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Low level arsenic contaminated water consumption and birth outcomes in Romania—An exploratory study

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ABSTRACT

Women are exposed to drinking water with low arsenic concentrations (<10.0 μg/L) worldwide, yet little work has been done to assess the risk. To begin to address this data gap, we conducted an exploratory study of birth outcomes in Timis County, Romania. We prospectively followed 122 women with singleton deliveries, for whom we constructed individual exposure indicators using self-reported water consumption weighted by arsenic measured in drinking water sources. There were no overall confounder-adjusted effects for arsenic exposure on birth outcomes. Yet, higher average arsenic (10 μg/L) was associated with a −2.45 lower birth weight Z-score ($P = 0.021$) and a −1.17 shorter birth length Z-score ($P = 0.029$) among smokers. Higher average iAs (10 μg/L) was also associated with smaller ponderal index in boys ($P = 0.023$). Our results suggest smoking may potentiate an otherwise benign arsenic exposure. A larger, more definitive biomarker-based study is needed to investigate the potential risks in conjunction with smoking.

1. Introduction

The extensive distribution of inorganic arsenic (iAs) in the earth's crust leads to local and regional contamination of ground drinking water supplies and widespread human exposure [1,2]. Following ingestion, iAs is well-absorbed by the mammalian gastrointestinal tract, and myriad adverse health effects have been characterized in association with chronic exposure [3,4]. Although less investigated to date, concern is growing with respect to an increased risk for adverse birth outcomes including preterm delivery and smaller neonatal size [3,5,6].

Inorganic arsenic crosses the human placenta, and accumulates in a developing fetus [7,8]. It is not only genotoxic [9,10] and anti-estrogenic [11,12], but studies also indicate a generalized inflammatory response to long-term iAs exposure [13], and increased inflammation in newborns following exposure *in utero* [14,15]; inflammation is a strong predictor of preterm delivery and fetal growth restriction [16]. Experimental and observational evidence also suggests that iAs accumulates in and disrupts placental function [15,17]. Altered vasculogenesis leading to dysplastic placental development has been reported *in vivo* following iAs treatment [18], as have changes in placental levels of reactive oxygen species *in vitro* [10,19]; abnormal placentation is also a strong predictor of preterm delivery and restricted fetal growth [20].

A growing body of epidemiologic evidence suggests increased risks for adverse birth outcomes in association with the use of highly iAs contaminated water sources by pregnant women (>10 μg/L), including the delivery of lower birth weight babies at term [21,22], in Argentina [23], Bangladesh [24,25], India [26,27], Mexico [28], and Taiwan [29]. Shorter birth length [28,30] and smaller head and chest circumferences [25] have also been reported among mothers with high level iAs exposure. Studies in populations presumed to have low iAs exposure (<10 μg/L [31]) reported lower birth weight or smaller size at birth in association with higher total

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blood or urine arsenic [32–36], although others reported no association [37,38]. However, the results of these prior studies were often limited by cross-sectional designs, small sample size and the absence of source data, or ill-timed exposure assessments that inappropriately incorporated less toxic organic arsenic compounds [39].

Millions of reproductively-aged women are likely to be exposed to low level drinking water iAs contamination (<10 μg/L) worldwide [40,41] and few data are available to characterize the risks [21,42]. There is a critical need for additional epidemiologic studies of the health effects of low level iAs exposure to help guide prevention efforts. In an initial step to address this gap, we conducted a preliminary cohort study by following up pregnant women who had previously participated as controls in a study of drinking water iAs exposure and pregnancy loss [43].

2. Material and methods

2.1. Study sample

The sampling frame comprised 150 women with ongoing singleton pregnancies, and receiving prenatal care services at Bega Hospital in Timisoara, Romania, between December 2011 and January 2013. Prenatal care is provided at no cost and a minimum of three visits is compulsory in Romania. All of the women resided in Timis County, Romania, an area known for low level iAs contamination of drinking groundwater sources [2]. The women were initially recruited as controls participants for our recent study of pregnancy loss, which was previously described in detail [43]. In brief, we recruited women with incident spontaneous pregnancy loss of 5–20 weeks completed gestation as cases ($n = 150$), and women with ongoing pregnancies matched by gestational age (±1 week) as controls ($n = 150$). Participants completed a physician-administered questionnaire regarding demographic, socioeconomic, and lifestyle factors, as well as medical, gynecologic, residential, and occupational histories, and provided blood and urine specimens. We also inquired as to water consumed through various means (*e.g.*, directly, bottled, tea, coffee, mixed beverages, soups) and used for cooking, and their sources and locations.

Shortly after participant enrollment, our team collected water samples from up to two residential drinking water sources reported by each study participant and transferred them on ice to the Environmental Health Center in Cluj-Napoca, Romania. We determined iAs in duplicate by hydride generation-atomic absorption spectrometry according to a strict quality control procedure as previously described [44]. The method detection limit was 0.5 μg/L. Focusing on periconception and early gestation as critical windows [45], we calculated three metrics for drinking water iAs exposure. The average iAs and peak iAs concentrations (μg/L) were defined as the mean iAs concentration measured in up to two residential drinking water sources and the higher of the two, respectively. The daily iAs exposure (μg/day) was defined as the average iAs concentration (μg/L) multiplied by the average water consumed from non-bottled sources daily (L/day), including direct consumption, mixed beverages, and soups.

In early 2014, we re-enrolled 124 control participants, having delivered 126 infants (two sets of twins), who consented for access to the study pregnancy birth record. The response rate was 95%; we could not contact $n = 15$, $n = 4$ had losses or terminations, and $n = 7$ refused to participate. At delivery, data are handwritten by the attending obstetrician on a standardized form, following good practice guidelines established by the Neonatology Association of Romania [46]; data are also collected in the rare event of a home birth. The birth record is linked to the maternal health records, and stored by surname in a secure area of Bega Hospital. The current sample includes 122 mothers with singleton live births.

2.2. Birth outcomes

We abstracted birth information from the medical record. We considered gestational age at delivery as continuous and as dichotomized at <37 weeks to indicate preterm delivery (PD) [47]. We expressed birth weight as Z-scores (BW-Z) relative to expected mean reference population values for gestational age [48], dichotomized at <10th% tile for the reference population distribution (small for gestational age (SGA)), and also dichotomized at <2500 g [47]. We considered birth length (BL-Z) and head circumference (HC-Z) at delivery as Z-scores relative to expected mean reference population values for gestational age [49], and dichotomized at <10th% tiles. We used ponderal index (PI-Z) as 100 x (birth weight/birth length^3), expressed as Z-scores relative to an expected median reference population value for gestational age [50], and dichotomized at <10th% tile.

2.3. Statistical analysis

We characterized distributions for birth outcomes, iAs exposure metrics, and for potential confounders. We natural log-transformed skewed distributions and identified outliers for further examination. We assessed unadjusted associations among predictors, outcomes, and potential confounders using ANOVA and linear correlation. We used Cox-proportional hazards models to accommodate medically-assisted deliveries indicated by Caesarean section, in confounder adjusted analyses of iAs exposure predicting gestational age at birth; exponentiated coefficients and their 95% CIs provided hazard ratios [51]. For other continuous outcomes we used multivariable linear regression to evaluate associations with iAs exposure, adjusted for confounders. For dichotomous outcomes, we used logistic regression models assess confounder-adjusted associations with iAs; exponentiation of model coefficients and corresponding 95% CIs provided odds ratios [52]. We entered maternal age in years [53], self-reported pre-pregnancy body mass index (BMI) in kg/m^2 [54], self-reported cigarette smoking during pregnancy as a dichotomous variable [55,56], and education as a trichotomous marker of socioeconomic status [57,58], into regression models contingent on literature evidence of confounding coupled to selection using directed acyclic graphs (DAGs) [59]. Due to convergence issues, logistic regression models for PD were adjusted for only maternal age and cigarette smoking. We also considered residential location as a covariate in the models, defined as urban vs. rural; to serve as a proxy for air pollution [60–63] and for exposure to drinking water chlorine disinfection byproducts (DBPs) [64].

We included product terms in additional linear regression models to assess interactions for continuous iAs exposure metrics with cigarette smoking, infant sex, and prior LBW delivery; factors likely to modify associations with continuous birth size outcomes. Interactions were tested using likelihood ratios and expressed as individual and joint effects [65].

We considered drinking water iAs metrics as continuous (to maximize study power) and as tertiles (to accommodate potential non-linear associations). Extreme outliers, defined as observations >3 interquartile ranges above the 75th% tile for the distribution were excluded from the analysis [66]. Observations with DFBetas $\geq |1|$ for the exposure were excluded as influential and regression models repeated [67]. Effects were expressed for 10 unit increases in iAs. Statistical significance was defined as $P < 0.05$ for main effects and $P < 0.10$ for product terms. Consistent with the exploratory aim of our study we made no adjustments for type-1 error inflation due to multiple-comparisons, in an effort to maximize sensitivity