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Environmental exposure to arsenic and cadmium during pregnancy and fetal size: A longitudinal study in rural Bangladesh

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ABSTRACT

Prenatal exposures to arsenic (As) and cadmium (Cd) have been associated with decreased size at birth. We here studied associations of prenatal As and Cd exposures with multiple fetal size parameters measured by ultrasound in gestational week (GW) 14 and 30 in a population-based mother–child cohort in rural Bangladesh. We measured As ($n = 1929$) and Cd ($n = 1616$) in urine during pregnancy. In the longitudinal evaluation of combined exposure, urinary Cd (UCd) showed an inverted U-shaped association (turning-point 1.5 μg Cd/L) with all fetal size parameters, while UAs showed no significant association. Cross-sectional analyses indicated that associations with UCd were somewhat stronger in early gestation. Stratification indicated stronger associations between UCd and fetal size in girls than in boys, and in poorer than in richer families, while UAs was weakly associated with fetal size in boys. In conclusion, particularly Cd, but also As, appeared to influence fetal development in a sex-dependent manner.

1. Introduction

The periods of embryonic, fetal and infant development are generally susceptible to nutritional imbalance, stress, and environmental hazards. Exposure to toxic agents during early child development may cause intrauterine growth retardation and fetal loss, disease and disability during childhood, and probably also impaired health much later in life [1,2]. There is increasing evidence that adverse effects on gene expression, particularly of imprinted genes, may affect fetal development [3]. Arsenic (As) and cadmium (Cd) are two highly toxic metals that have been associated with several adverse health outcomes [4,5]. The preponderance of available toxicity data concerns long-term exposure in adults, while there is limited data concerning potential adverse effects of early-life exposure.

Hundreds of millions of people world-wide are at risk of being exposed to inorganic As through drinking water and food, especially rice [4,6]. The main source of Cd exposure, besides tobacco smoking, is food, in particular cereals, vegetables and seafood [7]. Arsenic readily crosses the placenta [8,9] and there is evidence from studies in Bangladesh, India, Taiwan and Chile that exposure during pregnancy may increase the risk of fetal loss, low birth weight, infant morbidity and mortality [10–16]. In contrast, the placenta exerts a partial barrier toward Cd, but cord blood concentrations do increase with increasing maternal exposure [17]. The accumulation of Cd in the placenta has been associated with impaired hormone production, as well as decreased transfer of nutrients to the fetus, both which may indirectly affect fetal growth and development [17,18].

We have recently shown that fairly low level exposure to both As and Cd during pregnancy is associated with smaller size at birth [14,19]. Possibly, the timing of the toxic insult during pregnancy is critical for the related adverse effects, as tissues and organs develop at specific windows during gestation. Therefore, the aim of the present study was to elucidate the effect of prenatal exposure to As and Cd on several fetal size parameters, measured by ultrasound, in early and later gestation.

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2. Materials and methods

2.1. Study area and subjects

This longitudinal study of fetal size is nested into a population-based food and micronutrient supplementation trial in pregnancy (Maternal and Infant Nutrition Interventions of Matlab, MINIMat), carried out in Matlab, a rural area without any known industrial activity located approximately 50 km southeast of Dhaka, the capital city in Bangladesh. In Matlab, the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) is running a health and demographic surveillance system with community health research workers (CHRWs) that visit every household to update family status on a monthly basis. The majority of the 220,000 inhabitants in Matlab drink water from local tube-wells. About 70% of these tube-wells contained ground-water with As concentrations above the WHO guideline value of 10 μg/L, when tested in 2002–2003 [20]. Wells with As concentrations above the local standard of 50 μg/L were painted red and people, in particular pregnant women and children, were recommended to use water from green-painted wells with lower concentrations.

The MINIMat trial enrolled 4436 women in early pregnancy from November 2001 to October 2003. Pregnancy was identified by urine test, on average in gestational week (GW) 8, at the monthly home visit by the CHRW. Women with positive pregnancy tests were invited to the nearest health care center for further assessment and screening for enrollment into the MINIMat trial. The eligibility criteria for the trial were viable fetus, gestational age <14 weeks by ultrasound examination, no severe maternal illnesses, and consent to participate. Enrolled women were randomly assigned to one of three micronutrient supplementations, beginning in GW 14: 30 mg iron and 400 μg folic acid, 60 mg iron and 400 μg folic acid, or a multiple micronutrient tablet with 15 different vitamins and minerals (vitamin A, vitamin D, vitamin E, vitamin C, vitamin B-1, vitamin B-2, niacin, vitamin B-6, vitamin B-12, folic acid, iron, zinc, copper, selenium, and iodine), all together with either early (GW 9) or usual (GW 16) food supplementation [21,22].

Out of the 4436 pregnancies in the MINIMat trial 3625 resulted in live births. In total, 3267 women had singleton live birth with anthropometry measurements at birth. All these women had ultrasound measurements throughout pregnancy. We excluded 269 women (8.2%) who had more than 21 days difference between their last menstrual period (LMP) and ultrasound estimated LMP at 8–13 weeks; leaving 2998 women. Maternal exposure to As and Cd was based on concentrations in urine. Urine collection at GW 8 was initiated first in February 2002 and continued up to July 2003. In total, 1885 women had both measurements of urinary arsenic (UAs) at GW 8 and ultrasound measures at GW 14, whereas 1929 women had both UAs at GW 30 and ultrasound measures at GW 30. Maternal urinary Cd (UCd) had previously been measured in 1616 out of the 1697 women recruited from February 2002 to January 2003 and who had a singleton birth with birth anthropometry [14,19].

The study was approved by the ethical committee at ICDDR,B in Dhaka, Bangladesh, and the regional ethical committee at the Karolinska Institutet, Stockholm, Sweden. Informed written consent was obtained from the study participant at the enrollment in the MINIMat trial. In case abnormalities were found during any of the ultrasound examinations we referred the pregnant women to the district level public hospitals or to the private clinics for specialist consultation and further care.

2.2. Exposure assessment

Spot-urine samples were collected in the women's homes at GW 8 (range 6–14 weeks) in case of a positive result of the urine-based pregnancy test, and at the health clinics in GW 30 (range 24–40 weeks). Urine was transferred to 20 mL acid-washed polyethylene containers (Zinsser Analytic GmbH, Germany) [23], and stored at −70 °C at the central hospital in Matlab until being transported frozen to the Karolinska Institutet, Sweden, for analyses of As and Cd. Urinary concentrations of both As and Cd were adjusted to the mean specific gravity (1.012 g/mL) to compensate for the variation in urine dilution [24].

We measured the sum of urinary metabolites of inorganic As (hereafter called UAs) using hydride generation atomic absorption spectrophotometry (HG-AAS) as previously described [23,25]. UAs is a measure of the current exposure (whole body half-time about 3–4 days), but exposure via drinking water is usually associated with fairly constant intake levels. However, as our parallel project on screening of As concentrations in tube-wells in Matlab encouraged pregnant women to collect water from nearby tube-wells with less than 50 μg/L [20], we measured As in urine collected in both GW 8 and 30. The limit of detection (LOD) was 1.30 ± 0.27 μg/L, and only one sample was below LOD. For quality control purposes, certified reference materials (NIST 2670 urine HL with certified 480 ± 100 μg As/L and NIST 1643d water with certified 56.02 ± 0.73 μg As/L) were included in each analytical run. Because certified reference materials exist only for total As, we also compared the results with those of HPLC-HG-ICPMS, HPLC-HG-AFS and total UAs, which consistently showed very good agreement between our methods [26,27].

UCd is a biomarker of long-term exposure, reflecting the accumulated fraction in the kidney where Cd is efficiently retained [5]. UCd concentrations have previously been shown to be rather constant throughout pregnancy [28], and were therefore only measured in GW 8. UCd was measured by inductively coupled plasma mass spectrometry (ICPMS; 7500ce, Agilent Technologies, Japan) [19]. The LOD was <0.02 μg/L and no samples were below this concentration. For quality control we analyzed two commercial control materials (Seronorm™ Trace Elements Urine Blank, REF 201305, LOT OK4636; recommended value 0.31 ± 0.05 μg Cd/L and Seronorm™ Trace Elements Urine, REF 201205, LOT NO2525; recommended value 5.06 ± 0.22 μg Cd/L).

2.3. Fetal size parameters

The first ultrasound measurement was conducted at enrolment (GW 8–13) to determine gestational age. It included measurement of crown-rump length or, for larger fetuses, biparietal diameter (BPD). Thereafter, all women were invited for additional ultrasound examinations at GW 14 and 30. The women received a formal invitation to the health care center one week before the scheduled gestational week. In case the women were not able to attend at the scheduled date, they were asked to come either just before or after, and if this was not possible they were asked to attend the health care center sometime before the next scheduled visit. In this manner, all women were given maximum opportunity to participate in all the examinations, and the study obtained repeated longitudinal measurement data spreading throughout gestation (GW 14: range 11–18; GW 30: range 24–40).

The examinations were performed with portable ultrasound machines (SSA 320A Justavision-200, Toshiba, Tokyo, Japan with 3.5 MHz standard convex probe) together with ultrasound thermal printers. Each examination took approximately 10 min and included the following five measurements: BPD, occipito-frontal diameter (OFD), head circumference (HC), abdominal circumference (AC), and femur length (FL). We included three different measures of the head; HC which is commonly used in clinical practice as a measure of head development, as well as BPD and OFD which also reflect the deposition of bone minerals to form thicker posterior bone. All three parameters were measured directly by ellipsoid technique, according to the manufactures instructions. Before initiation of field data collection, a qualified ultrasound expert trained nine sonographers (paramedics) and one supervisor (medical doctor). The measurements were standardized to assure acceptable intra- and inter-observer variability, as described previously [29]. Measurement quality was documented by re-examination of 3% of the total cases by a doctor who did not have access to the previous results and without notification to the study sonographers.

We used fetal size reference values from the United Kingdom [30–33] to convert our fetal size parameters into *z*-scores. These references were chosen because they provide model-derived fetal biometry values for each gestational week with standard deviation for all fetal size parameters, which is usually not the case for other published international references on fetal size.

2.4. Other parameters

We measured weight (accurate to 10 g) and length at birth (accurate to 1 mm) [14]. Gestational age at birth was calculated by subtracting the date of the mothers reported LMP from the date of birth, and if the women had no LMP date due to amenorrhea prior to pregnancy, we used the date estimated by the ultrasound measurement [14]. We recorded maternal weight and height at enrolment. The families' socio-economic status (SES) was estimated by a wealth index, constructed from information on household assets [34].

2.5. Statistical methods

Statistical analyses were performed with STATA 11 (StataCorp LP, USA). A *p*-value <0.05 was considered statistically significant. Bivariate associations between maternal UAs (GW 8 and 30), UCd (GW 8) and the different fetal size parameters (GW 14 and 30) were assessed with Spearman's rank correlation (r_s). We also visually evaluated scatter plots of all the outcomes against exposure measures and examined the associations with Lowess moving-average fitted curves.

We used linear regression analyses to elucidate associations of maternal UAs and UCd with fetal size separately. UAs concentrations were $\log_2$-transformed because it provided the best fit for the data, as most of the associations were found to be non-linear (see Supplemental material, Fig. 1), and it fulfilled the requirements of normally distributed residuals in the unadjusted models. The visual examination with Lowess moving-average fitted curves also indicated non-linear associations between UCd and the different fetal size parameters (see Supplemental material, Fig. 2). We tested if there was a significant change in the slopes, using a linear spline model. The above described analyses were repeated, adjusting for variables that were or had previously been associated with the exposures and/or outcomes (maternal BMI at enrollment (GW 8), SES, birth order, and fetal sex). Because of multi-colinearity we did not include maternal education or maternal age in the models (education and SES $r_s = 0.62$, $p < 0.001$; age and birth order $r_s = 0.80$, $p < 0.001$). Instead, we included SES and birth order as these variables, in general, explained the variation more clearly. To adjust for multiple testing on a set of correlated outcomes (fetal size parameters) we conducted seemingly unrelated regression analyses. The obtained model estimates were then compared with the estimates from the linear regression analysis described above.

Because data on fetal size were collected longitudinally throughout pregnancy, we modeled the mean changes in these parameters over time using linear mixed effect models with random intercept using maximum likelihood estimation. Fetal size parameters were regressed against GW at measurement (estimated by