Maternal Arsenic Exposure and DNA Damage Biomarkers, and the Associations with Birth Outcomes in a General Population from Taiwan

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Abstract

Inorganic arsenic (iAs) is an established transplacental agent known to affect fetal development in animal studies. However, iAs has not been adequately studied in the general population with respect to iAs exposure during pregnancy and its impact on the health status of newborns. The aims of this study were to 1) elucidate the association between arsenic exposure and oxidative/methylated DNA damage in pregnant women, and 2) determine the association with birth outcomes. A birth cohort study of 299 pregnant mother-newborn pairs was recruited during 2001–2002 in Taiwan. We collected maternal urine samples during the 3rd trimester for measuring iAs and its metabolites. We used high-performance liquid chromatography/inductively coupled plasma mass spectrometry (HPLC-ICP-MS) for quantifications of the arsenic species. Liquid chromatography/tandem mass spectrometer (LC-MS/MS) was used to measure the 8-oxodG and N7-methylguanosine (N7-MeG) DNA damage biomarkers. Birth outcomes were collected to assess the associations with maternal arsenic exposure and the DNA damage biomarkers. Multiple regression analyses showed that maternal urinary iAs had positive associations with the methylated N7-MeG (beta = 0.35, p < 0.001) and oxidative 8-oxodG (beta = 0.24, p < 0.001) DNA damage biomarkers, and a decreased one-minute (1-min) Apgar score (beta = -0.23, p = 0.041). Maternal N7-MeG was also associated with a decreased 1-min Apgar score (beta = -0.25, p = 0.042). Mutual adjustment for iAs and N7-MeG showed an independent and significant prediction for a decreased 1-min Apgar score of iAs (beta = -0.28, p = 0.036). Maternal iAs exposure was associated with both maternal DNA damage and adverse newborn health. Maternal N7-MeG levels might be a novel biomarker for monitoring fetal health related to iAs.

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Introduction

Health status at birth is an important determinant of morbidity and mortality in early childhood [1] and of chronic disease in adulthood [2]. For example, birth weight is not only reflective of maternal health status, but also predictive of the probability for newborn survival, development, and long-term health [3]. The Apgar score is a routine for evaluating the physical condition of the newborn, including heart rate, respiratory effort, muscle tone, reflex irritability, and skin color shortly after delivery. A score ≥7 indicates that the condition of the newborn is good-to-excellent [4]. Otherwise, immediate extra medical care or even an intensive care unit admission would be necessary. Newborns with low birth weight (LBW) or low Apgar scores often develop various negative health consequences. Long-term effects of LBW include increased risk of cardiovascular disease, type 2 diabetes mellitus, and impaired reproductive function [5]. Several studies have shown that low Apgar scores may be associated with an increased risk of reduced cognitive function and increased learning difficulties later in life [6]. Arsenic is a well-known toxicant and carcinogen, and increasing evidence indicates that arsenic may adversely affect pregnancy outcomes and development of the newborn.

Arsenic easily crosses the placenta [7], and even moderate exposure to arsenic during pregnancy has been associated with adverse health outcomes in the fetus [8]. Studies have shown that prenatal arsenic exposure is inversely associated with birth weight in Bangladesh [9,10], and Inner Mongolia [11]. Prenatal arsenic exposure at low-to-moderate levels might also have effects on the fetus, but more evidence is needed [12].

Disrupted placentation [13] and endocrine disturbance [14] have been reported for arsenic-related adverse pregnancy outcomes. However, the mechanisms require further investigation.
Studies of mice have reported that arsenic leads to an increase in oxidative stress and a subsequent increase in 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) in tissues of tested animals [15,16]. Increased urinary 8-oxodG in pregnant women has been linked to arsenic exposure [17]. Recently, maternal DNA damage mediated by arsenic exposure has been proposed as one mechanism responsible for fetal programming [18]. There is, however, little information on the formation of methylated DNA damage induced by arsenic exposure. Arsenic-treated mice have an arsenic-related increase in hepatic N7-methylguanine (N7-MeG), a marker of methylated DNA damage that reflects the overall rate of DNA methylation [19,20], but this finding has not been confirmed in humans.

We sought to determine whether prenatal exposure to low-to-moderate levels of arsenic is associated with maternal oxidative/methylated DNA damage, and to evaluate the associations with birth outcomes. We found decreased Apgar score was associated with arsenic-related increase in hepatic N7-methylguanine (N7-MeG), a marker of methylated DNA damage that reflects the overall rate of DNA methylation [19,20], but this finding has not been confirmed in humans.

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Birth length might be correlated with the physical condition of the baby, such as body relaxation, and head and chest girth with the mode of delivery (i.e., normal spontaneous delivery, cesarean). In the present study, adverse birth outcomes in newborns were based on LBW and low Apgar scores. LBW newborns were defined as newborns with a birth weight <2,500 g. Newborns with low Apgar scores had a 1- or 5-min Apgar score <7 [4]. Information on demographic or socioeconomic factors, or other factors that confounded the associations between maternal arsenic exposure, maternal DNA damage, and newborn birth outcomes was collected. We used an administrative questionnaire to obtain the demographic data of the pregnant women, such as age, parity, education, and health-related data (medical history, as well as cigarette smoking and alcohol use before and after pregnancy).

Statistical analysis

We assessed the associations between maternal arsenic exposure (iAs, MMA, and DMA), maternal DNA damage (8-oxodG and N\textsuperscript{7}-MeG), and newborn health parameters (birth weight, birth length, head circumference, chest girth, and Apgar scores). Arsenic metabolites and DNA damage biomarker levels under LOD were recorded as one-half of the LOD values. All variables were assessed for normal distribution or natural logarithmic-transformation to approximate a normal distribution. Specifically, all urinary arsenic species levels were log-transformed, and the urinary levels of 8-oxodG and N\textsuperscript{7}-MeG were natural log-transformed to obtain a normal distribution. Covariates assessed included maternal age at delivery, mode of delivery, pre-pregnancy BMI, gestational age, newborn sex, prenatal alcohol consumption, and maternal education.

To assess differences in the characteristics between subjects who were followed and subjects lost to follow-up, we used an independent sample t-test for continuous variables and the \( \chi^2 \) method for categorical variables (see Supplemental Material, Table S1). Pearson correlations were used to explore the associations between maternal arsenic levels, DNA damage, newborn health status, and other covariates. Significantly correlated variables were further analyzed in multivariable regression models adjusting for potential confounders. Potential confounders were identified based on correlations (\( p<0.1 \)) with arsenic exposure and birth outcomes. There were no significant differences in the relationships in the birth outcomes and maternal iAs between the sexes; we combined data from female and male newborns to increase the statistical power.

Maternal age, pre-pregnant BMI, gestational age, mode of delivery, and newborn sex were included in the final model. We used birth outcomes as both continuous and binary variables in the linear regression model and the Cox’s proportional hazards model, respectively. Statistical significance was set at a \( p<0.05 \). For the Cox’s proportional hazards model estimates, we transformed continuous variables into a two-level scale to compare the above and below medians of the arsenic metabolites and DNA damage biomarkers. We calculated the 95% CIs of the relative risks from the corresponding regression coefficients and standard errors. All statistical analyses were performed using SPSS 18.0 software (SPSS, Inc., Chicago, IL, USA).

Results

General characteristics of the subjects

Table 1 presents the characteristics of pregnant women and the newborns by sex. Of the 299 newborns, 151 (51%) were male and 148 (49%) were female. Female newborns had significantly lower birth weights than the male newborns (3170 vs. 2905 gm, \( p=0.001 \)). Twenty-one (7%) newborns had a LBW (<2,500 g), 13 (61.9%) of which were female. Sixty-nine (23%) newborns had 1-min Apgar scores <7, of which 40 were male (\( p=0.15 \) for sex difference). In addition, there were 4 female newborns with low 5-min Apgar scores as compared with none in males. Only 5 (1.7%)
of women consumed alcohol during pregnancy. The characteristics for the excluded twins were similar to the included twins. Women lost to follow-up had a significantly lower average gestational age and greater income than the women who were followed. In addition, the mothers followed tended to be older and had slightly lower pre-pregnant BMIs than the mothers lost to follow-up ($p<0.1$). No significant differences existed in parity, newborn birth outcomes, arsenic levels, mode of delivery, or maternal education and life styles between the groups (see Supplemental Material Table S1).

Maternal arsenic exposure and DNA damage

The levels and distribution of arsenic metabolites in maternal urine are shown in Table 2. The median (5th–95th) levels of creatinine-adjusted urinary As$_3^+$, As$_5^+$, MMA, DMA, iAs, and tAs were 0.30 (0.06–1.77), 0.21 (0.05–2.95), 0.46 (0.07–5.82), 20.01 (1.43–69.94), 0.79 (0.18–3.96), and 22.26 (2.80–83.0) μg g$^{-1}$ cre$^{-1}$, respectively. Among all arsenic metabolites, the frequency of samples with arsenic metabolite levels above the LODs was 92%. The median (5th–95th) concentrations of creatinine-adjusted urinary 8-oxodG and N7-MeG were 3.48 (1.59–8.56) mg g$^{-1}$ cre and 12.88 (6.47–25.57) mg g$^{-1}$, respectively (Table 2).

Maternal arsenic exposure, oxidative/methylated DNA damage, and birth outcomes

Table 3 shows a significant positive correlation between maternal 8-oxodG levels and iAs ($r=0.24$, $p<0.001$), MMA ($r=0.16$, $p<0.001$), DMA ($r=0.13$, $p<0.05$), and tAs ($r=0.17$, $p<0.001$). Similarly, a significant positive correlation was shown between the maternal N$^7$-MeG levels and iAs ($r=0.33$, $p<0.001$) and the metabolites. In addition, a significant positive correlation was shown between maternal 8-oxodG levels and N$^7$-MeG.
A negative correlation of $-0.13$ ($p = 0.08$) was found between maternal iAs levels and the 1-min Apgar scores of newborns. A significant positive correlation was noted between the maternal MMA levels and the 1-min Apgar scores of newborns ($r = 0.15$, $p = 0.03$). A suggested negative correlation might exist between the maternal N$^7$-MeG levels and the 1-min Apgar scores of newborns ($r = -0.18$, $p = 0.06$). The maternal MMA and DMA levels were not significantly correlated with the birth outcomes of birth weight, length, and head and chest circumferences.

### Table 2. Distribution of creatinine-adjusted concentrations of urinary arsenic species (iAs, MMA, and DMA) and urinary DNA damage biomarkers (8-oxodG and N$^7$-MeG) for pregnant women in the present study versus two others with established arsenic-related effects $(n = 299)$.

| Exposure variables$^a$ | < LOD $^b$ | Percentile$^b$ |
|------------------------|------------|--------------|
|                        | $(n)$      | Min $^{5\text{th}}$ | $25\text{th}$ | $50\text{th}$ | $75\text{th}$ | $95\text{th}$ | Max $^c$ |
| As metabolites $(\mu g \text{ g cre}^{-1})$ | | | | | | | |
| As$^{3+}$ | 14 | 0.02 | 0.06 | 0.16 | 0.30 | 0.66 | 1.77 | 12.17 |
| As$^{5+}$ | 20 | 0.05 | 0.11 | 0.21 | 0.49 | 2.95 | 16.52 |
| MMA          | 10 | 0.05 | 0.07 | 0.18 | 0.46 | 2.03 | 5.82 | 43.89 |
| DMA          | 12 | 0.12 | 1.43 | 7.48 | 20.01 | 32.30 | 69.94 | 131.5 |
| iAs $(\Sigma \text{As}^{3+}, \text{As}^{5+})$ | - | 0.05 | 0.18 | 0.38 | 0.79 | 1.49 | 3.96 | 18.00 |
| tAs $(\Sigma \text{iAs, MMA and DMA})$ | - | 0.21 | 2.80 | 8.21 | 22.26 | 36.72 | 83.00 | 137.5 |

### DNA damage biomarkers

|                  | Min $^{5\text{th}}$ | $25\text{th}$ | $50\text{th}$ | $75\text{th}$ | $95\text{th}$ | Max $^c$ |
|------------------|----------------------|--------------|--------------|--------------|--------------|--------|
| 8-oxodG $(\mu g \text{ g cre}^{-1})$ | 0.24** | 0.16** | 0.13* | 0.17** | 1.00 | 1.00 |
| N$^7$-MeG $(\mu g \text{ g cre}^{-1})$ | 0.35** | 0.19** | 0.23** | 0.27** | 0.62** | 1.00 |

### Table 3. Pearson correlations between maternal inorganic arsenic and its metabolites levels, DNA damage in pregnant women, newborn health status, and other factors.

| Health parameter       | Maternal urinary As species $(\mu g \text{ g cre}^{-1})^a$ | Maternal urinary DNA damages $(\mu g \text{ g cre}^{-1})^b$ |
|------------------------|-----------------------------------------------------------|-----------------------------------------------------------|
|                        | iAs$^b$ | MMA$^b$ | DMA$^b$ | tAs$^b$ | 8-oxodG | N$^7$-MeG |
| Maternal urinary DNA damage$^c$ | | | | | | |
| 8-OhdG                 | 0.24** | 0.16** | 0.13* | 0.17** | 1.00 | 1.00 |
| N$^7$-MeG             | 0.35** | 0.19** | 0.23** | 0.27** | 0.62** | 1.00 |

### Pregnant women

|                        |                  | Maternal age (years) | Pre-pregnant BMI (Kg/m$^2$) |
|------------------------|------------------|----------------------|-----------------------------|
|                        |                  | 0.06                 | -0.02                       |
|                        |                  | 0.06                 | 0.12**                     |
|                        |                  | 0.05                 | 0.15                        |
|                        |                  | -0.03                | -0.05                      |
|                        |                  | -0.03                | -0.09                      |

|                        |                  | Mode of delivery | Birth weight (g) | Birth length (cm) | Head circumference (cm) | Chest girth (cm) | One-minute Apgar score | Five-minute Apgar score |
|------------------------|------------------|-----------------|-----------------|-----------------|------------------------|-----------------|------------------------|------------------------|
|                        |                  | 0.08             | 0.03            | -0.03           | 0.09                   | 0.04            | $-0.13^a$              | $-0.18^a$              |
|                        |                  | 0.03             | 0.03            | 0.05            | 0.05                   | 0.04            | 0.09                   | 0.11                   |

### Newborns

|                        |                  | Sex               | Gestational age (weeks) | Mode of delivery | Birth weight (g) | Birth length (cm) | Head circumference (cm) | Chest girth (cm) | One-minute Apgar score | Five-minute Apgar score |
|------------------------|------------------|------------------|-------------------------|-----------------|-----------------|------------------|------------------------|-----------------|------------------------|------------------------|
|                        |                  | 0.04             | -0.04                   | 0.08            | 0.03            | -0.03           | 0.09                   | 0.04            | $-0.13^a$              | $-0.18^a$              |

$r = 0.62$, $p<0.001$. A negative correlation of $-0.13$ ($p = 0.08$) was found between maternal iAs levels and the 1-min Apgar scores of newborns. A significant positive correlation was noted between the maternal MMA levels and the 1-min Apgar scores of newborns ($r = 0.15$, $p = 0.03$). A suggested negative correlation might exist between the maternal N$^7$-MeG levels and the 1-min Apgar scores of newborns ($r = -0.18$, $p = 0.06$). The maternal MMA and DMA levels were not significantly correlated with the birth outcomes of birth weight, length, and head and chest circumferences.

### Footnotes

$a$All urinary arsenic species in pregnant women were adjusted by creatinine and log-transformed.

$b$Pearson correlation coefficient; $^a$ $p<0.10$; $^p<0.05$; $^{**}p<0.01$. The $p$-value was bolded when $<0.1$.

$c$Urinary 8-oxodG and N$^7$-MeG in pregnant women were both adjusted by creatinine and natural log-transformed.

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Regression analyses identified positive associations between the maternal urinary level of iAs, MMA tAs, and the DNA damage biomarkers 8-oxodG and N7-MeG (Table 4). Maternal iAs levels had negative associations with 1-min Apgar scores (β-coefficient = −0.23, 95% CI: −0.29 – −0.18, p = 0.041). MMA had positive associations with the newborn birth weight (β = 0.25, 95% CI: 0.02–0.42, p = 0.034), and a marginally significant positive association with 1-min Apgar scores (β = 0.21, 95% CI: −0.01–0.44, p = 0.06). In addition, the DMA and tAs were not significantly associated with the 1-min Apgar score. The maternal urinary N7-MeG levels had negative associations with the birth length (β = −0.21, 95% CI: −0.36 – −0.13, p = 0.024) and 1-min Apgar score (β = −0.25, 95% CI: −0.63 – −0.15, p = 0.042). To determine whether or not the estimated effects of maternal iAs exposure on newborn health status was via methylated DNA damage, we performed a multivariable adjusted regression analysis to estimate the association between maternal iAs exposure and newborn health outcomes, controlling for N7-MeG and potential confounders (Table 5). The associations between maternal iAs and DNA damage biomarkers are shown in Table 4.

**Table 4.** Multiple linear regression (β-coefficient) for the associationa between birth outcomes with maternal urinary arsenic metabolitesb and DNA damage biomarkers (n = 299).

| Health parameter | Maternal urinary As species (µg g creatinine⁻¹) | Maternal urinary DNA damage (µg g creatinine⁻¹) |
|------------------|-----------------------------------------------|-----------------------------------------------|
| Pregnant women   | iAsb | MMAb | DMAb | tAsb | 8-oxodGc | N7-MeGc |
| 8-oxodGc         | β    | 0.24 | 0.16 | 0.08 | 0.13 | - | 0.64 |
| 95% CI           | 0.16–0.43 | 0.05–0.26 | −0.02–0.17 | 0.01–0.25 | - | 0.56–0.74 |
| p-value          | <0.001 | 0.03 | 0.123 | 0.028 | - | <0.001 |
| N7-MeGc          | β    | 0.35 | 0.17 | 0.19 | 0.27 | 0.62 | - |
| 95% CI           | 0.28–0.55 | 0.07–0.27 | 0.09–0.28 | 0.15–0.39 | 0.53–0.71 | - |
| p-value          | <0.001 | 0.001 | <0.001 | <0.001 | - | - |
| Newborns         | Birth weight (g) | β    | 0.01 | 0.25 | 0.10 | 0.17 | 0.05 | −0.16 |
| 95% CI           | −0.28–0.34 | 0.02–0.42 | −0.13–0.33 | −0.12–0.45 | −0.25–0.34 | −0.35–0.35 |
| p-value          | 0.845 | 0.034 | 0.396 | 0.312 | 0.745 | 0.062 |
| Birth length (cm) | β    | −0.19 | 0.398 | 0.06 | 0.18 | 0.04 | −0.21 |
| 95% CI           | −0.99–0.62 | −0.15–0.95 | −0.54–0.65 | −0.54–0.90 | −0.25–0.31 | −0.86 – −0.13 |
| p-value          | 0.648 | 0.157 | 0.854 | 0.626 | 0.886 | 0.024 |
| Head circumference (cm) | β    | 0.29 | 0.01 | −0.07 | −0.05 | −0.09 | −0.02 |
| 95% CI           | −0.22–0.81 | −0.35–0.35 | −0.45–0.32 | −0.47–0.46 | −0.59–0.39 | −0.81–0.76 |
| p-value          | 0.259 | 0.970 | 0.750 | 0.985 | 0.696 | 0.053 |
| Chest girth (cm) | β    | 0.11 | 0.28 | 0.14 | 0.12 | 0.24 | 0.46 |
| 95% CI           | −0.46–0.68 | −1.22–0.69 | −0.37–0.49 | −0.41–0.64 | −0.29–0.78 | −0.11–1.02 |
| p-value          | 0.699 | 0.169 | 0.798 | 0.655 | 0.376 | 0.116 |
| One-minute Apgar score | β    | −0.23 | 0.21 | 0.19 | 0.24 | 0.13 | −0.25 |
| 95% CI           | −0.29 – −0.18 | −0.01–0.44 | −0.04–0.43 | −0.05–0.52 | −0.16–0.43 | −0.63 – −0.15 |
| p-value          | 0.041 | 0.060 | 0.104 | 0.102 | 0.390 | 0.042 |
| Five-minute Apgar score | β    | 0.02 | 0.09 | 0.14 | 0.15 | 0.04 | −0.09 |
| 95% CI           | −0.19–0.24 | −0.07–0.24 | −0.02–0.30 | −0.04–0.34 | −0.16–0.25 | −0.15–0.29 |
| p-value          | 0.861 | 0.269 | 0.083 | 0.125 | 0.692 | 0.413 |

*aMultiple linear regression model was adjusted for maternal age, pre-pregnant BMI, mode of delivery, gestational age, and newborn sex; β, regression coefficient; 95% CI, 95% confidence interval. The p-value was bolded when <0.1.
*bAll maternal urinary As species were adjusted by creatinine and log-transformed.
*cUrinary 8-oxodG and N7-MeG in pregnant women were both adjusted by creatinine and natural log-transformed.

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levels and 1-min Apgar scores remained significant after adjusting for N7-MeG.

Cox’s proportional hazard model demonstrated that the maternal iAs level over the median was associated with the risk of decreased Apgar scores (RR = 1.14, 95% CI: 1.03–2.32, p = 0.012; Table 6). The relative risks for decreased Apgar scores was significantly increased (OR = 1.88, 95% CI: 1.07–2.15, p = 0.03) for pregnant women with urinary N7-MeG levels over the median. Relative risks for decreased Apgar scores associated with maternal N7-MeG levels were 1.92 (95% CI: 0.98–2.01, p = 0.07) after adjusting for iAs and other potential confounders.

### Discussion

This is the first study to simultaneously assess the effects from maternal arsenic exposure and maternal oxidative and methylated DNA damage on the health status of newborns. The concentrations of urinary tAs in this study were significantly lower than previous studies conducted in Bangladesh (median urinary tAs, 717.5 μg g⁻¹ cr) [26] and Chile (median urinary tAs, 40.4 μg g⁻¹ cr) [27]. Adverse birth outcomes and decreased 1-min Apgar scores were associated with increased maternal levels of iAs and N7-MeG in the general population.

We observed a significantly increased relative risk of low Apgar scores associated with maternal urinary iAs levels after adjusting for N7-MeG. The critical mechanism underlying prenatal arsenic exposure is still not clear. Disrupted placentation [13] and endocrine disturbance [14] have been reported for arsenic-related adverse pregnancy outcomes. Animals treated with high doses of arsenic have reported severe early effects, such as neural tube effects [28]; however, the levels of arsenic exposure relevant to humans await further investigation. DNA damage was reported to be induced by iAs, as shown by increased concentrations of the biomarkers of oxidized DNA adducts (8-oxodG) in the brains of mice [29] and in the urine of women in the early stages of pregnancy [17]. In the present study, urinary 8-oxodG and N7-MeG levels were significantly associated with concentrations of maternal urinary arsenic species. N7-MeG was significantly associated with decreased birth length and 1-min Apgar scores after adjustment for maternal age, pre-pregnant BMI, mode of delivery, gestational age, and newborn sex. Thus, N7-MeG appeared to be a more sensitive biomarker than 8-oxodG for maternal DNA damage related to newborn adverse outcomes. This suggests that maternal iAs might cause both DNA damage and adverse newborn health independent of the DNA damage.
We first reported that the association between iAs and N7-MeG.  
N7-MeG has been established as a marker of human exposure to 
methylating agents and smoking [30]. The induction of N7-MeG is 
believed to occur via the direct toxicity of arsenic on enzyme 
activities, such as DNA repair enzymes. It has been shown that low 
concentrations of iAs are able to inhibit the expression and activity 
of poly(ADP-ribose) polymerase (PARP) enzymes [31], the 
enzymes responsible for base excision repair pathways, which 
increase unrepaired DNA lesions [32]. Urinary N7-MeG is derived 
from transfer RNA (tRNA) turnover [33]. The current association 
between arsenic exposure and increased urinary N7-MeG levels 
might be due to upregulation of alternative repair pathways of 
methylated DNA damage, such as base excision repair, resulting in 
the formation of N7-MeG. A previous report indicated that a 
variety of methylated purines and pyrimidines are found in tRNA, 
thus degradation of tRNA might result in the release of all minor 
methylated bases [34]. A recent in vivo study showed that arsenic 
exposure induces tRNA modification in Saccharomyces cerevisiae, and 
may subsequently increase cellular levels of N7-MeG [35]. An 
improper alteration in tRNA modification may lead to disorders in 
embryonic development, cell proliferation, and differentiation in 
mice [36], and developmental abnormalities in Caenorhabditis elegans 
[37].

DNA methylation is an important mechanism of fetal 
programming during fetal development [2,10]. Long-term expo-
sure to arsenic in utero has been associated with changes in DNA 
methylation, which may have severe consequences for the 
development of fetal health effects [10]. Urinary N7-MeG could 
be used as a biomarker for the alteration of DNA methylation due 
to arsenic [38]. It has been shown that high arsenic exposure in 
pregnant mice significantly reduces DNA methylation in offspring 
[39]. We suggest that iAs might induce adverse birth outcomes. 
Further evaluation is needed in a larger sample to understand 
whether or not prenatal low iAs exposure results in altering DNA 
methylation in newborns.

Maternal urinary DMA was not significantly associated with 
maternal 8-oxodG nor were 1-min Apgar scores. This might result 
from organic arsenic as a source of DMA and tAs: A large 
population study showed that seafood intake was a major 
determinant of increased urine concentrations of DMA and total

Table 6. Relative risks (RR) and 95% confidence interval (95% CI) for low birth weight and decreased one-minute Apgar score in relation to maternal urinary arsenic and DNA damage biomarker levels (n = 299).

| Relative risk | Low birth weight (<2500 g) | Decreased Apgar score (<7) | p-valued | n | RR (95% CI) | p-valued | n | RR (95% CI) |
|---------------|---------------------------|---------------------------|-----------|---|------------|-----------|---|------------|
| Arsenic metabolites (µg g⁻¹ cre) | | | | | | | | |
| iAs | | | | | | | | |
| >0.38 | 11 | 1.04 (0.68–1.59) | 0.522 | 37 | 1.14 (1.03–2.32) | 0.012* | | |
| ≤0.38 | 10 | 1.0 | | 32 | 1.0 | | | |
| MMA | | | | | | | | |
| >0.46 | 8 | 0.56 (0.23–1.31) | 0.131 | 26 | 0.59 (0.28–1.17) | 0.156 | | |
| ≤0.46 | 13 | 1.0 | | 44 | 1.0 | | | |
| DMA | | | | | | | | |
| >20.01 | 7 | 0.55 (0.24–1.30) | 0.124 | 22 | 0.46 (0.22–0.95) | 0.035* | | |
| ≤20.01 | 14 | 1.0 | | 47 | 1.0 | | | |
| DNA damage biomarkers (µg g⁻¹ cre) | | | | | | | | |
| 8-oxodG | | | | | | | | |
| >3.48 | 12 | 1.31 (0.54–3.13) | 0.355 | 32 | 0.84 (0.41–1.74) | 0.713 | | |
| ≤3.48 | 9 | 1.0 | | 38 | 1.0 | | | |
| N7-MeG | | | | | | | | |
| >12.88 | 13 | 1.59 (0.88–2.86) | 0.060** | 45 | 1.88 (1.07–2.15) | 0.030* | | |
| ≤12.88 | 8 | 1.0 | | 24 | 1.0 | | | |
| ARRc | | | | | | | | |
| iAs | | | | | | | | |
| >0.38 | 11 | 1.02 (0.66–2.26) | 0.625 | 37 | 1.21 (1.10–2.45) | 0.006* | | |
| ≤0.38 | 10 | 1 | | 32 | 1 | | | |
| N7-MeG | | | | | | | | |
| >12.88 | 13 | 1.49 (0.89–1.96) | 0.342 | 45 | 1.92 (0.98–2.01) | 0.07** | | |
| ≤12.88 | 8 | 1 | | 24 | 1 | | | |

*The continuous variables were transformed into a two-level scale using medians, which represented the high or low levels arsenic/DNA damage biomarkers exposure, to calculate relative risks (RR).

**Adjusted for potential confounders (maternal age, mode of delivery, pre-pregnant BMI, gestational age, and newborn sex).

#Adjusted for iAs or N7-MeG and potential confounders.

Cox’s proportional hazards model; p<0.10; *p<0.05 for RR significance above 1. p-value was bolded when less than 0.1.

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arsenic [40]. Current study area residents in Taiwan regularly consume seafood and fish from the surrounding Pacific Ocean [41]. These foods might contain substantial amounts of arseno-sugars or arsenolipids, which could metabolized as DMA [41].

The main strengths of this study include a study design that allows findings on the longitudinal capture of biomarkers for inorganic arsenic exposure to relate to birth outcomes, monitoring biomarkers of oxidative damage, and sufficient sample size. In addition, none of the study subjects smoked cigarettes, which otherwise would have been a major confounder because smoking is associated with several adverse outcomes of pregnancy, including LBW [42] and increased N2-MeG levels [30].

The current study was limited by the lack of paternal data, thus early contributions from the male complement due to arsenic-induced damage cannot be evaluated and warrant further studies. Furthermore, iAs has a short half-life and the efficiency of inorganic arsenic methylation to DMA was increasing since the 1st trimester of pregnancy [43]. We only measured urinary arsenic species once during the third trimester, which likely introduced a substantial degree of exposure measurement misclassification towards null hypothesis.

In this study, there were no significant differences in the association of birth outcomes with maternal iAs between sexes; we combined data from female and male newborns to increase the statistical power. Future work might recruit more subjects to further verify the sex difference. We assessed arsenic and biomarkers of DNA damage using the same spot urine and thus temporality cannot be evaluated. However, we do not think the converse is likely to occur (i.e., greater oxidation/methylation metabolism increases arsenic elimination).

Conclusions

This is the first study to report significant associations between arsenic-induced N2-MeG levels of pregnant women and an increased risk of adverse birth outcomes in newborns. These findings strongly emphasize that maternal N2-MeG may be a sensitive and effective biomarker for newborn health, particularly after early-life arsenic exposure. Further studies are necessary to understand the potential health effects of arsenic-related DNA modifications or rRNA modifications in newborns.

Supporting Information

Table S1 Demographic characteristic of followed and lost-to-follow-up subjects.

(DOC)

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Author Contributions

Conceived and designed the experiments: SLW YCT. Performed the experiments: WCC YTC JW YCT. Analyzed the data: WCC. Contributed reagents/materials/analysis tools: WCC YTC JW YCT. Wrote the paper: WCC SLW. Providing clinical materials: THY. Advising the 1st author: CYC SLW.

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