and informed consent along with blood and urine samples at up to four study visits (median 10, 18, 26, and 35 weeks gestation) [11]. Gestational dating was based on last menstrual period with verification by first trimester ultrasound. Retrospectively, 130 women who delivered live, singleton, preterm births were selected from this population along with 352 random controls for a study designed to assess the relationship between phthalate exposure during pregnancy and preterm birth.

2.2. Urinary phthalate metabolite and BPA measurement

In 2011 maternal urine samples were extracted from $-80\,°C$ storage for analysis of 9 phthalate metabolites, including mono-2-ethylhexyl phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-benzyl phthalate (MBzP), mono-n-butyl phthalate (MBP), mono-isobutyl phthalate (MiBP), mono-ethyl phthalate (MEP), and mono-(3-carboxypentyl) phthalate (MCPP), as well as total (free plus conjugated) BPA. Analysis was performed by NSF International (Ann Arbor, MI) using high performance liquid chromatography and tandem mass spectrometry methods described in detail elsewhere [12]. Values below the limit of detection (LOD) were replaced with the LOD divided by the square root of 2 [13]. To adjust for urine dilution, specific gravity was measured in all samples at the time of analysis. For di-2-ethylhexyl phthalate (DEHP) metabolites (including MEHP, MEHHP, MEOHP, and MECPP), we additionally created a summed measured based on nanomolar concentrations for modeling purposes ($\sum$DEHP) [11].

2.3. Plasma angiogenic marker measurement

Biomarkers of angiogenic function were measured in plasma samples from all pregnant women in the parent cohort study [8]. Measurement was performed using ARCHITECT immunoassays by Abbott Laboratories (Abbott Park, IL). Total (free and bound) soluble fms-like tyrosine kinase-1 was detected from 0.10 to 150 ng/mL and free PlGF was detected from 1 to 1500 pg/mL. As with exposure measures, levels below the LOD were replaced with the LOD divided by the square root of 2 [13]. In addition to examining these biomarkers individually, we also examined a ratio of sFlt-1 to PlGF as an increase in this measure is thought to be a stronger predictor than either measure alone for placental disorders like preeclampsia [14].

2.4. Statistical analysis

All statistical analyses were performed using R version 3.0.2. The goal of the present analysis was to examine the relationship between urinary phthalate metabolites and BPA and circulating biomarkers of placental function in a generalizable population of pregnant women. This was a secondary goal to the primary aim of the study, to examine the relationship between urinary phthalate metabolites and preterm birth in a nested case-control study. Thus, for all statistical analyses, we utilized inverse probability weightings created from the probability of selection from the parent study population for cases (90.1 percent) and controls (33.9 percent) [15]. This adjustment negates the effect of oversampling preterm births and makes results generalizable to pregnant women in the base cohort population, regardless of birth outcome [16].

Angiogenic biomarker levels by demographic covariates and by study visit were examined using selected percentiles, and differences in levels between groups were tested using linear mixed models (LMM) with random intercepts and slopes. To examine the relationships between exposures and angiogenic biomarkers, we created LMM with one angiogenic biomarker predicted by one phthalate metabolite (or BPA) per model with adjustment for subject-specific random intercepts and slopes. Both exposure and outcome biomarkers were right skewed and hence natural log transformed for analysis. Crude models were created with adjustment for gestational age at sample collection and urinary specific gravity only. Full models additionally included covariates that were significantly associated with exposures and outcomes in bivariate analyses and that altered effect estimates by greater than 10 percent. Because of the log transformation of the exposure and outcome variables, beta estimates and standard errors obtained from regression models were converted to percent change in angiogenic biomarker in association with an interquartile range (IQR) increase in exposure for interpretability. Because this was an exploratory analysis, we did not correct for multiple comparisons.

As a sensitivity analysis, we also created models with an interaction term between the exposure and the visit of sample collection to investigate whether relationships were stronger at any particular time point during pregnancy.

3. Results

In the nested case-control population, there were 1611 time points where subjects (N = 457) had both exposure and angiogenic biomarker measurements. The present analysis was restricted to these measures. sFlt-1 was above the detection limit in all but 6 samples; PlGF was detected in all samples measured. Phthalate metabolites were highly detectable (most metabolites >99%) [3] and BPA was slightly less so (83%). Other characteristics of urinary phthalate metabolites and BPA, including distributions overall, by study visit, and by demographic characteristics are presented in detail elsewhere [11,15,17].

Selected percentiles of plasma angiogenic biomarkers measured in all samples by demographic characteristics are presented in Table 1. Based on LMM, PlGF levels were significantly higher in mothers who were African American or Other race/ethnicity compared to White, who had public compared to private health insurance providers, who had previously had a child, and who had a term pregnancy. Levels were significantly lower in mothers who were obese (BMI >30 kg/m^2) compared to mothers with lower BMI. Few differences by demographic characteristics were observed for sFlt-1, but mothers who were obese or parous had significantly lower plasma concentrations.

Distributions of sFlt-1 and PlGF in all samples and by study visit are presented in Table 2. Levels were similar to those observed in the parent population study [8]. For sFlt-1, concentrations were slightly but significantly higher at visits 2–4 compared to visit 1, and for PlGF levels were markedly increased later in pregnancy. Results from LMM adjusted for urinary specific gravity and gestational age only are presented in Table 3. An IQR increase in BPA was associated with a 7.08% increase in sFLT-1 (95% CI = 2.04, 12.4, p = 0.006) but no associations with phthalate metabolites were detected. For PlGF, inverse associations were observed for the oxidized DEHP metabolites MEHHP, MEOHP, and MECPP as well as $\sum$DEHP, and the associations for MECPP and $\sum$DEHP were statistically significant. However, a positive association was observed between PlGF and MEP. Associations between exposures and the ratio of sFlt-1/PlGF were stronger in magnitude than for either individual measure alone. A positive association was observed with DEHP metabolites and also with BPA.