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Estimating effects of arsenic exposure during pregnancy on perinatal outcomes in a Bangladeshi cohort

Original Research Article

Title: Estimating direct and indirect effects of arsenic exposure during pregnancy on maternal weight gain, gestational age, and birth weight in a Bangladeshi birth cohort

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Running Head: Arsenic and birthweight

ABSTRACT

Background: The relationship between arsenic and birth weight is not well understood.

Objective: To evaluate the causal relationship between prenatal arsenic exposure and birth weight considering the potential mediation effects of gestational age (GA) and maternal weight gain (MWG) during pregnancy using structural equation models (SEMs).

Methods: A prospectively enrolled cohort of pregnant women was recruited in Bangladesh from 2008-2011. Arsenic was measured in personal drinking water at the time of enrollment (<16 GA, N=1,140) and in toenails collected ≤1 month postpartum (N=624) using inductively coupled plasma mass spectrometry. SEMs estimated the direct and indirect effects of arsenic on birth weight with GA and MWG considered as mediating variables.

Results: Every unit increase in natural log water arsenic was indirectly associated with decreased birth weight (β=-19.17 grams, 95% CI: -24.64, -13.69) after adjusting for other risk factors. This association was mediated entirely through GA (β=-17.37 grams, 95% CI: -22.77, -11.98) and MWG during pregnancy (β=-1.80 grams, 95% CI: -3.72, 0.13). When exposure was modeled using toenail arsenic concentrations, similar results were observed. Every increase in natural log toenail arsenic was indirectly associated with decreased birth weight (β=-15.72 grams, 95% CI: -24.52, -6.91) which was mediated through GA (β=-13.59 grams, 95% CI: -22.10, -5.07) and MWG during pregnancy (β=-2.13grams, 95% CI: -5.24, 0.96).

Conclusion: Arsenic exposure during pregnancy was associated with lower birth weight. The effect of arsenic on birth weight appears to be mediated mainly through decreasing gestational age and to a lesser extent by lower maternal weight gain during pregnancy.

INTRODUCTION

Arsenic-contaminated drinking water is a global health concern.[1] A naturally occurring element, inorganic arsenic can dissolve in groundwater and lead to human exposure if the contaminated aquifer is used as a source of drinking water or to irrigate crops that accumulate arsenic such as rice.[2,3] This is particularly problematic in Bangladesh where public health interventions intended to reduce the incidence of waterborne disease switched the primary drinking water source from surface water to groundwater.[4] It is estimated that 46% of the population in Bangladesh is exposed to arsenic concentrations above the World Health Organization's (WHO) drinking water recommendation of 10 µg/L, and 27% are exposed to levels above the Bangladesh government's recommendation of 50 µg/L.[5] There are at least 19 other countries, including Taiwan, Mexico, Chile, Argentina, Vietnam, Laos, India, China, Romania, and the United States, that have groundwater aquifers that are naturally-contaminated with arsenic at levels exceeding the WHO drinking water recommendation.[6] Many of these aquifers are positioned below densely-populated regions, leading to millions of people being chronically exposed to arsenic.[1]

Bangladesh is also among the top ten countries with the highest preterm birth rate (<37 weeks of gestation).[7] For instance a large cohort study of 32,126 pregnant women in rural Bangladesh reported a preterm birth of 22.3%.[8] Additionally, Bangladesh it is estimated that the incidence of low birthweight babies is 31-47% which is among the highest in the world.[9] There are several recommended behavioral, nutritional, clinical, and health systems interventions that have been shown to reduce the preterm birth rate, however, none of these address common environmental risk factors such as arsenic. Many studies have shown that arsenic can cross through the placenta leading to fetal exposure.[10-12,13] There is epidemiological evidence that

exposure to elevated levels of arsenic in drinking water is related to higher rates of spontaneous abortion[14,15] and neonatal death.[16,17] Several studies have also examined the relationship between arsenic exposure and birth weight, with mixed results. An ecological study in Taiwan observed that infants (N=3,872) born into arsenic-exposed villages (ranging from 0.15 µg/L to 3.59 mg/L) were, on average, 29 grams (95% CI 13.6–44.6 g) lighter than infants (N=14,387) born into non-arsenic-exposed villages (<0.9 µg/L) after adjusting for confounders.[18] Whereas a different ecological study performed in Mongolia observed that infants (N=9,890) born in arsenic-exposed villages (>100 µg/L) were, on average, 50 grams heavier than infants born in non-arsenic exposed villages (<20 µg/L).[16] Prospective cohort studies conducted in Chile and Bangladesh, however, observed a dose-dependent relationship between arsenic measured in drinking water,[19] maternal hair,[20] or maternal urine[21] on lower mean birth weight although the magnitude and statistical significance of the reported relationship differed between studies. Interestingly, the largest of these prospective studies (N=1,578) only observed a negative relationship between exposure and birth weight when maternal urinary arsenic concentrations were <100 µg/L.[21]

Additionally, arsenic exposure may exacerbate factors that can contribute to low birthweight including gestational age and maternal health[22]. For instance, arsenic exposure is related to increased risk of nausea and vomiting during pregnancy[19] which in turn may decreased maternal weight gain during pregnancy[22] and contribute to poor maternal nutritional status particularly among populations that experience chronic nutritional stress [23-26] Arsenic exposure has also been shown to increase the risk of premature birth.[27] Structural equation models provide an opportunity to examine highly intercorrelated factors that may lie on the pathway between arsenic exposure and birthweight including factors such as maternal weight gain during pregnancy.[28,29] This approach has also been widely used to account for gestational age which is a

strong predictor of birthweight and has been shown in many studies to be an important

intermediate in the causal pathway between an exposure and birthweight.[30,31] Therefore, we

examined the direct and indirect effects of arsenic exposure on birthweight in a population-based

birth cohort recruited in Bangladesh using a causal pathway approach with structural equation

models. Specifically, we hypothesized that arsenic exposure would be associated with decreased

maternal weight gain during pregnancy and decreased gestational age and that these two

variables would mediate the effect between arsenic exposure and reducedbirthweight.

METHODS

Study Population and Subject Selection

We established a prospective birth cohort in the Sirajdikhan and Pabna Sadar Upazilas of

Bangladesh. The objective of this cohort was to observe the effects of chronic moderate arsenic

exposure on reproductive outcomes. These districts were selected as the study areas because 1) a

national survey conducted by the British Geological Survey indicated that the average

concentration of arsenic in the groundwater in these areas was more moderate than other regions

in Bangladesh and spanned a wide range of concentrations;[5] 2) Dhaka Community Hospital

Trust (DCH) operates rural health clinics in these districts that offer prenatal care and promote

arsenic awareness by encouraging people to drink water only from wells that comply with the

Bangladesh drinking water arsenic standard of ≤ 50 µg/L; and 3) the clinics serve

demographically similar populations.[32]

Women were eligible to participate in the study if they were 18 years or older with a

singleton pregnancy ≤ 16 weeks' gestation confirmed by ultrasound at the time of enrollment,

planned to continue receiving prenatal care through DCH, had used the same drinking water

source for at least the six months prior to enrollment, and intended to live at the same household throughout pregnancy. This analysis used data from participants (N=1,613) who were enrolled into the cohort between January 2008 to June 2011. After exclusion due to loss of contact with participants (n=123), study withdrawal (n=125), stillbirth (n=75), miscarriage (n=132), missing drinking water sample at enrollment and/or missing information on environmental tobacco smoke (n=2), and non-singleton pregnancy (n=4) the sample size was 1,153.

Ethical Consideration

Study protocols were approved by the Human Research Committees at Dhaka Community Hospital, Harvard School of Public Health and Oregon State University. The cohort was recruited in villages where DCH has actively engaged in arsenic-awareness campaigns and safe water options were available. All participants were able to request a technician to test their water for arsenic using a field test kit and were given the results immediately. Additionally, all participants were informed if their water samples contained arsenic above the Bangladesh standard after analysis by inductively coupled plasma mass spectrometry. Participants were also provided with free prenatal vitamins throughout their pregnancies. Free transportation to a DCH hospital was available to all participants in case of a pregnancy-related emergency. Consent documents were provided to participants in Bengali and read aloud by trained staff. All participants provided consent prior to participation in the study.

Exposure Assessment

Arsenic was measured in maternal drinking water samples (N=1,140) at the time of enrollment and has been described previously.[22] Briefly, nitric acid preserved water samples were analyzed for total arsenic concentrations by inductively coupled plasma-mass spectrometry following US EPA method 200.8 (Environmental Laboratory Services, North Syracuse, New

York). The samples that were below the limit of detection (LOD) of 1 µg As/L (N= 252) were re-assigned half the value of the LOD for statistical analysis.

Toenail clippings were collected from participants at ≤ 1 month postpartum. Arsenic concentrations in these nails reflect the cumulative exposure across the prenatal period since it can take several months to up to 1 year for nails to grow to the free edge of the plate where they can be collected.[33] Samples were sonicated in 1% Triton X-100 solution (Sigma-Aldrich, Inc.) and rinsed repeatedly with Milli-Q water (Millipore Corporation, Billerica, MA) to remove external contamination prior to microwave acid digestion using Trace Select Ultra Pure nitric acid (HNO_3) (Sigma-Aldrich, Inc.). Digested samples were diluted with Milli-Q water and analyzed for total arsenic using an inductively coupled plasma mass spectrometer (Perkin-Elmer Model DRC-II 6100, Norwalk, CT). The reported arsenic concentrations were blank-corrected and then further corrected for systemic error by normalizing the sample concentrations using the arsenic concentration of the batch-specific certified human hair reference material (CRM Hair; Shanghai Institute of Nuclear Research, Academia Sinica, China). Of the 641 toenail samples with arsenic measurements available, samples were excluded if the mass was ≤ 5 mg (n=3) and/or if the relative standard deviation ≥25% (n=8). One sample was below the sample LOD (which ranged from 0.09 ng/g–0.7 ng/g) and was re-assigned half the value of the LOD for statistical analysis. This left a total of 629 samples included in this analysis.

Birth weight and Covariates

Women were followed throughout their pregnancies with three scheduled clinical visits which occurred at the time of enrollment, approximately 28 weeks gestational age (GA), and ≤1 month post-delivery. During these clinical visits trained interviewers used structured questionnaires to collect socio-demographic, medical, and environmental information. After their

first clinical visit which occurred at the time of enrollment, trained health care providers visited participants in their homes once per month to distribute prenatal vitamins, record symptoms, weigh participants and measure their blood pressure. All births were attended by trained health care workers. Birth weight was measured on a pediatric scale which was calibrated before each measurement and rounded to the nearest 10 grams. Length and head circumference were measured using standard protocols. Approximately 46% of birth anthropometry was measured at a hospital or clinic with the remainder occurring at the participant's home. The same survey instruments and staff were used to collect information in the participants home, clinic and hospital.

Maternal weight gain over the follow-up period (kg/week) was calculated by subtracting weight obtained prior to delivery from weight measured at the time of enrollment divided by the amount of weeks of follow up. Birth gestational age (weeks) was estimated from ultrasound measurements collected at the time of enrollment. Other covariates that were considered as potential confounders included infant sex, maternal education (illiterate, primary or secondary), body mass index at the time of enrollment (kg/m^2), exposure to secondhand tobacco smoke (yes/no), chewing betel nut (yes/no), birth type (cesarean/vaginal), birth location (home/clinic-hospital) and maternal age in years (continuous).

Statistical Analysis

Descriptive statistics were computed for all variables. Arsenic concentrations were skewed and subsequently transformed to their natural log. T-test or analysis of variance (ANOVA) was used to compare mean birth weight across categories of all covariates in bivariate analyses. To evaluate for homogeneity of variances, Levene's test was performed for all bivariate comparisons, and a histogram of birth weight indicated no gross violations for the normality

assumption. Multivariate linear regression models were used to evaluate the association between ln arsenic and birth weight adjusting for other covariates (e.g. infant sex, maternal education, secondhand tobacco smoke exposure, entry BMI and maternal age). All numerical variables were model as is with the exception of both exposures which were natural log-transformed.

SEM were used to evaluate the direct relationship between variables (a_1, b_1, c_1, a_2, b_2, c_2) as well as the direct effect of arsenic on birth weight (c') controlling for all mediators (Figure 1). The potential indirect effect of arsenic exposure through gestational age on birth weight ($a_1 \bullet b_1$), as well as, the potential indirect effect through maternal weight gain ($a_2 \bullet b_2$) were calculated. The total effect of arsenic exposure on birth weight mediated through both mediators was calculated ($a_1 \bullet b_1 + a_2 \bullet b_2 + c'$), as well as the total mediated effect ($a_1 \bullet b_1 + a_2 \bullet b_2$). The results from these partial and total effects with respect to each mediator on the outcome of birthweight are then reported in Table 2 and 3. Furthermore, we assumed the variance for birth gestational age and maternal weight gain were correlated and modeled them accordingly. After evaluating the proposed mediation pathways, we further adjusted models for the direct effect of the other covariates (e.g. infant sex, maternal education, secondhand tobacco smoke exposure, entry BMI, birth type, birth location, and maternal age; Figure 2). Confidence intervals and standard errors were computed from 10,000 bootstrap samples for all SEM models. Model fit was evaluated using the comparative fit index (CFI), the root mean square error of approximation (RMSEA), overall model χ^2 p-value, the Tucker-Lewis non-normed index and the standardized root mean squared residual. Modification indices based on χ^2 improvement were used to optimally tune the final model fit. The R-squared for all endogenous variables was also estimated. The maximum likelihood estimation (MLE) method was used to estimate all parameters. Statistical significance was evaluated using a cut off value of $\alpha \leq 0.05$, and all tests performed were two-tailed. All

statistical analyses were performed in STATA (Version 12.1, StataCorp LP. College Station, Texas).

RESULTS

Overall, the average birth weight in this population was 2,836 grams (standard deviation: 415 grams; range: 800 grams – 4,800 grams). As anticipated, arsenic exposures were relatively modest with a median concentration of 2.3 µg/L in drinking water at the time of enrollment (interquartile range: 0.9 µg/L, 36 µg/L) but spanned a wide range (<LOD – 1,400 µg/L). Toenail arsenic was strongly correlated with drinking water exposure ($\sigma_{spearman}$=0.49, p-value<0.001). The median toenail arsenic concentration was of 1.46 µg/gram (interquartile range: 0.76 µg/g, 3.73 µg/g). Other population characteristics and how they are associated with birth weight are presented in Table 1. Birth weight was greater among males, infants not exposed to secondhand tobacco smoke, infants who were born to mothers with higher BMI at study enrollment, infants born to mothers with higher educational attainment, born at a clinic or hospital, cesarean births and infants born to mothers who gained the most weight during pregnancy. Birth weight also increased with gestational age (ρ=0.41, P<0.001). Parity, expressed as a continuous variable or as a binary variable (uniparious vs multiparous) was not associated with birthweight.

Initial SEM were developed that included both the direct and indirect effects of arsenic in drinking water and toenail arsenic on birth weight as described in Figure 1. As initially hypothesized, these models showed that arsenic exposure was significantly correlated with gestational age (a_1) and maternal weight gain (a_2), and in turn, gestational age (b_1) and maternal weight gain (b_2) were significantly correlated with birth weight. Additionally, maternal BMI at the time of enrollment was significantly correlated with maternal weight gain (c_1) and birth

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weight (c_2) (Figure 2). Yet, the estimated direct effect of arsenic on birth weight controlling for both mediators (c') was non-significant. The observed structural relationships were consistent regardless of whether arsenic was measured in drinking water (Figure 1A) or toenail (Figure 1B). These initial models supported the hypothesis that gestational age and maternal weight gain were on the causal pathway between arsenic exposure and birth weight. Contrary to our hypothesis, however, these models indicated no significant direct effect of arsenic exposure on birth weight. These results suggest that the observed effect of arsenic exposure on birth weight in this population was completely mediated by its effect on gestational age and maternal weight gain during pregnancy for the follow up time.

To account for potential confounding, the relationship between arsenic exposure and birth weight was adjusted for the direct effects of the newborn's sex, mother's education, environmental tobacco smoke exposure, mother's age, birth location, birth type and BMI at enrollment (Figure 2). The modification indices suggested that maternal BMI at time of study enrollment also had an indirect effect on birth weight which was mediated through maternal weight gain during pregnancy. Birth type was only indirectly associated with birth weight through gestational age and maternal weight gain. The direct effect of maternal age at enrollment on birth weight was not significant. However, age at enrollment had a significant indirect effect on birth weight that was mediated through maternal weight gain. Therefore, the indirect pathways of maternal age and BMI at enrollment were added to all final models. The adjusted indirect effects of arsenic exposure in drinking water or maternal toenails on birth weight (grams) mediated through gestational age (weeks) and maternal weight gain (kg/week), as well as the indirect effect of entry BMI, and maternal age on maternal weight gain, are presented in Tables 2 and 3, respectively. Specifically, arsenic exposure directly reduced gestational age

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(β_{water}=-0.23 weeks, 95% CI: -0.28, -0.17; β_{toe}=-0.26 weeks, 95% CI: -0.40, -0.13) and directly reduced maternal weight gain during pregnancy (β_{water}=-0.009 kg/week, 95% CI: -0.01, -0.006; β_{toe}=-0.009 kg, 95% CI: -0.01, -0.0002). In turn, gestational age (weeks) directly increased birth weight (β_{water}=75.94 g, 95% CI: 63.46, 88.41; β_{toe}=51.19 g, 95% CI: 29.63, 72.74); as did maternal weight gain during pregnancy (kg/week) (β_{water}=191.33 g, 95% CI: 2.0, 395.26; β_{toe}=241.3 g, 95% CI: -85.85, 568.36). Similar to our initial SEM models, there was no significant direct effect of arsenic exposure on birth weight. These partial direct effects show the negative relationships between arsenic and maternal health characteristics (e.g. gestational age and maternal weight gain during the follow up period). Yet these maternal characteristics ultimately had a strong positive relationship with birth weight, as does maternal education and body mass index at the time of enrollment.

The total mediated effect of arsenic (accounting for the direct and indirect effects) was distributed among the two mediating pathways as described in Table 4. The vast majority of this indirect effect was mediated through birth gestational age (β_{water}=-17.37 g, 95% CI: -22.77, -11.98; or $\beta_{toenail}$=-13.59 g, 95% CI: -22.10, -5.07) and to a lesser extent through maternal weight gain during pregnancy (β_{water}=-1.80 g, 95% CI: -3.72, 0.13; or $\beta_{toenail}$ =-2.13 g, 95% CI: -5.24, 0.96). Thus, the total indirect effect of arsenic exposure mediated completely through gestational age in weeks and maternal weight gain during pregnancy in kg/week suggested that birth weight would decrease by approximately 16 to 19 grams after adjusting for other risk factors for every unit increase in natural log-transformed arsenic in drinking water or maternal toenails. The fit of the final adjusted SEM conformed to all model fit statistics (Table 5).

As an additional sensitivity analysis, we restricted on term pregnancy (37-42 weeks of gestational age). As expected, the mediated association of arsenic on birth weight through

gestational age was attenuated for term births in this restricted analysis (Table 6). If arsenic decreases birth gestational age as hypothesized, the stratified analysis further supports its mediated effect and our initial hypothesis since the mediating effect of maternal weight gain strengthens and the indirect effect through birth gestational age is attenuated in term pregnancies.

DISCUSSION

Structural equation models (SEM) provide useful insights into the biological mechanism underlying life-course epidemiology. Additionally, SEMs has been used previously in perinatal epidemiological studies to interpret the causal structure between exposures mediated through gestational age and perinatal outcomes. By integrating an *a priori* understanding of how arsenic-related reproductive toxicity was influenced by gestational age and maternal weight gain during pregnancy, we constructed SEM that allows testing of the direct, indirect, and total effects of arsenic on birth weight while appropriately controlling for correlated risk factors. This analysis demonstrated that prenatal arsenic exposure was significantly associated with decreased birth weight in a dose-dependent manner. Specifically, we observed that for every doubling in arsenic exposure measured in maternal drinking water or maternal toenails, birth weight decreased by approximately 22 grams. This effect, however, was completely mediated by gestational age and maternal weight gain during pregnancy, suggesting that these mediators are part of the causal pathway for arsenic-related reproductive toxicity. Moreover, the indirect effect of arsenic was greatest on gestational age and to a lesser extent on maternal weight gain during pregnancy which can indicate maternal health during pregnancy in this rural population.

The results from these SEM are consistent with several epidemiological studies that report a significant relationship between arsenic exposure and decreased birth weight. [18,

34][20, 21] Interestingly, it is only the studies that were conducted in populations with relatively low-level exposure levels that observed significant negative effects of arsenic on birth weight. It is possible that arsenic's reproductive toxicity is dose-dependent and causally related to factors that influence fetal growth and survival since high levels of arsenic exposure have been shown to be related to increased rates of spontaneous abortion[14], [15] and neonatal death. [16], [17] Our study also shows a strong negative causal relationship between prenatal arsenic exposure and gestational age. It would be useful if future studies examined the relationship between arsenic exposure and gestational age as a continuous variable instead of using a clinical definition of preterm birth (<37 weeks gestational age) to further explore this notion.

Our study has several strengths. We used data from a prospectively enrolled study in which drinking water arsenic exposure was measured in personal drinking water samples early in pregnancy and in maternal toenails providing an estimate of internal dose over the prenatal period. Therefore, the proposed temporal arrangement between the exposure, mediator and outcome is valid. Our use of two exposure indices also minimized the potential for exposure misclassification since arsenic exposure measured in drinking water at the time of enrollment would reflect the participants initial exposure and arsenic exposure measured in maternal toenails after delivery would provide an integrated measure of exposures that occurred in the prior 9 to 12 months which would span the entire pregnancy. The arsenic exposures measured in this population are relevant to many other populations that have more modest exposure levels, such as the United States. All women received the same level of prenatal health care since DCH is one of the few health care providers in these catchment areas which would minimize bias and confounding by unmeasured factors related to prenatal care. Also, maternal weight gain was estimated by using data collected monthly, albeit this variable does not include a

final maternal weight at delivery. Gestational age was estimated consistently in this cohort. However, we relied solely on ultrasound measurements to estimate gestational age since few women were able to recall the date of their last menstrual period. Ultrasound measurements are the gold standard for estimating gestational age when taken in early pregnancy but may not be as accurate when used in the second trimester.[35] Thus, if gestational age at enrollment into the cohort was associated with arsenic exposure, this could be a source of bias. We carefully examined this issue and saw no correlation between gestational age at enrollment and ln-transformed arsenic in drinking water (spearman's ρ=0.009, P=0.75) or ln-transformed arsenic in toenails (spearman's ρ=-0.004, P=0.92). Furthermore, we examined whether women who had arsenic concentrations in their water above and below the Bangladesh drinking water standard of 50 µg/L had different gestational ages at enrollment and found no difference between these groups (rank-sum P-value=0.35). Additionally, we conducted a sensitivity analysis where we stratified our cohort by gestational age at the time of study enrollment ($\leq$12 weeks of pregnancy compared to 13-16 weeks of pregnancy). Overall the results were consistent between the two strata suggesting that the results for early and late enrollment during pregnancy in this cohort did not influence the estimated overall indirect effects of arsenic observed in adjusted models (See Supplemental Table 1). Thus, the potential for bias due to ultrasound calculated gestational age is most likely minimal. We were also able to control for many other positive and negative confounders in this analysis including maternal body mass index at the time of enrollment and maternal age at the time of pregnancy. However, we acknowledge that unmeasured confounding may be present in our analysis since we were unable to adjust for some important covariates such as interpregnancy interval since the vast majority of participants did not provide this information.

This study does have some weaknesses. Namely, we there were many missing maternal toenail samples due to a lag in the on-going laboratory analysis. Toenail samples were randomly selected by the laboratory for analysis and laboratory technicians were blinded to the concentration of arsenic in the participant's drinking water. However, the sub-group that included toenail measurements had slightly higher arsenic exposure in their drinking water (54.4 µg/L vs 39.98 µg/L, p<0.001) and slightly lower maternal weight gain during pregnancy (0.31 kg/weeks vs 0.34 kg/weeks, p<0.001) but no difference in birthweight (2851g vs 2860g, p=0.26) or gestational age (37.9 weeks vs 37.9 weeks, p=0.27). Since arsenic exposure is negatively associated with maternal weight gain, the estimated mediating effect of maternal weight gain is likely greater in this subset of the population that included toenail arsenic measurements. Additionally, we were unable to control for maternal micronutrient deficiencies, although we note that all women were provided with prenatal vitamins throughout their pregnancy and based on monthly interviews with the participants and pill count compliance with taking these vitamins was very good. Also, it is likely that some maternal weight gain during pregnancy was missed given the staggered enrollment into this cohort, the strong correlations between maternal weight gain and gestational age, and the inability to weigh all participants just prior to birth. We attempted to minimize this misclassification of maternal weight gain by normalizing weight gain by the individual's follow up time in the cohort but recognize that there may be error in this term. Additionally, maternal BMI at enrollment is also likely to be inflated with regards to gestational age at the time of enrollment. To account for the relationship between gestational age and maternal weight gain, we correlated the residual variance between these two variables that is not explained by arsenic exposure even though we could not incorporate this issue into the causal model structure.

In conclusion, we observed a negative association between prenatal arsenic exposure and birth weight. However, this effect was completely mediated through birth gestational age and maternal weight gain during pregnancy. Taking into account this causal pathway, the direct effect of arsenic exposure on birth weight was not significant. Given that birth weight is an extremely important predictor of infant mortality and overall health, it is important that public health interventions focus on reducing arsenic exposure in this population. We also encourage investigators to consider alternative methods to modeling birth weight that take into account direct and indirect effects in order to move beyond the discovery of risk factors.

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Figure 1. Effect sizes for the initial conceptual SEM model are presented. This model

hypothesized that natural log-transformed arsenic water (A) or toenail (B) directly effects (c′)

birth weight (grams) after adjusting for gestational age (weeks) and maternal weight gain(kg).

The partial effects of arsenic on gestational age (a_1) and maternal weight gain (a_2), as well as, the

partial effects of gestational age (b_1) and maternal weight gain (b_2) on birth weight are presented.

The total indirect effects of arsenic on birth weight mediated by gestational age ($a_1 \cdot b_1$) and by

maternal weight gain ($a_2 \cdot b_2$) were highly significant. Error terms (ε) of the mediators were

significantly correlated. ($^{**}p<0.01$, $^{***}p<0.001$, *NS*: Non-Significant)
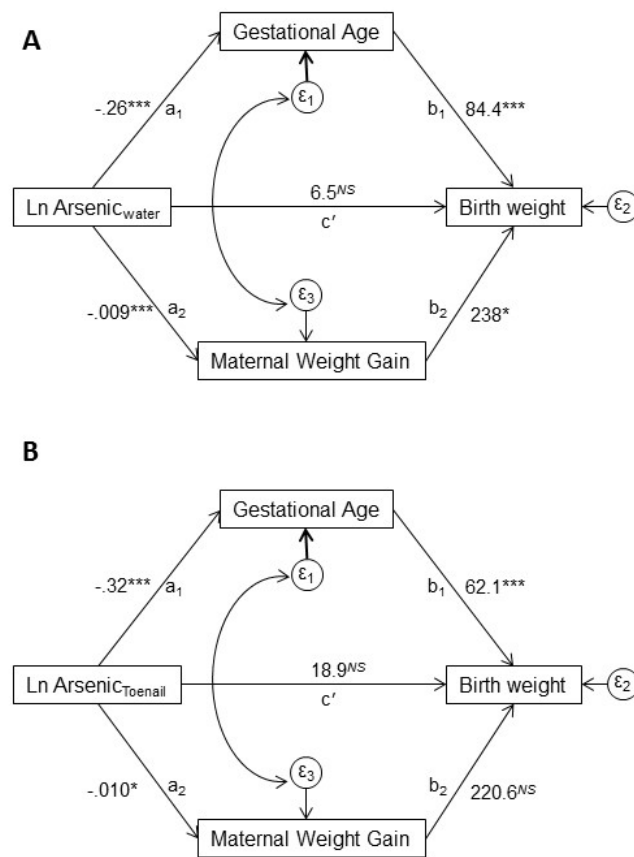
Figure 2. Final SEM models for the indirect effect of log-transformed arsenic measured in maternal drinking water (A) or toenail (B) on birth weight (grams) that is completely mediated through gestational age (weeks) and maternal weight gain (kg/week) adjusting for other risk factors. *p<0.05, **p<0.01, ***p<0.001, *NS*: Non-Significant
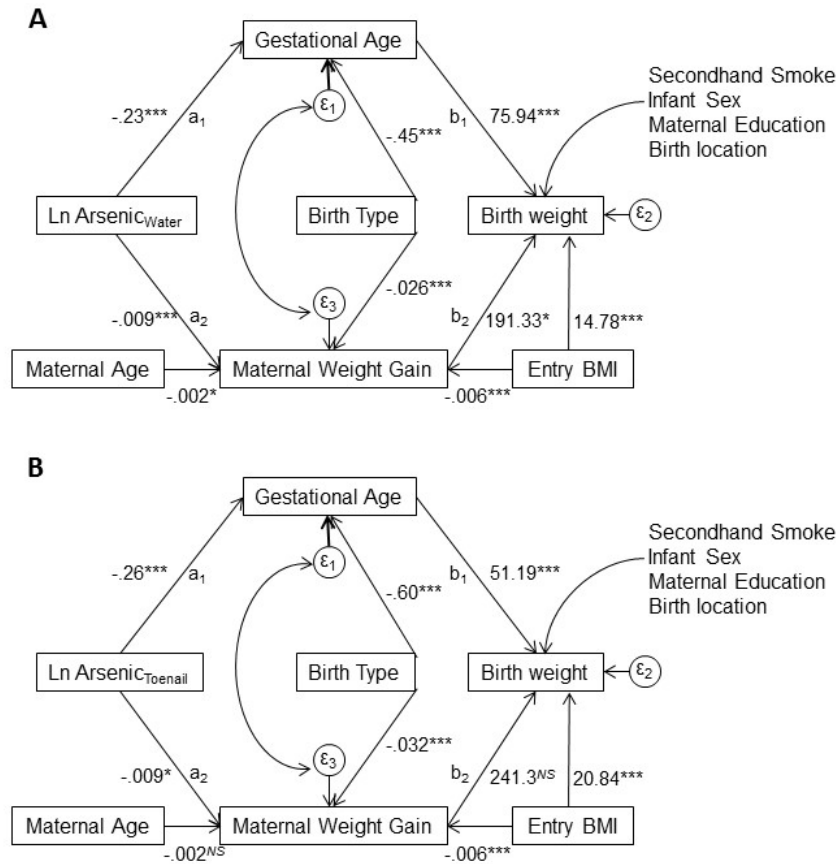
Table 1. Description of selected characteristics and their relationship with birth weight

(N=1,153).

	N (%)	Mean Birth Weight (SD) in grams
Birth Gender		
Male	584 (50.65%)	2,873 (400)
Female	569 (49.35%)	2,799 (425)
Birth Location		
Home	624 (54.17%)	2,731 (410)
Hospital/Clinic	528 (45.83%)	2,952 (388)
Missing	*6*	*----*
Birth Type		
Vaginal	746 (64.70%)	2,769 (423)
Cesarean	407 (35.30%)	2,959 (362)
Maternal Betel/Tobacco chewing		
No	1,130 (98.95%)	2,838 (413)
Yes	12 (1.05%)	2,647 (371)
Missing/Refused	*11*	*----*
Maternal Education		
Secondary	614 (53.25%)	2,867 (413)
Primary	373 (32.35%)	2,843 (408)
Illiterate	166 (14.40%)	2,708 (408)
Secondhand Tobacco Smoke		
Yes	478 (41.49%)	2,795 (410)
No	674 (58.51%)	2,865 (415)
Missing	*1*	*----*
Entry BMI		
Underweight	324 (28.10%)	2,772 (427)
Normal	719 (62.36%)	2,835 (391)
Overweight	98 (8.50%)	3,039 (478)
Obese	12 (1.04%)	2,983 (287)
Age Group (years)		
18-20	459 (39.81%)	2842.7 (394)
21-25	425 (36.86%)	2868.5 (430)
26-41	269 (23.33%)	2835.8 (423)
Gestational Age (Weeks)		
22-36 Weeks	254 (22.03%)	2,563 (505)
37-39 Weeks	609 (52.82%)	2,903 (355)
40-42 Weeks	290 (25.15%)	2,936 (332)
Maternal Weight Gain/Time of follow up (Kg/Week)		
0.04 to 0.27	390 (34.09%)	2,787 (415)
0.27 to 0.35	379 (33.13%)	2,816 (412)

0.36 to 0.93	375 (32.78%)	2,907 (405)
Missing	8	----
Drinking Water Arsenic (µg/L)		
0.50-1.4	396 (34.35%)	2,871 (348)
1.45-18	373 (32.35%)	2,844 (407)
18.4-1400	384 (33.30%)	2,793 (478)
Toenail Arsenic (µg/g)		
0.19-0.93 µg/g	210 (33.39%)	2,878 (422)
0.94-2.62 µg/g	210 (33.39%)	2,812 (440)
2.63-34.77 µg/g	209 (33.23%)	2,855 (427)
Missing	*524*	

Table 2. Direct and indirect effects for the effect of natural log transformed arsenic concentrations in drinking water (µg/L) on birth weight (grams) that is mediated by gestational age (weeks) and maternal weight gain (kg) based on 10,000 bootstraps samples (N=1,140).

Outcome	Direct Effects (95 % CI)	Indirect Effect (95 % CI)	Total Effect (95 % CI)
Gestational Age			
Ln(Arsenic$_{water}$)→Gestational age	-0.23*** (-0.28 , -0.17)	----	-0.23*** (-0.28 , -0.17)
Birth Type (cesarean)→Gestational age	0.45*** (0.22 , 0.67)	-----	0.45*** (0.22 , 0.67)
Maternal Weight Gain (Kg/Week)			
Ln(Arsenic$_{water}$)→Maternal weight gain	-0.009*** (-0.01 , -0.006)	-----	-0.009*** (-0.01 , -0.006)
Entry BMI→Maternal weight gain	-0.006*** (-0.008 , -0.004)	-----	-0.006*** (-0.008 , -0.004)
Maternal Age→Maternal weight gain	-0.002* (-0.003, -0.0001)	-----	-0.002* (-0.003, -0.0001)
Birth Type (cesarean)→Maternal weight gain	0.026*** (0.012 , 0.039)	-----	0.026*** (0.012 , 0.039)
Birth Weight			
Maternal Weight Gain→Birth weight	191.33* (2.0, 395.26)	----	191.33* (2.0, 395.26)
Maternal age→Birth weight	NS	-0.29^{NS} (-0.73 , 0.14)	-0.29^{NS} (-0.73 , 0.14)
Maternal Education→Birth weight	86.98** (26.77 , 147.19)	-----	86.98** (26.77 , 147.19)
Entry BMI→Birth weight	14.78*** (7.61 , 21.96)	-1.18^{NS} (-2.49 , 0.13)	13.60*** (6.37, 20.84)
Secondhand Smoke→Birth weight	-32.32 ^{NS} (-75.55 , 10.91)	-----	-32.32 ^{NS} (-75.55 , 10.91)

Gender (Female)→Birth weight	-80.46*** (-122.99, -37.94)	-----	-80.46*** (-122.99, -37.94)
Ln(Arsenic$_{water}$)→ Birth weight	NS	-19.17*** (-24.64 , -13.69)	-19.17*** (-24.64 , -13.69)
Gestational age→ Birth weight	75.94*** (63.46 , 88.41)	-----	75.94*** (63.46 , 88.41)
Birth Type (cesarean)→ Birth weight	NS	38.88*** (19.59 , 58.17)	38.88*** (19.59 , 58.17)
Birth Location (clinic/hospital)→ Birth weight	141.44*** (99.21 , 183.67)	-----	141.44*** (99.21 , 183.67)

*p<0.05, **p<0.01, ***p<0.001, *NS*: Non-Significant.

Table 3. Direct and indirect effects for the effect of natural log transformed arsenic concentrations in maternal toenail (μg/g) on birth weight (grams) that is mediated by gestational age (weeks) and maternal weight gain (kg) based on 10,000 bootstraps sample (N=624).

Outcome	Direct Effects (95 % CI)	Indirect Effect (95 % CI)	Total Effect (95 % CI)
Gestational Age			
Ln(Arsenic$_{Toenail}$)→Gestational age	-0.26*** (-0.40 , -0.13)	----	-0.26*** (-0.40 , -0.13)
Birth Type (cesarean)→Gestational age	0.60*** (0.31 , 0.90)	-----	0.60*** (0.31 , 0.90)
Maternal Weight Gain (Kg/Week)			
Ln(Arsenic$_{Toenail}$)→Maternal weight gain	-0.009* (-0.01 , -0.0002)	-----	-0.009* (-0.01 , -0.0002))
Entry BMI→Maternal weight gain	-0.006*** (-0.009 , -0.003)	-----	-0.006*** (-0.009 , -0.003)
Maternal Age→Maternal weight gain	-0.002^{NS} (-0.003, -0.0006)	-----	-0.002^{NS} (-0.003, -0.0006)
Birth Type (cesarean)→Maternal weight gain	0.032*** (0.015 , 0.049)	-----	0.032*** (0.015 , 0.049)
Birth Weight			
Maternal Weight Gain→Birth weight	241.3^{NS} (-85.85, 568.36)	----	241.3^{NS} (-85.85, 568.36)
Maternal age→Birth weight	NS	-0.36^{NS} (-1.05 , 0.32)	-0.36^{NS} (-1.05 , 0.32)
Maternal Education→Birth weight	113.57** (42.21 , 184.93)	-----	113.57** (42.21 , 184.93)
Entry BMI→Birth weight	20.84*** (10.39 , 31.28)	-1.45^{NS} (-3.51 , 0.61)	19.38*** (42.21, 184.93)
Secondhand Smoke→Birth weight	-50.50^{NS} (-108.9 , 7.96)	-----	-50.50^{NS} (-108.9 , 7.96)

Gender (Female)→Birth weight	-89.20** (-147.03, -31.36)	-----	-89.20** (-147.03, -31.36)
Ln(Arsenic$_{Toenail}$)→ Birth weight	NS	-15.72*** (-24.52 , -6.91)	-15.72*** (-24.52 , -6.91)
Gestational age→ Birth weight	51.19*** (29.63 , 72.74)	-----	51.19*** (29.63 , 72.74)
Birth Type (cesarean)→ Birth weight	NS	38.74** (16.21 , 61.26)	38.74** (16.21 , 61.26)
Birth Location→ Birth weight	122.01*** (64.64 , 179.40)	-----	122.01*** (64.64 , 179.40)

*p<0.05, **p<0.01, ***p<0.001, *NS*: Non-Significant.

Table 4. Indirect effects of natural log transformed arsenic mediated through gestational age and mother's weight gain on birth weight (grams) based on 10,000 bootstrap sample after adjusting for infant sex, maternal education, secondhand tobacco smoke exposure, entry BMI, maternal age, birth type and birth location.

Mediation pathway	β coefficient	95% CIs	P-value
Drinking water (µg/L)			
Ln(Arsenic)→Gestational age→Birth weight	-17.37	(-22.77, -11.98)	<0.001
Ln(Arsenic)→Maternal weight gain→ Birth weight	-1.80	(-3.72, 0.13)	0.067
Toenail arsenic (µg/g)			
Ln(Arsenic)→Gestational age→ Birth weight	-13.59	(-22.10, -5.07)	0.002
Ln(Arsenic)→Maternal weight gain→ Birth weight	-2.13	(-5.24, 0.96)	0.179

Table 5: Fit indices for the final SEM models (Figure 2) that describe the indirect effect of arsenic exposure on birth weight that is completely mediated through birth gestational age and maternal weight gain during pregnancy.

Index	Criterion for Good Fit	Fitted Values Water Arsenic	Fitted Values Toenail Arsenic
χ^2 p-value	>0.05	0.12	0.31
Root Mean Square Error of Approximation (RMSEA)	<0.05	0.02	0.01
Comparative Fit Index	>0.95	0.99	0.99
Tucker-Lewisnon-normed Fit Index	>0.90	0.98	0.98
Standardized Root Mean Squared Residual	>0.05	0.01	0.02

Table 6. A sensitivity analysis that stratifies on term pregnancies and non-term pregnancies. The Indirect effects of natural log transformed arsenic mediated through gestational age and mother's weight gain on birth weight (grams) based on 10,000 bootstrap sample after adjusting for infant sex, maternal education, secondhand tobacco smoke exposure, entry BMI, maternal age, birth type and birth location.

Mediation pathway	Overall β Coefficient (95% CI)	Term pregnancy β Coefficient (95% CI)	Non-term pregnancy β Coefficient (95% CI)
Drinking water arsenic (µg/L)	N=1,140	N=889	N=251
Ln(Arsenic)→Gestational age→Birth weight	-17.37 (-22.77, -11.98)	-1.72 (-3.79, 0.35)	-4.28 (-17.14, 8.58)
Ln(Arsenic)→Maternal weight gain→ Birth weight	-1.80 (-3.72, 0.13)	-3.88 (-6.10, -1.72)	0.99 (-1.78, 3.78)
Toenail arsenic (µg/g)	N=624	N=485	N=139
Ln(Arsenic)→Gestational age→ Birth weight	-13.59 (-22.10, -5.07)	1.42 (-2.21, 4.06)	-4.55 (-34.12, 43.23)
Ln(Arsenic)→Maternal weight gain→ Birth weight	-2.13 (-5.24, 0.96)	-3.13 (-7.49, 1.23)	2.03 (-6.11, 10.17)