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# Urinary total arsenic and arsenic methylation capacity in pregnancy and gestational diabetes mellitus: A case-control study

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## Abstract

Previous studies suggest arsenic exposure may increase the risk of gestational diabetes mellitus (GDM). However, prior assessments of total arsenic concentrations have not distinguished between toxic and nontoxic species. Our study aimed to investigate the relationships between inorganic arsenic exposure, arsenic methylation capacity, and GDM.

Sixty-four cases of GDM and 237 controls were analyzed for urinary concentrations of inorganic arsenic species and their metabolites (arsenite (As3), arsenate (As5), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA)), and organic forms of arsenic. Inorganic arsenic exposure was defined as the sum of inorganic and methylated arsenic species (iSumAs). Methylation capacity indices were calculated as the percentage of inorganic arsenic species [iAs% = (As3 + As5)/iSumAs, MMA% = MMA/iSumAs, and DMA% = DMA/iSumAs]. Multivariable logistic regression was performed to evaluate the association between inorganic arsenic exposure, methylation capacity indices, and GDM.

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Declarations of interest

The authors have no conflict of interest to declare.

We did not observe evidence of a positive association between iSumAs and GDM. However, women with GDM had an increased odds of inefficient methylation capacity when comparing the highest and lowest tertiles of iAs% (adjusted odds ratio (aOR) = 1.48, 95% CI 0.58–3.77) and MMA% (aOR = 1.95 (95% CI 0.81–4.70) and a reduced odds of efficient methylation capacity as indicated by DMA% (aOR = 0.62 (95% CI 0.25–1.52), though the confidence intervals included the null value.

While the observed associations with arsenic methylation indices were imprecise and warrant cautious interpretation, the direction and magnitude of the relative measures reflected a pattern of lower detoxification of inorganic arsenic exposures among women with GDM.

**Keywords**

Arsenic; Inorganic arsenic; Methylation; Gestational diabetes

## 1 Introduction

Gestational diabetes mellitus (GDM), defined as glucose intolerance with onset or first recognition during pregnancy [1], is associated with a continuum of adverse perinatal and maternal outcomes. Pregnant women diagnosed with GDM have increased risk of late intrauterine fetal death, fetal macrosomia, congenital malformations, neonatal hypoglycemia, jaundice, cesarean delivery, maternal hypertension and subsequent development of type 2 diabetes [2]. Children born to mothers with GDM are also at increased risk of obesity, glucose intolerance, and the development of diabetes during late childhood or early adulthood [2]. The national prevalence of GDM in the U.S. was 6.0% in 2016, according to National Vital Statistics Birth Data [3]. While GDM risk factors such as maternal age and obesity are well recognized [4, 5], arsenic, an environmental contaminant and endocrine-disrupting metal, is also suspected to play a role in GDM development [6–13].

Arsenic is a naturally occurring element that is widely distributed across the Earth's surface and is typically found in the environment in its inorganic form. Arsenic-related toxicities are generally attributed to inorganic arsenic exposures, which have been associated with adverse human health effects including various cancers, skin disorders, and peripheral vascular disorders [14]. Exposure to inorganic arsenic occurs primarily through drinking water, while seafood intake is the major source of organic arsenic, which is generally less toxic [15]. Once arsenate (As5) from drinking water or food is absorbed in the human body, it is subsequently reduced to arsenite (As3) and undergoes methylation to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) in the liver, before being excreted through urine [16]. Inorganic arsenic species (As3 and As5) and their metabolic intermediates are toxic for humans [17]. Low arsenic methylation capacity characterized by higher proportions of MMA (MMA%) and lower proportions of DMA (DMA%) has been identified as a risk factor for multiple cancers and cardiovascular disease, while faster or more complete methylation indicated by lower MMA% and higher DMA% has been associated with increased risk of diabetes [18]. Previous U.S. studies have observed an increased prevalence of type 2 diabetes associated with low level arsenic exposure, measured

as total urinary concentrations [19, 20]. Lower MMA% was also associated with increased insulin resistance in a prospective cohort study among American Indians [21], suggesting that exposure to metabolic intermediates of inorganic arsenic may be etiologically-relevant for diabetes development.

To date, a small but growing number of epidemiologic studies have indicated that arsenic exposure may be associated with the development of GDM [6–11]. Methods of arsenic exposure assessment have varied across studies and included levels of total arsenic in blood [6, 8, 11], toenails [9], meconium [7], and inorganic arsenic exposure via tap water [9, 10]. Few prior GDM studies have evaluated urinary measures of inorganic arsenic species with limited or no reported assessment of arsenic methylation capacity [12, 13]. The present study aimed to address the existing research gap by examining associations between urinary inorganic arsenic concentrations, arsenic methylation capacity, and GDM.

## 2 Materials and Methods

### 2.1 Study design and population

To evaluate the association between urinary biomarkers of arsenic body burden during pregnancy and GDM, we analyzed stored urine specimens from a clinic-based case-control study of pregnant women with and without GDM who received prenatal care at the University of Oklahoma Health Sciences Center (OUHSC) Women's Clinic and High Risk Pregnancy Clinic. Patients were recruited for study participation between August 2009 and May 2010 following glucose screening routinely administered between 24 and 28 weeks of pregnancy. Eligible individuals included pregnant women without pre-existing diagnosis of type 1 or type 2 diabetes, ≥ 18 years old who were attending their first prenatal visit following glucose screening. Participation was restricted to English or Spanish speakers and women who resided within the 9 counties surrounding the clinic location (Oklahoma, Kingfisher, Logan, Lincoln, Pottawatomie, Cleveland, Canadian, McClain and Grady counties). GDM cases (n = 64) had a blood glucose level ≥ 135 mg/dL during a 1-hour 50-g glucose challenge test (GCT), and ≥ 2 values exceeding standard diagnostic thresholds (fasting: 95 mg/dL, 1 hour: 180 mg/dL, 2 hour: 165 mg/dL, 3 hour: 145 mg/dL) during a 3-hour 100-g oral glucose tolerance test (OGTT) or with initial GCT screening ≥ 200 mg/dL [1]. All patients receiving a GDM diagnosis were approached for participation during their first post-diagnostic visit at the High Risk Pregnancy Clinic. Unmatched controls (n = 237) were selected from patients who tested negative for GDM (GCT < 135 mg/dL or OGTT with < 2 values exceeding the diagnostic threshold). As patients who screened negative attended their post-screening visit at the Women's Clinic, they were approached consecutively for study recruitment. Consent was obtained from these participants to collect and store their urine specimens for future study. Participants also underwent a short interview to report demographic, behavioral, and medical characteristics. This study was approved by the OUHSC Institutional Review Board.

### 2.2 Urinary arsenic measurement

Urine samples were collected upon enrollment in sterile polypropylene containers and stored in a −20°C freezer. A 2-mL urine sample of each study participant was shipped frozen to

the Centers for Disease Control and Prevention (CDC), National Center for Environmental Health, Division of Laboratory Sciences in November 2011 for analysis of total arsenic and seven inorganic and organic species. Urinary total arsenic was measured by inductively coupled plasma-dynamic reaction cell mass spectrometry (ICP-DRC-MS). Analyses of speciated arsenic included measurements of inorganic-related arsenic species (As3, As5, MMA and DMA) and organic forms of arsenic species (arsenobetaine (AsB), arsenocholine (AsC) and trimethylarsine (TMO)), measured separately with high-performance liquid chromatography (HPLC) coupled to ICP-DRC-MS as described previously [22]. The limit of detection (LOD) for total arsenic, As3, As5, MMA, DMA, AsB, AsC, and TMO were 1.25, 0.48, 0.87, 0.89, 1.80, 1.19, 0.28, and 0.25 μg/L, respectively. Prior to analysis and summing of arsenic species to calculate arsenic methylation indices, total arsenic and arsenic species concentrations less than the LOD were assigned a value equal to the LOD divided by the square root of two (LOD/√2) [23].

### 2.3 Arsenic exposure and methylation capacity variables

Urinary arsenic concentrations may be subject to variation due to urinary dilution. Therefore, specific gravity (SG) was measured at the time of collection using a calibrated hand-held refractometer. Total arsenic and arsenic species concentrations were SG-adjusted using the formula: [(1.016−1)/(measured SG−1)] [24]. Measured SG was the SG of each urine sample, and 1.016 represented median SG value in our study population.

To assess inorganic arsenic exposure, we examined four strategies to account for organic contributions to total arsenic concentrations, as implemented in previous research [20, 25, 26]. First, we evaluated total arsenic and adjusted for AsB, a marker of seafood intake, as a covariate in the model. Second, we excluded the participants with detectable AsB concentrations from our analysis in an effort to control for recent seafood consumption. Third, we subtracted AsB and AsC concentrations, as measures of organic arsenic in diet, from total arsenic. Fourth, we summed the urinary concentrations of the four inorganic-related arsenic species (iSumAs), including the inorganic (As3 and As5) and methylated arsenic (MMA and DMA) species.

Measures of arsenic methylation capacity were examined among the 237 (46 cases, 191 controls) who had one or more inorganic and methylated arsenic species with concentrations above the LOD, given estimates of arsenic methylation patterns would not be accurately estimated in the presence of non-detectable inorganic arsenic exposure. We calculated the proportion of the arsenic species in urine [inorganic arsenic percentage (iAs%) = (As3 + As5)/iSumAs; MMA% = MMA/iSumAs; DMA% = DMA/iSumAs] [27]. An increased iAs% and increased MMA% each reflect an inefficient methylation capacity and prolonged exposure to more toxic species; whereas, higher DMA% reflects a more efficient methylation capacity [28]. In addition, we examined the secondary methylation index (SMI) defined as the ratio of DMA to MMA, where higher values reflect greater methylation capacity [27]. Although the primary methylation index (PMI) defined as the ratio of MMA to iAs was also of interest as a measure of methylation capacity [27], these calculations were insufficient for further examination given high proportion of non-detectable concentrations for MMA and iAs.

Due to the low proportion of detectable arsenic species, we classified concentrations according to detectable levels at or above the median of the distribution among controls, concentrations below the median, or non-detectable concentrations. For measures of inorganic arsenic exposure and arsenic methylation capacity, we categorized concentrations into tertiles based on the distribution of levels among controls and used the lowest tertile as the reference group.

### 2.4 Covariates measurement

Covariates were obtained from the questionnaire completed at enrollment. Factors evaluated as potential confounders included maternal age (assessed as a continuous measure, categories of < 25, 25–29, 30–34, and ≥ 35 years, and as a binary measure of < 30 and ≥ 30 years), race/ethnicity (Non-Hispanic White, African American, Hispanic, Asian, and Native American and as a binary measure of Hispanic and non-Hispanic), educational level (less than high school, high school, and more than high school), annual household income (≤ $9,999, $10,000–29,999, and ≥ $30,000), parity (0 and ≥1), and history of GDM diagnosis (yes/no). We also examined parity and history of GDM combined as a categorical variable with three categories indicating nulliparous, parous without GDM history, and parous with GDM history. Using self-reported pre-pregnancy height and weight, we calculated the pre-pregnancy body mass index (BMI) ($kg/m^2$). Pre-pregnancy BMI was classified as normal (BMI < 25), overweight (BMI 25–29.9) or obese (BMI ≥ 30) and assessed as a binary measure of obese and not obese. Active smoking status (yes/no) was defined as urinary cotinine concentration over 15 ng/mL [29] or self-report of current smoking status. Analysis of urinary cotinine was conducted using high resolution capillary-column gas chromatography with split/splitless injection, a fused silica capillary column and a thermionic specific detector (LOD: 1.0 μg/L).

### 2.5 Statistical analysis

Summary statistics (medians, interquartile range (IQR), percentages) were reported for demographics, lifestyle factors, and GDM history among GDM cases and controls and compared using chi-square tests for categorical variables and Wilcoxon rank-sum tests for continuous variables. Due to skewed distributions, the arsenic species and total arsenic concentrations were summarized using geometric mean (GM) concentrations (95% CI). The GM was not reported for the species that had a detectable proportion below 40%.

Multivariable logistic regression was then performed estimating odds ratios (ORs) and 95% confidence intervals (CIs) to evaluate the associations between inorganic arsenic exposure, arsenic species, arsenic methylation capacity indices, and GDM.

Potential confounding variables were evaluated using a manual forward selection approach to identify factors that changed the exposure ORs by 10% or more when the covariates were added to models assessing total arsenic and DMA%. We used the forward selection approach as an alternative to the backward deletion strategy, given the number of events in this study population were too limited to begin the confounding assessment with a model that simultaneously included all potential confounders [30]. When the change in the exposure OR was similar for different specifications of the same covariate and met the

criterion for confounding, we selected the measure with the fewest categories in order to maximize the number of covariates that could be supported by the number of GDM events in our logistic regression models. For instance, the ORs adjusted for race/ethnicity using either the binary measure (Hispanic or non-Hispanic) or the five original race/ethnicity categories resulted in similar percent change calculations both greater than 10%. Thus, we adjusted for race/ethnicity by using the binary race/ethnicity measure in our models. Age (<30 or ≥30 years), race/ethnicity (Hispanic or non-Hispanic), pre-pregnancy BMI (obese or not obese), current smoker (yes or no), and history of GDM (nulliparous or parous without GDM history versus parous with GDM history) met the criterion for confounding and were controlled for in the adjusted models. To further examine whether associations with indices of arsenic methylation capacity were independent of arsenic exposure levels, we evaluated separate models that additionally controlled for total arsenic concentrations (continuous measure). Adjusted ORs were not reported for arsenic species with cell counts less than five.

Given prior studies have suggested that pre-pregnancy obesity may potentially modify the association between arsenic concentrations and GDM [9–11], we explored effect modification by adding interaction terms for obesity status and the arsenic exposure indices to the models. A p-value < 0.05 for the interaction terms was used to conclude the presence of interaction. All statistical analyses were performed using SAS 9.4 (Cary, NC).

## 3 Results

Demographics, lifestyle factors, and GDM history are presented in Table 1. GDM cases tended to be older than controls (Median (IQR): 30.0 (9.0) vs. 24.0 (7.0) years, $p < 0.01$), with the majority of cases age 30–34 and most controls under age 25. A higher percentage of cases were Hispanic, had an educational level less than high school, and were less likely to be a current smoker compared to controls. The pre-pregnancy BMI of cases was higher than controls (Median (IQR): 30.18 (9.65) vs. 25.82 (10.17) $kg/m^2$, $p < 0.01$), and more than half of the cases had a pre-pregnancy BMI that was classified as obese. Parity did not differ between groups. Among parous women, 31% of cases reported having a GDM history compared to only 4.9% among controls ($p<0.01$).

Table 2 summarizes the percentage of observations above the LOD, GM concentrations (95% CI), and distribution percentiles among cases and controls for unadjusted and SG-adjusted urinary total arsenic and arsenic species concentrations. Except for total arsenic and DMA, we found the detectable proportions of As3, As5, MMA, AsB, AsC, and TMO were relatively low. Overall, total arsenic was detected in 97% of women, and 79% had at least one inorganic-related arsenic species that was higher than the LOD. DMA was the predominant arsenic species detected in 75% of women.

In Table 3, we evaluated the associations between SG-adjusted inorganic arsenic exposure, arsenic species, and GDM. No statistically significant associations with GDM were observed for total arsenic or iSumAs, even when we restricted analyses to women without detectable AsB or subtracted AsB and AsC concentrations from total arsenic (Table 3). Similarly, no evidence for association was observed for the individual arsenic species.

Associations between arsenic methylation capacity and GDM are shown in Table 4. ORs presented compare the upper and lower tertiles of inefficient arsenic methylation capacity, measured as iAs% and MMA%. ORs for efficient arsenic methylation capacity were also estimated comparing levels of DMA% and SMI among cases and controls for the upper and lower tertiles. We observed an increased odds of elevated iAs% levels among cases compared to controls, with the confidence interval including the null value (adjusted OR 1.48, 95% CI 0.58–3.77). ORs for MMA%, were in the same positive direction but were also statistically non-significant (adjusted OR 1.95, 95% CI 0.81–4.70). When examining markers of more efficient arsenic methylation capacity, the odds of being in the upper tertile of DMA% decreased 38% in cases compared to controls (adjusted OR 0.62, 95% CI 0.25–1.52). The OR for SMI similarly depicted an inverse relationship with GDM, and the confidence interval reflected a similar range of potential effects exceeding 1.0 (adjusted OR 0.55, 95% CI 0.23–1.33). Adjustment for total arsenic concentrations slightly attenuated the observed associations, but did not substantively change the conclusions. We observed no evidence of interaction between the arsenic indices and obesity status ($p > 0.05$).

## 4 Discussion

The results of this study expand the scope of evidence assessing the association between inorganic arsenic exposures and GDM in a population characterized by low-level arsenic exposure. Our assessment of arsenic methylation capacity indicates that detoxification capacity may be an important aspect of arsenic exposure assessment when evaluating associations with GDM. However, we were unable to rule out the lack of association as the null value was included in the effect estimate confidence intervals. We employed several strategies to measure arsenic exposure and took urinary dilution into account by adjusting exposure levels for specific gravity. Our study population had median unadjusted urinary total arsenic (5.74 μg/L), iSumAs (3.42 μg/L), and DMA (3.18 μg/L) levels that were less than median levels for US women participating in the National Health and Nutrition Examination Survey (NHANES) 2009–2010 (total arsenic: 7.63 μg/L, iSumAs: 5.64 μg/L, and DMA: 3.28 μg/L) [26]. The present study found no association between total arsenic and GDM after examining different strategies that accounted for the contribution of organic arsenic species from the diet. This finding was similar to previous studies examining associations between summed inorganic-related arsenic species exposure and GDM in low- to middle-level arsenic-exposed populations [9, 12]. We did observe a pattern of an increased odds of inefficient arsenic methylation capacity among cases compared to controls, through statistically non-significant. In contrast, in a study of Chilean pregnant women with low to moderate arsenic exposure (median concentrations of iSumAs: 14.95 μg/L) [12], mean estimates of arsenic methylation capacity measures (iAs%, MMA%, DMA%) were similar between GDM cases and non-GDM controls, but measures were not adjusted for confounding. Moreover, the reported mean values of arsenic methylation capacity among the controls of the Chilean study (iAs%: 8.5, MMA%: 8.6, DMA%: 82.8, and SMI: 14.6) were different when compared to the controls in our study (iAs%: 22.23, MMA%: 12.04, DMA%: 65.74, and SMI: 7.12). The study's lower detection limits and potential population differences in exposure and arsenic methylation capacity may be reasons for the inconsistencies observed. A Canadian pregnancy cohort using

DMA concentrations as a proxy for total inorganic arsenic reported an increased odds of GDM among women in the highest tertile of DMA concentrations [13]. However, due to the variations in the proportions of different arsenic metabolites in urine, excluding the contribution of As3, As5, and MMA for the measure of inorganic arsenic might not be an appropriate indicator of inorganic arsenic exposure [31]. This finding also contradicts previous evidence that increased methylation capacity reflects low retention of arsenic in tissues and decreases the risk of arsenic-related disease [28].

Currently, six epidemiological studies have reported evidence that arsenic exposure is related to increased risk of GDM. The earliest research was conducted among pregnant women residing near the Tar Creek Superfund site, an area contaminated with metals from mining waste in Ottawa County, Oklahoma [6]. In this population, a 2.79-fold increased odds of impaired glucose tolerance (IGT) was observed in pregnant women with postpartum blood total arsenic concentrations in the highest quartile of exposure (2.09–24.07 μg/L) (95% CI 1.13–6.87) relative to women in the lowest quartile (0.23–0.92 μg/L). Using blood samples to measure biomarkers of arsenic exposure, two cohort studies conducted in Canada and China measured exposure during the first trimester [8, 11]. In the Maternal-Infant Research on Environmental Chemicals (MIREC) study of Canada, pregnant women with the highest quartile of whole blood arsenic concentrations (≥ 1.3 μg/L) were found to have a 3.7-fold increased odds of GDM (95% CI 1.4–9.6) when compared to those with the lowest quartile (< 0.5 μg/L) [8]. Among the Ma'anshan Birth Cohort (MABC) study in China, the odds of GDM gradually increased with increasing quartile of serum arsenic concentrations with a significant trend [11]. In addition, other biomarkers such as those found in toenails, meconium, and water have also been used to explore the link between arsenic exposure and GDM in pregnancy. Peng et al. conducted a nested case-control study that reported an increased GDM prevalence in Chinese pregnant women as quartiles of meconium arsenic concentrations increased [7]. Arsenic measured in maternal toenails two weeks postpartum has also shown a positive association with GDM in the New Hampshire Birth Cohort [9]. An ecological study provided evidence that arsenic exposure from drinking water was associated with GDM [10]. The odds of developing GDM were 1.62 times higher among pregnant women who used tap water that contained arsenic concentrations ≥ 10 μg/L compared to < 10 μg/L, (95% CI 1.01–2.53). Although previous results lend support to the association between maternal arsenic exposure and hyperglycemia status during pregnancy, these studies were unable to distinguish between inorganic arsenic and organic arsenic and, thus, were susceptible to potential exposure misclassification.

The evaluation of different arsenic biomarkers may lead to discrepant results. Although drinking water is a source of inorganic arsenic exposure, ingestion of both inorganic and organic arsenic can occur through consumption of seafood, rice, cereal, mushrooms, and poultry [32]. Therefore, relying only on water arsenic to characterize arsenic exposure may underestimate exposure levels and bias associations toward the null. In addition, using ecological measures of arsenic in area water sources may not reflect individual arsenic level, leading to potential measurement error. In contrast, using total arsenic in blood may overestimate exposure levels because it would include AsB and AsC from seafood, which are the nontoxic forms of organic arsenic [15]. Our study and those of Farzan et al. [9] and Munoz et al. [12] assessed total urinary inorganic arsenic levels by summing As3,

As5 and its metabolites MMA and DMA in urine, which is a more direct measurement of inorganic arsenic exposure that is not affected by organic arsenic from seafood [31]. Exposure assessment using urinary biomarkers of arsenic speciation additionally offers the opportunity to examine markers of inorganic arsenic metabolism.

Various biological specimens containing arsenic reflect different etiologically relevant time windows based on the time of sample collection and methods of exposure assessment. The half-life of absorbed arsenic in the human body is about 4 days and is widely different in the blood (a few hours) and urine (4 days) [31]. Although arsenic concentrations in blood and urine remain only a short time after absorption, the values could remain relatively stable reflecting chronic arsenic exposure if pregnant women are continuously and steadily exposed to arsenic and have no change in their lifestyle [31, 33]. Ettinger et al. reported that for pregnant women in the MIREC cohort, blood arsenic median levels were significantly higher in the first trimester (0.82 μg/L) compared to the third trimester (0.69 μg/L) [34]. Xia et al. found only first trimester serum arsenic measures to be associated with GDM [11]. Thus, to avoid measurement error attributed to lifestyle change, it is optimal to collect the blood or urine before the diagnosis of GDM. The present study assessed urinary arsenic exposure at the clinical visit immediately following mid-pregnancy glucose screening. Thus, our urinary arsenic assessment may not reflect exposures that occurred within the etiologically relevant window for GDM. The null associations observed in our study could possibly be attributed to exposure misclassification.

Previous experimental studies indicated that arsenic may trigger diabetes. In vivo research observed that mice exposed to 50 ppm sodium arsenite for eight weeks developed glucose intolerance and had decreased plasma insulin levels when treated with 5 ppm arsenic trioxide in drinking water for six consecutive weeks [35, 36]. Results supported by in vitro studies reported that arsenite exposure led to a decrease in insulin mRNA expression involved in insulin signal transduction or induced pancreatic β-cell apoptosis followed by reduced insulin secretion [35–37]. Until now, only two studies have been conducted in pregnant animals. Results of a study conducted in mice that were intraperitoneally injected with 9.6 mg/kg sodium arsenate on gestational day 7.5 and 8.5 showed glucose intolerance and a higher homeostatic model assessment for insulin resistance (HOMA-IR) than controls [38]. However, a difference in insulin levels was not observed between the injected mice and the control groups. In a study by Bonaventura et al. [39], pregnant rats that had been treated with 50 mg/L of sodium arsenite in drinking water from gestational day 1 to postpartum showed glucose intolerance and decreased insulin secretion on days 16 and 17 of pregnancy. Conversely, fasting glucose and insulin and HOMA-IR did not differ between treated and control groups. This study revealed that arsenic may alter the pancreatic beta cell and cause glucose imbalance and enhanced risk of GDM [39]. Findings from two gestational animal models were conflicting, probably because of differences in animal species, route, and concentrations. Most studies conducted on high arsenic levels in animals are incomparable with current U.S. maximum contaminant level (10 μg/L) of low-level human exposures. Future animal studies are needed to clarify the mechanisms of chronic low-level arsenic exposure in GDM.

With a wide range of glucose abnormalities, some studies combined IGT and GDM into one category [6, 7], but other studies combined IGT with normal glucose [10, 11]. IGT represents the status of pre-diabetes that falls between normal glucose and GDM. Whether IGT is combined with the normal glucose or the GDM groups, these two scenarios would make the GDM and non-GDM groups more similar with regard to glucose dysregulation. In a study by Farzan et al., increased toenail arsenic concentrations were linked to the increased risk of GDM but not when IGT and GDM were combined [9]. Thus, the association between arsenic and GDM would be underestimated when IGT is combined with the GDM groups. In our study, 41 of our 237 controls had an initial screening GCT > 135 mg/dL prompting referral for a diagnostic OGTT. Only 7 of these controls were classified as IGT according to a single abnormal value on the 3-hour OGTT. Although the numbers are limited, the inclusion of women with IGT in the control group may have biased comparisons toward the null.

Several studies revealed that pre-pregnancy BMI may potentially modify the effect of arsenic on GDM. Mechanisms proposed for this joint effect include increased arsenic methylation efficiency in obesity and lowered arsenic retention causing increased arsenic excretion [40, 41]. BMI could also possibly be related to other dietary factors associated with arsenic burden [42, 43]. By using water arsenic estimates, Marie et al. and Farzan et al. showed that the association between water arsenic levels and GDM could be enhanced in women who had high pre-pregnancy BMI [9, 10], whereas Xia et al.'s study reported that the risk of blood arsenic on GDM was only observed in women with normal weight [11]. Prior research indicated that high maternal pre-pregnancy BMI was a risk factor for GDM [44, 45]. Also, overweight female adults reportedly have lower toenail arsenic than those with normal weight [46]. In our study, we observed no meaningful difference when the association between inorganic arsenic exposure and GDM was stratified by obesity status. This result is consistent with those reported by a Chilean study which demonstrated that obesity status did not modify the association between urinary inorganic arsenic and GDM [12]. Previous studies have not sufficiently investigated the potential interaction between pre-pregnancy BMI and arsenic on the risk of GDM. Further studies with larger sample sizes are needed to explore potential effect measure modification of the association between arsenic methylation capacity and GDM by pre-pregnancy BMI.

There were several limitations to the present study. We assessed urinary arsenic concentrations with single spot urine at a time proximate to blood glucose testing. Because arsenic is rapidly metabolized and excreted from the body, urinary measurements would primarily reflect exposures in the days immediately preceding specimen collection. However, a previous study on the intra-individual variability of urinary arsenic exposure indicated that measurements were stable over time and may be relatively well characterized by the use of a single sample [47]. The case-control study design of our study does not establish the temporality of the relationship between urinary arsenic concentrations, methylation capacity, and GDM. Urinary arsenic concentrations and methylation capacity may have been distorted by GDM development. Further, residual confounding by uncontrolled factors such as diet and exercise might have influenced our results [48]. In addition, detectable proportions of most arsenic species were relatively low in this study. Our study used the substitution of LOD/√2 for measures below the LOD, which may be

prone to biased estimation compared to a more complex multiple imputation procedure [49]. Furthermore, when an individual has more than one imputed value for arsenic species measured at levels below the LOD, the calculations of arsenic methylation indices may not accurately reflect patterns of methylation in the presence of low levels of arsenic exposure [21]. Our use of exposure tertiles, however, may serve to diminish the impact of this measurement error as individuals within the high, medium and low ranges of the distributions are grouped together. Future research will benefit as laboratory techniques advance to achieve more sensitive detection limits [50] and methods of complex multiple imputation procedures are considered in the context. Lastly, the sample size of the present study was small and limited statistical power, the precision of point estimates, and the ability to simultaneously assess multiple confounders. This is particularly noteworthy when our analyses of total arsenic concentrations excluded participants with detectable AsB concentrations and in analyses of the association between arsenic methylation capacity and GDM, which further reduced the sample size. Despite these limitations, this study advances the limited knowledge of arsenic exposure in the context of GDM development. We incorporated analytic advances in arsenic speciation to improve exposure assessment by better isolating arsenic concentrations from inorganic compounds. Moreover, we corrected the urinary arsenic metabolites by specific gravity, which could be considered a robust measure for adjustment of urine concentration to assess urinary excretion of substances [51].

## 5 Conclusions

In summary, while the observed associations with arsenic methylation indices were imprecise, the direction and magnitude of the relative measures reflected a pattern of lower detoxification of inorganic arsenic exposures among women with GDM. Our study, however, did not observe evidence of positive associations with urinary concentrations of individual arsenic species, total arsenic, or the sum of inorganic-related arsenic species. These findings support the need for refining exposure assessments to exclude organic arsenic intake and to consider the role of methylation capacity in studies of arsenic and GDM. To further improve exposure assessment, additional studies should incorporate repeated measures of arsenic species during pregnancy and investigate the etiologically relevant time window of inorganic arsenic exposure.

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**Table 1.**

Distribution of demographics, lifestyle factors, and GDM history for cases and controls.

| Variables | Cases (n = 64) N (%) | Controls (n = 237) N (%) | *P*-value[c] |
|---|---|---|---|
| Age (years) | | | < 0.01 |
| < 25 | 18 (28.1) | 134 (56.5) | |
| 25 – 29 | 12 (18.8) | 63 (26.6) | |
| 30 – 34 | 24 (37.5) | 24 (10.1) | |
| ≥ 35 | 10 (15.6) | 16 (6.8) | |
| Race/Ethnicity | | | < 0.01 |
| Non-Hispanic White | 16 (25.0) | 70 (29.5) | |
| African American | 6 (9.4) | 79 (33.3) | |
| Hispanic | 40 (62.5) | 70 (29.5) | |
| Other | 2 (3.1) | 18 (7.6) | |
| Educational level | | | 0.02 |
| Less than high school | 32 (50.0) | 73 (30.8) | |
| High school | 18 (28.1) | 89 (37.6) | |
| More than high school | 14 (21.9) | 75 (31.7) | |
| Annual household income | | | 0.45 |
| ≤ $9,999 | 26 (40.6) | 117 (49.4) | |
| $10,000 – $29,999 | 31 (48.4) | 96 (40.5) | |
| ≥ $30,000 | 7 (10.9) | 24 (10.1) | |
| Pre-pregnancy BMI ($kg/m^2$)[a] | | | < 0.01 |
| Normal (≤ 24.99) | 13 (20.6) | 107 (45.5) | |
| Overweight (25.0–29.99) | 17 (27.0) | 52 (22.1) | |
| Obese (≥ 30.0) | 33 (52.4) | 76 (32.3) | |
| Active smoker[b] | | | 0.04 |
| No | 55 (85.9) | 175 (73.8) | |
| Yes | 9 (14.1) | 62 (26.2) | |
| Parity | | | 0.62 |
| 0 | 13 (20.3) | 55 (23.2) | |
| ≥1 | 51 (79.7) | 182 (76.8) | |
| Self-reported history of GDM | | | < 0.01 |
| Nulliparous | 13 (20.3) | 55 (23.2) | |
| Parous without GDM history | 35 (54.7) | 173 (73.0) | |
| Parous with GDM history | 16 (25.0) | 9 (3.8) | |

[a] Pre-pregnancy BMI data was missing for one case and two controls.

[b] Self-reported currently smoking or urinary cotinine over 15 ng/mL for active smoker.

[c] *P*-value based on chi-square test.

**Table 2.**

The percentage of observations above LOD, GM (95% CI), and distribution percentiles for urinary total arsenic and arsenic species concentrations for cases and controls.

| | | | Unadjusted (μg/L) | | | | SG-adjusted (μg/L) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | % > LOD | GM (95% CI) | P50 | P75 | P90 | GM (95% CI) | P50 | P75 | P90 |
| Total As | Cases | 96.9 | 6.03 (4.83–7.53) | 5.39 | 10.20 | 19.45 | 6.76 (5.57–8.20) | 5.59 | 10.79 | 17.33 |
| | Controls | 99.2 | 6.56 (5.80–7.41) | 5.74 | 9.88 | 21.15 | 7.32 (6.55–8.18) | 6.04 | 10.29 | 20.47 |
| iSumAs[a] | Cases | | 4.83 (4.05–5.76) | 3.02 | 6.46 | 10.90 | 4.62 (3.87–5.52) | 3.13 | 6.05 | 8.88 |
| | Controls | | 4.63 (4.19–5.12) | 3.42 | 5.56 | 10.27 | 4.46 (4.04–4.94) | 3.28 | 5.74 | 10.24 |
| As3 | Cases | 10.9 | - | < LOD | < LOD | 0.49 | - | < LOD | < LOD | 0.37 |
| | Controls | 14.8 | - | < LOD | < LOD | 0.56 | - | < LOD | < LOD | 0.44 |
| As5 | Cases | 18.8 | - | < LOD | < LOD | 1.16 | - | < LOD | < LOD | 1.24 |
| | Controls | 15.6 | - | < LOD | < LOD | 1.70 | - | < LOD | < LOD | 1.70 |
| iAs[b] | Cases | | 1.37 (0.91–2.07) | < LOD | 0.50 | 1.61 | 1.24 (0.81–1.91) | < LOD | 0.37 | 1.56 |
| | Controls | | 1.37 (1.09–1.73) | < LOD | 0.55 | 1.88 | 1.36 (1.02–1.82) | < LOD | 0.38 | 1.78 |
| MMA | Cases | 9.4 | - | < LOD | < LOD | < LOD | - | < LOD | < LOD | < LOD |
| | Controls | 11.0 | - | < LOD | < LOD | 0.98 | - | < LOD | < LOD | 0.63 |
| DMA | Cases | 68.8 | 4.22 (3.53–5.03) | 2.66 | 4.37 | 10.70 | 4.00 (3.32–4.82) | 2.59 | 4.52 | 8.10 |
| | Controls | 76.4 | 4.26 (3.90–4.65) | 3.18 | 4.86 | 8.16 | 3.93 (3.59–4.30) | 2.90 | 4.41 | 7.25 |
| AsB | Cases | 25.0 | - | < LOD | 0.66 | 6.40 | - | < LOD | 0.70 | 7.46 |
| | Controls | 23.2 | - | < LOD | < LOD | 5.98 | - | < LOD | < LOD | 5.72 |
| AsC | Cases | 1.6 | - | < LOD | < LOD | < LOD | - | < LOD | < LOD | < LOD |
| | Controls | 1.3 | - | < LOD | < LOD | < LOD | - | < LOD | < LOD | < LOD |
| TMO | Cases | 1.6 | - | < LOD | < LOD | < LOD | - | < LOD | < LOD | < LOD |
| | Controls | 2.5 | - | < LOD | < LOD | < LOD | - | < LOD | < LOD | < LOD |

Abbreviations: Total As, urinary total arsenic; iSumAs, Sum of urinary inorganic-related arsenic species; As3, arsenite; As5, arsenate; iAs, inorganic arsenic; MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; AsB, arsenobetaine; AsC, arsenocholine; TMO, trimethylarsine oxide; SG, specific gravity; LOD, limit if detection; GM, geometric mean.

One participant was unavailable for As3, As5, MMA, DMA, AsB, AsC, and TMO, due to insufficient sample.

The detectable proportions of As3, As5, MMA, AsB, AsC, and TMO were below 40%; thus, the GM was not summarized for these species.

[a] iSumAs = As3 + As5 + MMA + DMA

[b] iAs = As3 + As5

**Table 3.**

Associations between SG-adjusted inorganic arsenic exposure, arsenic species, and GDM.

| Variables | Cases N (%) | Controls N (%) | Unadjusted OR (95% CI) | Adjusted OR (95% CI) |
|---|---|---|---|---|
| Total As (μg/L)[c] | | | | |
| ≤ 4.72 | 22 (34.4) | 79 (33.3) | 1.00 | 1.00[e] |
| 4.73 – 8.07 | 21 (32.8) | 79 (33.3) | 0.95 (0.49–1.87) | 0.71 (0.32–1.57) |
| ≥ 8.08 | 21 (32.8) | 79 (33.3) | 0.95 (0.49–1.87) | 0.77 (0.33–1.79) |
| Total As (μg/L)[a,c] | | | | |
| ≤ 4.32 | 20 (41.7) | 61 (33.7) | 1.00 | 1.00[f] |
| 4.33 – 6.32 | 14 (29.2) | 60 (33.2) | 0.71 (0.33–1.54) | 0.44 (0.17–1.13) |
| ≥ 6.33 | 14 (29.2) | 60 (33.2) | 0.71 (0.33–1.54) | 0.70 (0.29–1.71) |
| Total As (μg/L)[b,c] | | | | |
| ≤ 3.42 | 23 (37.1) | 78 (33.5) | 1.00 | 1.00[f] |
| 3.42 – 5.95 | 20 (32.3) | 78 (33.5) | 0.87 (0.44–1.71) | 0.74 (0.34–1.63) |
| ≥ 5.95 | 19 (30.6) | 77 (33.1) | 0.84 (0.42–1.66) | 0.67 (0.30–1.49) |
| iSumAs (μg/L)[c] | | | | |
| ≤ 4.54 | 23 (35.9) | 79 (33.5) | 1.00 | 1.00[f] |
| 4.55 – 6.75 | 17 (26.6) | 79 (33.5) | 0.74 (0.37–1.49) | 0.66 (0.29–1.49) |
| ≥ 6.76 | 24 (37.5) | 78 (33.1) | 1.06 (0.55–2.03) | 0.73 (0.34–1.61) |
| As3 (μg/L)[d] | | | | |
| Non-detectable | 57 (89.1) | 201 (85.2) | 1.00 | |
| ≤ 0.50 | 3 (4.7) | 18 (7.6) | 0.59 (0.17–2.07) | - |
| ≥ 0.51 | 4 (6.3) | 17 (7.2) | 0.83 (0.27–2.56) | - |
| As5 (μg/L)[d] | | | | |
| Non-detectable | 52 (81.3) | 199 (84.3) | 1.00 | 1.00[f] |
| ≤ 2.04 | 7 (10.9) | 19 (8.1) | 1.41 (0.56–3.53) | 1.24 (0.39–3.93) |
| ≥ 2.05 | 5 (7.8) | 18 (7.6) | 1.06 (0.38–3.00) | 1.62 (0.50–5.22) |
| iAs (μg/L)[d] | | | | |
| Non-detectable | 47 (73.4) | 170 (72.0) | 1.00 | 1.00[f] |
| ≤ 1.19 | 9 (14.1) | 33 (14.0) | 0.99 (0.44–2.21) | 1.26 (0.48–3.33) |
| ≥ 1.20 | 8 (12.5) | 33 (14.0) | 0.88 (0.38–2.03) | 1.07 (0.40–2.91) |
| MMA (μg/L)[d] | | | | |
| Non-detectable | 58 (90.6) | 210 (89.0) | 1.00 | |
| ≤ 1.00 | 2 (3.1) | 13 (5.5) | 0.56 (0.12–2.54) | - |
| ≥ 1.01 | 4 (6.3) | 13 (5.5) | 1.11 (0.35–3.55) | - |
| DMA (μg/L)[d] | | | | |
| Non-detectable | 20 (31.3) | 55 (23.3) | 1.00 | 1.00[f] |

| Variables | Cases N (%) | Controls N (%) | Unadjusted OR (95% CI) | Adjusted OR (95% CI) |
|---|---|---|---|---|
| ≤ 3.46 | 20 (31.3) | 90 (38.1) | 0.61 (0.30–1.24) | 0.70 (0.30–1.62) |
| ≥ 3.47 | 24 (37.5) | 91 (38.6) | 0.73 (0.37–1.43) | 0.55 (0.24–1.24) |
| AsB (μg/L)[d] | | | | |
| Non-detectable | 48 (75.0) | 181 (76.7) | 1.00 | 1.00[f] |
| ≤ 4.13 | 8 (12.5) | 28 (11.9) | 1.08 (0.46–2.52) | 0.79 (0.29–2.20) |
| ≥ 4.14 | 8 (12.5) | 27 (11.4) | 1.12 (0.48–2.62) | 0.67 (0.23–1.93) |

Abbreviations: Total As, urinary total arsenic; iSumAs, Sum of urinary inorganic-related arsenic species; As3, arsenite; As5, arsenate; iAs, inorganic arsenic; MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; AsB, arsenobetaine; SG, specific gravity.

Adjusted ORs were not reported for arsenic species with cell counts less than five.

[a] Excludes subjects with detectable levels of AsB.

[b] Subtracts AsB and AsC concentrations from Total As.

[c] Concentrations split at the tertile of control distribution.

[d] Women with non-detectable concentrations served as referent group; detectable concentrations split at the median of control distribution.

[e] Adjusted for age, race/ethnicity, pre-pregnancy BMI, smoking status, self-reported history of GDM, and AsB.

[f] Adjusted for age, race/ethnicity, pre-pregnancy BMI, smoking status, and self-reported history of GDM.

**Table 4.**

Association between arsenic methylation capacity indices and GDM.

| Variables | Cases N (%) | Controls N (%) | Unadjusted OR (95% CI) | Adjusted OR (95% CI)[e] | Adjusted OR (95% CI)[f] |
|---|---|---|---|---|---|
| iAs%[a] | | | | | |
| ≤ 16.74 % | 16 (34.8) | 64 (33.5) | 1.00 | 1.00 | 1.00 |
| 16.74 – 23.09 % | 16 (34.8) | 64 (33.5) | 1.00 (0.46–2.17) | 1.57 (0.63–3.90) | 1.50 (0.60–3.78) |
| ≥ 23.09 % | 14 (30.4) | 63 (33.0) | 0.89 (0.40–1.97) | 1.48 (0.58–3.77) | 1.44 (0.56–3.68) |
| MMA%[b] | | | | | |
| ≤ 9.94 % | 15 (32.6) | 64 (33.5) | 1.00 | 1.00 | 1.00 |
| 9.94 – 13.82 % | 11 (23.9) | 64 (33.5) | 0.73 (0.31–1.72) | 1.12 (0.42–3.00) | 1.08 (0.40–2.96) |
| ≥ 13.82 % | 20 (43.5) | 63 (33.0) | 1.35 (0.64–2.88) | 1.95 (0.81–4.70) | 1.85 (0.74–4.63) |
| DMA%[c] | | | | | |
| ≤ 62.53 % | 17 (37.0) | 63 (33.0) | 1.00 | 1.00 | 1.00 |
| 62.53 – 71.89 % | 13 (28.3) | 64 (33.5) | 0.75 (0.34–1.68) | 0.84 (0.34–2.06) | 0.84 (0.34–2.06) |
| ≥ 71.89 % | 16 (34.8) | 64 (33.5) | 0.93 (0.43–1.99) | 0.62 (0.25–1.52) | 0.64 (0.26–1.61) |
| SMI[d] | | | | | |
| ≤ 4.47 | 19 (41.3) | 64 (33.5) | 1.00 | 1.00 | 1.00 |
| 4.47 – 6.59 | 11 (23.9) | 63 (33.0) | 0.59 (0.26–1.34) | 0.60 (0.24–1.48) | 0.61 (0.25–1.51) |
| ≥ 6.59 | 16 (34.8) | 64 (33.5) | 0.84 (0.40–1.78) | 0.55 (0.23–1.33) | 0.58 (0.23–1.43) |

Abbreviations: iAs%, inorganic arsenic percentage; MMA%, monomethylarsonic acid percentage; DMA%, dimethylarsinic acid percentage; SMI, secondary methylation index.

[a] iAs% = iAs / iSumAs

[b] MMA% = MMA / iSumAs

[c] DMA% = DMD / iSumAs

[d] SMI = DMA / MMA

[e] Adjusted for age, race/ethnicity, pre-pregnancy BMI, smoking status, and self-reported history of GDM.

[f] Adjusted for age, race/ethnicity, pre-pregnancy BMI, smoking status, self-reported history of GDM, and Total As.