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Season-dependent concentrations of urinary phthalate metabolites among Chinese pregnant women: Repeated measures analysis

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ABSTRACT

In utero exposure to phthalates may have adverse effects on pregnant women and their offsprings. Therefore, the exposure level of these substances among individuals, particularly among sensitive population, is of concern. The objective of the present study is to characterize urinary concentrations of phthalate metabolites at multiple time points during pregnancy in Chinese women. A total of 3455 pregnant women were included from Ma'anshan Birth Cohort in China. Spot urine samples in the morning (8:00–10:00) and questionnaires were obtained at three separate visits (approximately in 10, 26, and 34 gestational weeks). Seven phthalate metabolites from urine samples were analyzed, including monomethyl phthalate (MMP), monoethyl phthalate (MEP), monobutyl phthalate (MBP), mono benzyl phthalate (MBzP), mono-2-ethylhexyl phthalate (MEHP), mono (2-ethyl-5-oxohexyl) phthalate (MEOHP) and mono (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP). Geometric means of concentrations were ranged from 0.05 to 41.0 ng/mL for all the metabolites mentioned above. No individual exposure level was above the 95th percentiles for all the seven phthalates. On the three separate visits, 0.5%, 0.9% and 1.2% of the participants had coexposure to above the 75th percentiles for all metabolites. Taken these visits together, a total of 29 urine samples had concentrations above the 95th percentiles, while 3.0%–5.6% of urine levels were above 75th percentiles for at least one specific phthalate metabolite. We observed moderate intraclass correlation coefficients (ICCs) ranging from 0.44 to 0.56 for MBzP, MEHP and MEP, and lower ICCs, from 0.28 to 0.32, for MMP, MBP, MEOHP and MEHHP. Sampling season was associated with concentrations of all phthalate metabolites, showing heavier exposure was more likely to occur during summer. In summary, phthalate exposure is prevalent in Chinese pregnant women. However, throughout pregnancy coexposure to multiple phthalates at the upper percentile of exposure is infrequent. Mild to moderate temporal stability indicates that a single measurement in spot urine collected in the morning (8:00–10:00) seems not enough to describe throughout pregnancy phthalate exposure. Urinary levels vary by sampling seasons, which should be taken into consideration in future analyses.

1. Introduction

Phthalates are a large class of synthetic compounds and highly produced for over 50 years. Phthalates are extensively used as plasticizers in daily life polyvinyl chloride (PVC) products, such as food packaging materials, personal care products, children's toys, medical and pharmaceutical articles (Dodson et al., 2012; Wittassek et al., 2011). The amount of PVC products used worldwide annually is around 35 million tonnes, and about 40% of which is consumed in Asia where China accounts for the majority (Andrady and Neal, 2009). Phthalates can easily leach out into surrounding environment, because they are not chemically bound to the polymer of the products. Therefore, humans are constantly exposed to phthalates through ingestion, inhalation and dermal absorption (Wittassek et al., 2011). And their metabolites are detectable in various biospecimens, including urine, serum, breast milk, placental tissue, amniotic fluid and umbilical blood (Frederiksen et al., 2007; Hines et al., 2009; Jensen et al., 2012; Yan et al., 2009).

Phthalates are well-known environmental endocrine disruptors. It has been shown that in utero exposure to phthalates potentially increase adverse health risks in pregnant women and their offsprings. For instance, epidemiological studies revealed that in utero exposure to some phthalates was associated with pregnancy complications (e.g. spontaneous abortion and increased blood pressure) (Jukic et al., 2016; Mu et al., 2015; Toft et al., 2012; Werner et al., 2015), adverse birth outcomes (e.g. preterm birth, low birth weight and decreased

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birth length) (Ferguson et al., 2014; Huang et al., 2009; Latini et al., 2003; Meeker et al., 2009; Minatoya et al., 2017; Philippat et al., 2012; Wolff et al., 2008; Zhang et al., 2009), and impaired children growth (Valvi et al., 2015) and neurodevelopment (e.g. lower IQ, problems with attention, hyperactivity and poorer social communication) (Miodovnik et al., 2014). Furthermore, during the critical period of foetal/child development such as in utero period and the first few years of life, exposure to environmental chemicals will increase disease risk later in life (Barouki et al., 2012). Therefore, much more attention should be paid on exposure levels of phthalates in pregnant women.

Currently, the urinary concentrations of phthalate metabolites in pregnant women have been reported across several countries and areas. For instance, the Health Outcomes and Measures of the Environment study (HOME) in the United States (Yolton et al., 2011), the Polish Mother and Child Cohort study (REPRO_PL) in Poland (Polanska et al., 2014), the Mothers and Children's Environmental Health study (MOCEH) in Korea (Kim et al., 2011), the Early Life Exposure in Mexico to Environmental Toxicants cohort study (ELEMENT) (Téllez-Rojo et al., 2013), and the Taiwan Maternal and Infant Cohort study (Lien et al., 2015) have analyzed urinary concentrations of several phthalate metabolites in pregnant women. In the mainland of China, limited studies have investigated urinary phthalate levels in this population. The Healthy Baby Cohort (HBC) study measured six phthalate metabolites in 293 urine samples from pregnant women at delivery (Zhu et al., 2016). Our previous Ma'anshan Birth Cohort (MABC) study analyzed seven phthalate metabolites in 3103 urine samples of pregnant women during early pregnancy (5–14 weeks gestation) (Gao et al., 2016). However, the exposure level was described by single urine sample measurement in the two studies mentioned above. The aim of our present study is to further investigate the concentrations and profiles of phthalate metabolites using urine samples from multiple time points during pregnancy.

2. Method

2.1. Study participants and sampling

This study was based on MABC study. The cohort study was a population-based prospective study, aimed to evaluate the impacts of maternal phthalate exposure on adverse pregnancy outcomes, child health and development. In brief, 3474 pregnant women, who were >18 years old and <14 gestational weeks, were consecutively recruited from May 2013 to September, when they came to Ma'anshan Maternal and Child Health Care Center for their first antenatal care visit. Gestational weeks were calculated using last menstrual period data, or estimated by ultrasound if their menstruation were irregular (Carey et al., 2000). At baseline (mean 10 gestational weeks), questionnaires and spot urine samples in the morning (8:00–10:00; within 7 days after completing questionnaires) were obtained. Afterwards, women were followed up during their second (mean 26 gestational weeks) and third (mean 34 gestational weeks) trimesters of pregnancy to complete followed-up questionnaires and provide urine samples. Three questionnaires were used to collect demographic data and three urine samples were provided to measure phthalate metabolites.

Among 3474 women, 19 participants were excluded because of unavailable urine sample. 3455 women with at least one urinary sample were ultimately enrolled. Of these, 243 (7.0%), 350 (10.1%) and 2862 (82.4%) women provided one, two and three urine samples, respectively. The sampling seasons were defined as follows: spring (March–May); summer (June–August); fall (September–November); and winter (December–February) (Riala et al., 2009).

2.2. Phthalate metabolites analyses

Urine samples were analyzed with previous methodology with modifications (Gao et al., 2015; Wang et al., 2013). Solid phase extraction followed by high-performance liquid chromatography-tandem mass spectrometry (6410LC-MS, Agilent Technologies Co., Santa Clara, CA, USA) analysis was used. Seven phthalate metabolites, including monomethyl phthalate (MMP), monoethyl phthalate (MEP), monobutyl phthalate (MBP), mono benzyl phthalate (MBzP), mono-2-ethylhexyl phthalate (MEHP), mono (2-ethyl-5-oxohexyl) phthalate (MEOHP) and mono (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) were measured. Concentrations below the limit of detection (LOD) were assigned with LOD/√2. Creatinine adjustment was used to correct for urine dilution. Urine creatinine was measured using a creatinine assay kit through picric kinetic method (Jiancheng Bioengineering Institute, Nanjing, China).

2.3. Statistical analysis

Geometric means (GMs) and their 95% confidence intervals (95% CIs) were used to describe the distributions of both unadjusted and creatinine-adjusted concentrations of urinary phthalate metabolites. Using percentile values, the coexposure patterns of the seven phthalate metabolites at each study visit, and the single exposure to a specific metabolite across all the three visits were evaluated. Spearman's rank correlations were performed to assess relationships between unadjusted and creatinine-adjusted concentrations of phthalate metabolites. Intraclass correlation coefficients (ICCs) and their 95% CIs were used to estimate between- and within-person variability (i.e., temporal reliability). ICCs > 0.75 suggested strong reproducibility, 0.4 to 0.75 suggested moderate reproducibility, and <0.4 suggested weak reproducibility (Rosner, 2011). Finally, the metabolite concentrations of all urine samples were compared between categories of demographic variables and seasons by linear mixed models with random effects.

All statistical analyses were performed with SPSS software (Version 13.0), and $p < 0.05$ was considered statistically significant with the two-tailed tests.

3. Results

Demographic characteristics of the participants are shown in Table 1. The means of maternal age and pre-pregnancy BMI were 26.2 years and 20.9 kg/m^2, respectively. Most women were highly educated (79.4% above high school), primiparas (88.2%), urban residents (60.3%), employed (57.9%) and nondrinkers (92.0%). 73.6% of the women had household income above 2500 yuan per month capita. 47.6% of the participants were passive smokers. 90% of the 9477 investigations reported no application of sunscreen products. 83.2% reported spending more time indoors. The proportions of sample collections in four seasons were almost equal.

Statistical analyses were performed for both unadjusted and creatinine-adjusted concentrations of urinary phthalate metabolites, and the results were highly consistent (Spearman's r: 0.61–0.92, $p < 0.05$). The distributions of the seven phthalate concentrations were shown in Table 2. The detectable frequency ranged from 49.9% to 99.9%. The highest concentration was MBP, followed by MMP, MEOHP, MEHHP, MEP, MEHP and MBzP. Mann-Whitney U tests found significant differences of metabolites' concentrations across three visits (all $p < 0.001$, data not shown).

Figs. 1 and 2 showed the percentages of individuals whose exposures were greater than certain percentile of the exposure distribution. For all the seven metabolites, most women were exposed to concentrations below the 25th percentiles at each study visit (63%, 61% and 60%, respectively) (Fig. 1). At any visit, no individual was exposed to concentrations above the 95th percentiles (data not shown). Regarding the urines that were collected from the three visits, 45%–50% of all metabolite (except MBzP) concentrations were above the 25th percentile. 29 (0.8%) samples were above the 95th percentile for at least one specific phthalate metabolite (Fig. 2).