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Maternal exposure to bisphenol A and anogenital distance throughout infancy: A longitudinal study from Shanghai, China

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ABSTRACT

Background: Bisphenol A (BPA) is one of the most common endocrine-disrupting compounds (EDCs) with a ubiquitous presence. Both animal and human studies have reported the association between maternal exposure to BPA and anogenital distance (AGD) in offspring. However, the results are conflicting and the longitudinal effect is unknown. We aimed to examine the effect of maternal exposure to BPA on AGD in offspring in a longitudinal birth cohort from birth to 1 year of age.

Methods: BPA was assayed using urine samples collected at 12–16 gestational weeks from 982 pregnant participants who later delivered infants. Infants' AGDs (AGDap [anus-penis] and AGDas [anus-scrotum] for boys, AGDac [anus-clitoris] and AGDaf [anus-fourchette] for girls) were measured at birth, and at 6 and 12 months of age. Multiple linear regression analysis was conducted to examine the associations between maternal exposure to BPA and offspring's AGDs. Then generalized estimating equation (GEE) model was applied to make use of the repeated measurements of AGDs and examine the overall effect of maternal exposure to BPA.

Results: Compared to boys with undetected maternal BPA, those with detected BPA were more likely to have shorter AGDap and AGDas at 6 and 12 months. However, the differences were statistically significant for AGDap and AGDas only at 12 months (2.87 and 4.12 mm shorter, respectively). In GEE models, similar patterns were observed. Boys in the higher quartiles were more likely to have shorter AGDap and AGDas than those in the first quartile. However, statistically significant differences were only observed in boys in the third quartile. For girls, these associations were not observed regardless of the timing of measurements (at birth, 6 months and 12 months).

Conclusions: Maternal exposure to BPA was associated with shortened AGDap and AGDas in boys at age 12 months but not in girls, which suggests a gender specific effect of BPA exposure on offspring's development.

1. Introduction

Bisphenol A (BPA) is one of the most common endocrine-disrupting compounds (EDCs) with a ubiquitous presence in the environment. Human exposure to BPA can occur from dietary and nondietary sources (Michalowicz, 2014; Legeay and Faure, 2017; Geens et al., 2009; Skinner, 2016). Animal studies have shown that BPA acts as an endocrine disruptor, with estrogenic and anti-androgenic effects. BPA exposure was associated with increased risks of obesity (Lee et al., 2003), recurrent miscarriage (Rochester, 2013), male infertility (Chapin et al., 2008) and maternal and fetal thyroid functions (Chevrier et al., 2013). BPA can be detected in cord serum and neonatal urine, indicating that children may be exposed to BPA during the fetal period *via* maternal exposure. Because fetal development is a time period of rapid cell proliferation and differentiation, tissue development and organ growth, maternal exposure to BPA may be of particular concern. The associations between maternal exposure to BPA and offspring's health outcomes in humans have been reported, including increased risks of low birth weight, preterm birth, smaller size for gestational age and neurobehavioral disorders (Harris et al., 2017; Pergialiotis et al., 2017; Miao et al., 2011a,b). Maternal exposure to BPA was also found to have specific effects on reproductive health of offspring, including reproductive hormones, sexual maturation (Watkins et al., 2017), sperm concentration and motility (Hart et al., 2018).

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A sensitive marker used in the evaluation of reproductive toxicology of exogenous chemicals is anogenital distance (AGD), which refers to the distance from the anus to the genitals and is sexually dimorphic in humans (Hsieh et al., 2008). In rodents, AGD has been shown to be determined by fetal androgen action during early stages of fetal development (Welsh et al., 2008). Thus, reduced AGD may be indicative of insufficient testosterone during the early stages of development of reproductive organs (Welsh et al., 2008). In humans, shorter AGD has been observed among males with hypospadias and cryptorchidism (Cox et al., 2017; Jain and Singal, 2013). Moreover, AGD is considered to be a biomarker of various human reproductive disorders, such as poor sperm quality (Mendiola et al., 2011). It has been identified by US Environmental Protection Agency as one of the end-points for reproductive toxicity studies in humans (Hsieh et al., 2008; Jain and Singal, 2013; Eisenberg et al., 2012).

Effects of maternal exposure to BPA on AGD have been evaluated in animal studies. One study found that gestational plus lactation exposure to high dose BPA (0.25 mg/kg/d) decreased male AGD in rats (Christiansen et al., 2014). However, male AGD was not altered following gestational plus lactation exposure to low-dose BPA ($\leq$0.20 μg/kg/d) (Howdeshell et al., 2008; Kobayashi et al., 2010, 2012). To date, only a few studies have examined the relationship between maternal exposure to BPA and AGD in humans. An occupational cohort study from China revealed that maternal exposure to BPA during pregnancy was associated with shortened AGD in male offspring. There was also a dose-responsive relationship of increased exposure to BPA levels in pregnancy with greater magnitude of shortened AGD in male offspring (Miao et al., 2011a,b). However, the evidence from this study was limited by the high occupational exposure and smaller sample size. To our knowledge, only one birth cohort study has examined the relationship between maternal exposure to BPA and AGD in female infants at birth, and the results showed that higher BPA exposure was significantly associated with shorter AGD in female offspring. However, this study included only female newborns, and the longitudinal effect at a later stage is unknown (Barrett et al., 2017). In the present study, we therefore aimed to examine the effect of maternal exposure to BPA on AGD of offspring in a longitudinal birth cohort from birth to 1 year of age.

2. Methods

2.1. Study population and recruitment

We used data from the Shanghai-Minhang Birth Cohort Study (S-MBCS). Women who visited Minhang Maternal and Child Health Center in Shanghai for their first prenatal care at 12–16 gestational weeks were recruited from April to December 2012. The eligibility criteria were as followings: being native Chinese and residents of Shanghai, having no history of major chronic diseases, planning to deliver in this hospital and being willing to attend the specified interviews during pregnancy and after delivery. All mothers gave their written informed consent for themselves and their children. Information on women's demographic characteristics, medical history and lifestyle factors including smoking and drinking habits were obtained by face-to-face interview, using a structured questionnaire at enrollment. One single spot urine sample from each participating woman was collected at recruitment and frozen at −80 °C until they were shipped on ice to the laboratory.

2.2. BPA measurements

Total BPA concentration in urine (free plus conjugated species) was measured based on the modified high-performance liquid chromatography (HPLC) described by He et al. (2009). Briefly, urine samples were treated with phosphorous acid buffer/β-glucuronidase for hydrolyzation, and were subsequently extracted twice using ether (HPLC grade, Dikma). The supernatants were collected and evaporated with nitrogen gas. The residue was dissolved in 60% acetonitrile and analyzed using HPLC equipment. Blank samples were randomly included during each HPLC analysis and no BPA was detected. Inter-assay coefficients of variation for the BPA measurements was 2.2% to 6.9% and the intra-assay coefficients was 4.5% to 7.7%. The Limit of Detection (LOD) of BPA in our study was 0.31 μg/L, which was calculated with the method recommended by EPA (EPA, 2004) and comparable to the published research (Calafat et al., 2008). We calculated the LOD by the following formula: $t_{(n-1,\ a=0.99)}(S)$ (n represents the actual number of injections, S represents standard deviation). The corresponding detection concentration was calculated based on the standard curve. Adjustment for creatinine was performed as urine BPA level divided by creatinine level to avoid errors introduced by diluted or concentrated urine samples.

2.3. Follow-up and outcome measure

From medical birth records, we obtained information on birth outcome, including infants' gender, birth weight and gestational weeks. Infants were then followed at ages 6 and 12 months by a home visit. AGD was measured at birth and each follow-up visit. Information on feeding patterns was also collected by self-designed questionnaire. Two different measures of AGD were made using a Vernier caliper that reads in increments of 0.1 mm. In boys, AGDs were measured as AGDap (from the center of the anus to the cephalad insertion of the penis) and AGDas (from the center of the anus to the posterior base of the scrotum). In girls corresponding AGDs were measured as AGDac (from the center of the anus to the top of the clitoris) and AGDaf (from the center of the anus to the posterior fourchette) (Sathyanarayana et al., 2015). The examining physicians who conducted AGD measurements were blinded to the exposure status of the participants. In addition, two examiners took independent measurements on 15 male and 15 female newborns on the same day using the same method as described previously, and no statistically significant difference was noted (Xia et al., 2018).

2.4. Statistics analyses

Demographic characteristics, including maternal age at delivery, pre-pregnancy body mass index (BMI, weight in kilograms divided by squared height in meters), nationality, education, occupation, family income, maternal history of gynecological disease, menstrual regularity, prepregnancy passive smoking, paternal smoking, maternal intake of folic acid and vitamins in early pregnancy and infants' gender, birth weight, gestational weeks at birth, full and any breastfeeding duration were tabulated according to maternal urine BPA (detected, undetected). Chi-square test was used to compare the distributions of BPA exposure for categorical variables and *t*-tests for continuous variables. Multiple linear regression models were used to examine the associations between maternal exposure to BPA (detected, undetected) and offspring's AGDs at birth, 6 months and 12 months, respectively. The associations between maternal exposure to BPA and offspring's AGDs differences from birth to 12 months were also calculated (shown in Supplemental material, Table S2). In the models, maternal age, prepregnant BMI, gestational age at delivery, maternal education (senior high school or below, undergraduate, postgraduate), maternal passive smoking (Yes, No) and paternal smoking (Yes, No) (Xia et al., 2018; Liu et al., 2016) were adjusted for. In addition, birth weight was adjusted for when analyzing the association of AGD at birth, and body weight at the corresponding visit when analyzing the association at age 6 and 12 months, respectively (Salazar-Martinez et al., 2004; Swan et al., 2005). To evaluate the dose-responsive effect, children were further divided into four groups according to the quartiles of maternal creatinine-adjusted BPA level. Multiple linear regression models were then used to examine the associations between maternal BPA exposure (first quartile, second quartile, third quartile and fourth quartile of maternal BPA concentrations) and AGDs in offspring. In order to make