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Urinary Arsenic Metabolism and Birth Outcomes in Tacna, Peru, 2019: A Prospective Cohort Study

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Urinary Arsenic Metabolism and Birth Outcomes in Tacna, Peru, 2019: A Prospective Cohort Study

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Abstract

Arsenic exposure during pregnancy might affect foetal development. Arsenic metabolism may modulate the potential damage to the foetus. Tacna has the highest arsenic exposure levels in Peru. However, this region has the highest birth weight in Peru. It is not known if arsenic exposure is affecting maternal-perinatal health in Tacna. The study aimed to evaluate the association between urinary arsenic metabolism and birth outcomes, specifically birth weight and gestational age at birth in Tacna, Peru. A prospective cohort study was conducted, involving 158 pregnant women in Tacna, Peru, during January-November 2019. Participants were enrolled in their second trimester and followed-up until birth. Urine samples were collected in the second and third trimester. Urine samples were analyzed for total arsenic concentration and its species. Generalized estimating equations (GEE) analysis was used to evaluate the association of interest. Inter-differences in arsenic toxicokinetics, calculated with principal component analysis (PCA) was included as an interaction term. Analysis was stratified by pregnancy trimester. The median total urinary arsenic (tAs) concentration was 33.34 µg/L. Inorganic arsenic (iAs) and Dimethylarsinic acid (DMA) were higher in the second trimester. Dimethylarsinic acid (DMA) was the predominant component (84.78% of total urinary arsenic). No significant association was found between urinary arsenic exposure and birth weight or gestational age at birth. The association was not affected by arsenic metabolism. Stratified analyses by pregnancy trimester also showed no significant associations. Urinary arsenic was not associated with birth weight, and this null relationship remained unaffected by arsenic toxicokinetic differences reflected in urine.

Keywords: Birth weight, Foetal development, Gestational age, Toxicity, Pregnant women, Latin America

Introduction

Arsenic is a naturally occurring element found in the earth's crust, soil, water, and air. It is a toxic substance and a known carcinogen, causing skin, lung, bladder, and kidney cancers.[1] Arsenic is also known to have adverse effects on foetal and infant health.[2] Pregnant women who are exposed to high levels of arsenic are at an increased risk of adverse birth outcomes, including stillbirth, preterm birth (<37 weeks of gestational age), low birth weight (<2500 g at term), and congenital abnormalities.[3] In recent years, there has been growing concern about the impact of arsenic exposure on maternal and child health.

The ingestion of water containing high concentration of arsenic is one of the most common routes of exposure. It is estimated that 107 countries around the world are affected by high levels of arsenic in water [4], with groundwater being the most common source, although high levels are also found in surface water.[5] Arsenic concentration in water can be very heterogeneous even in a same country, such as Bangladesh, with arsenic levels ranging from 90 to 4730 µg/L in tube-well water. [6] In Chile, at Bahía de Camarones, which is located near the city of Arica (border with Peru), drinking water inorganic arsenic levels of 48.7 – 1252 µg/L have been found, composed particularly of As^{V}.[7] A study from our group has determined that around two-thirds of the Tacna (a province in southern Peru) pregnant women population is exposed to inorganic arsenic levels higher than 10 µg/L in tap water, of which 50% were exposed to >50 µ/L. [8] However, Tacna, despite the arsenic exposure context, it has showed the highest birth weight in Peru[9, 10], as well as the lowest small for gestational age prevalence. [10]

Urinary arsenic and its metabolites are commonly used as biomarker of arsenic exposure in epidemiological studies.[11] Arsenic and its metabolites are excreted primarily in urine, and urinary arsenic levels have been shown to correlate with the internal dose of arsenic exposure.[11] Several studies have reported a significant association between maternal urinary arsenic levels and adverse birth outcomes, although the findings have been inconsistent across studies.[3, 12] It is important to note that individuals have varying proficiencies in metabolizing arsenic, and this could modulate the potential damage to the fetus.[13]

Given the potential health risks associated with arsenic exposure during pregnancy, there is a need for further research to better understand the impact of arsenic on maternal and child health. This study aims to evaluate the association between urinary arsenic metabolism and birth outcomes, specifically birth weight and gestational age at birth.

Materials and methods

Study design and study area

We conducted a longitudinal cohort study during January-November 2019, in which a total of 158 pregnant women that lived in the province of Tacna, in their second trimester of pregnancy who attend to their antenatal care-controls were enrolled and followed-up until birth. The province of Tacna is in southern Peru, with a total area of 8,170 km^2, and it is characterized for its desertic geography.

Enrolment of participants and follow-up

The recruitment of the pregnant women is described elsewhere.[8] In brief, a total of 16 health establishments within the 5 most populated districts in the province of Tacna were selected for the enrolment to take place. We were granted authorization to consult the prenatal health care record that included information about the date of last antenatal care consultation, gestational age by the time of consultation, age, address, and telephone number.

To be considered as a potential participant for the study, the women were 18-40 years-old, lived in Tacna for at least 5 years, and were pregnant for <24 weeks by the time of the recruitment. Eligible women were recruited via telephone call. Those invited to participate in the study were then visited in their homes or in the health establishment a total of 2 times for urine sampling. A final visit was scheduled after birth, in which data from their baby was collected, such as birth weight and gestational age at birth.

Urine sampling and arsenic quantification

One urine sample was taken in the second and third trimester of pregnancy. During the recruitment the women were given two sterile plastic flasks for urine specimen collection. They were asked to avoid consuming fish or seafood for the last three days prior the sampling. They were instructed in how to do the self-collection of the sample, indicating that they should eliminate the first few millilitres of the morning void. Once the sample was collected, participants were asked to store it in the freezer until the research personnel were able to collect them. The samples were transported at 4°C to the laboratory for storage. Samples were homogenized and then aliquoted in cryovials of 2 mL, and stored at -20°C. For arsenic quantification and speciation, the samples were delivered on dry ice to the LEADER laboratory at Emory University in Atlanta, GA, USA. Procedure is described elsewhere.[14]

Statistical Analysis

Descriptive statistics were used to display median with interquartile range for non-normal distributed data. Categorical variables are presented as absolute and relative frequencies. Arsenic species concentrations and their relative percent (%) are presented.

Relative percent of the species were calculated as follows:

$$\%iAs = \frac{[As^{III}] + [As^{V}]}{[As^{III}] + [As^{V}] + [MMA] + [DMA]}$$

$$\%MMA = \frac{[MMA]}{[As^{III}] + [As^{V}] + [MMA] + [DMA]}$$

$$\%DMA = \frac{[DMA]}{[As^{III}] + [As^{V}] + [MMA] + [DMA]}$$

where:

$[iAs]$: Inorganic arsenic concentration in urine

$[As^{III}]$: Arsenite concentration in urine

$[As^V]$: Arsenate concentration in urine

$[MMA]$: Monomethylarsonic acid concentration in urine

$[DMA]$: Dimethylarsinic acid concentration in urine

To compare total urinary arsenic and arsenic species concentration between the second and third trimester of pregnancy, we used Wilcoxon's sign-rank test. We used Student's t-test for paired observations to compare if %iAs, %MMA and %DMA was different between pregnancy trimesters, after the normal distribution evaluation of the differences. We performed a principal component analysis (PCA) to characterize the main sources of variability in the urinary arsenic data and its species (arsenic toxicokinetics differences between pregnant women). The PCA was conducted on the concentration of urinary inorganic arsenic (iAs), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). The principal components correlations and eigenvectors can be found in **Supplementary Material 1**.

Arsenic exposure was considered as the residuals of the following model to remove the influence of organic arsenic from seafood on urinary total arsenic: [15, 16]

$$tAs = \beta_1 * Asb + \beta_2 * Asb^2 + constant$$

Where. -

tAs: Total urinary arsenic (µg/L)

Asb: Arsenobetaine (µg/L)

Generalized estimating equations (GEE) with Gaussian family analysis was employed to evaluate the association between arsenic and birth weight, and whether this association was affected by arsenic toxicokinetic differences between pregnant women. This same approach was applied to examine the association with gestational age at birth but scaling the variable arsenic exposure dividing it by 1000 for better interpretation, since coefficients were small. GEE analysis was then stratified by newborn sex. An analysis stratified by pregnancy trimester was performed using linear regression. Regression models were adjusted for mother's age, pregestational body mass index and mother's education level (as a proxy for socioeconomic status). All statistical analyses were conducted using STATA 17.0 software with a significance level of $p < 0.05$.

Ethical aspects

The study protocol was approved by Universidad Peruana Cayetano Heredia IRB (R-29420-20). Informed consent was obtained from each participant.

Results

The study sample of pregnant women had a mean age of 28.15 years at the time of recruitment, and mean body mass index of 26.73 kg/m^2 before pregnancy. Only five women (3.13%) declared to be smokers during pregnancy and 13 consumed alcohol (8.16%). Thirty-six of the women (22.50) were single mothers, and the sample had a high proportion of women with higher education (38.13%). In **Table 1** we present the

distribution of urinary arsenic species concentrations as median and interquartile range (IQR). Median total urinary arsenic (tAs) was 33.34 µg/L and ranged between 2.50 – 167.48 µg/L. We observed variation in tAs across visits, being lower in visit 2. DMA was the most present arsenic component (84.78%). Water arsenic concentration distribution in the second and third trimester can be found in **Supplementary Material 2**, indicating that for the third trimester, pregnant women were mostly exposed to levels ≤10 µg/L (51.83% vs 29.56% in the second trimester), and there was a positive significant correlation between water arsenic and urinary DMA concentration in both trimesters.

Table 1. Urinary arsenic species concentration and relative content across pregnancy.

Arsenic specie (µg/L)	Total		Second trimester		Third trimester		p-value[£]
	Median	IQR	Median	IQR	Median	IQR	
tAs	33.34	30.58	41.57	33.95	28.32	20.67	<0.001
AsIII	1.57	1.57	2.08	1.9	1.24	1.03	<0.001
AsV	1.36	1.3	1.36	1.36	1.36	1.21	0.553
iAs	2.99	2.8	3.54	2.99	2.68	2.03	0.001
MMA	2.1	1.79	2.17	2.07	2.07	1.35	0.165
DMA	28.36	26.86	35.55	29.06	23.36	16.75	<0.001
Asb	2.37	2.55	2.64	3.05	2.09	2.24	0.002
%iAs*	8.85	2.72	8.3	2.59	9.49	2.73	<0.001
%MMA*	6.37	2.21	5.41	1.87	7.47	2.06	<0.001
%DMA*	84.78	4.05	86.28	3.56	83.03	3.89	<0.001

tAs: Total urinary arsenic.

Asb: Arsenobetaine.

MMA: Monomethylarsonic acid.

DMA: Dimethylarsinic acid.

IQR: Interquartile range

[£] Wilcoxon's sign-rank for total arsenic and arsenic species concentration; and paired Student's t-test for arsenic species relative content (%).

*Mean and standard deviation instead of median and IQR are showed for %iAs, %MMA and %DMA.

Mean birth weight was 3618 ± 477.38 grams. As seen in **Table** 2, there was no significant association between urinary arsenic and birth weight (adjusted β=0.16, 95%CI -1.07 ; 1.39, p=0.800). The interaction between urinary arsenic and arsenic toxicokinetics difference between women (PCA Score 1) showed a reduction in birth weight, nonetheless, this was non-significant (adjusted β=-0.05, 95%CI -0.76 ; 0.65, p=0.882).

Table 2. Association between urinary arsenic and interaction with arsenic metabolism with birth weight.

Variable	Unadjusted	95% CI	Adjusted	95% CI
Urinary Arsenic	0.04	-1.27 ; 1.36	0.16	-1.07 ; 1.39
Score 1[α]	0.62	-16.09 ; 17.33	1.27	-14.11 ; 16.65
Urinary arsenic*Score 1	-0.10	-0.89 ; 0.69	-0.05	-0.76 ; 0.65
Mother's age	4.40	-7.81 ; 16.60	3.63	-7.97 ; 15.23
Pregestational BMI	**23.76**	**9.59 ; 37.92**	**20.65**	**6.94 ; 34.35**
Education				
Elementary		Ref.		Ref.
Secondary	305.65	-1.68 ; 612.97	**371.28**	**72.67 ; 669.93**

Tertiary	212.99	-101.37 ; 527.36	**312.73**	**8.70 ; 616.77**

Residuals were calculated from the model $tAs \sim \beta_1(Arsenobetaine) + \beta_2(Arsenobetaine)^2$
Models were adjusted for mother's age, mother's education level, pre-gestational body mass index.
95% CI: 95% Confidence Interval.
[α]Score 1 (arsenic toxicokinetics difference between women), obtained from principal components analysis, is higher when %DMA is lower, meaning a reduced metabolic capability.
BMI: Body mass index
Bold letters indicate a $p<0.05$.

Regarding gestational age at birth, as seen in **Table 3**, we found a non-significant increase of 0.02 weeks (95%CI -2.37 ; 2.40, p=0.989), while the interaction term presented a decrease, although not significant, in gestational age at birth (β=-0.17, 95%CI -1.53 ; 1.19, p=0.802).

Table 3. Association between urinary arsenic and interaction with arsenic metabolism with gestational age at birth

Variable	Unadjusted	95% CI	Adjusted	95% CI
Urinary Arsenic	-0.08	-2.48 ; 2.32	0.02	-2.37 ; 2.40
Score 1[α]	0.01	-0.02 ; 0.04	0.01	-0.02 ; 0.04
Urinary Arsenic*Score 1	-0.19	-1.63 ; 1.24	-0.17	-1.53 ; 1.19
Mother's age	**-0.03**	**-0.06 ; -0.004**	**-0.03**	**-0.06 ; -0.001**
Pregestational BMI	**-0.04**	**-0.07 ; -0.01**	-0.03	-0.07 ; 0.003
Education				
Elementary	Ref.		Ref.	
Secondary	0.62	-0.12 ; 1.36	0.48	-0.27 ; 1.23
Tertiary	0.55	-0.21 ; 1.31	0.32	-0.45 ; 1.08

Residuals were calculated from the model $tAs \sim \beta_1(Arsenobetaine) + \beta_2(Arsenobetaine)^2$
Models were adjusted for mother's age, mother's education level, pre-gestational body mass index.

95% CI: 95% Confidence Interval.
BMI: Body mass index.
Bold letters indicate a $p<0.05$.
[α]Score 1 (arsenic toxicokinetics difference between women), obtained from principal components analysis, is higher when %DMA is lower, meaning a reduced metabolic capability.

In the stratified analysis by newborn sex, no significant association was found between arsenic exposure or the interaction term related to arsenic toxicokinetic differences and birth weight. However, for gestational age at birth, a significant association (p=0.041) was observed for males, indicating that each increase of 1000 units in urinary arsenic exposure is associated with an increase of 7.36 weeks in gestational age at birth (**Table 4**).

Table 4. Association between urinary arsenic and interaction with arsenic metabolism with birth weight and gestational age at birth stratified by newborn sex.

Newborn sex	Regression term	Birth weight		Gestational age at birth	
		Adjusted	p-value	Adjusted	p-value
Male	Urinary Arsenic	2.79 (-0.02 ; 5.60)	0.052	7.36 (0.30 ; 14.42)	0.041
	Urinary Arsenic * Score 1[α]	0.36 (-1.41 ; 2.13)	0.689	-2.79 (-8.84 ; 3.26)	0.364

Female	Urinary Arsenic	-0.47 (-4.27 ; 3.32)	0.806	-6.92 (-16.62 ; 2.77)	0.160
	Urinary Arsenic * Score 1	-1.41 (-3.79 ; 0.98)	0.245	0.95 (-4.83 ; 6.72)	0.745

Regressions were adjusted for mother's age, pregestational body mass index and education.

Coefficients for gestational age at birth are scaled (Urinary arsenic/1000).

[α]PCA Score 1 (arsenic toxicokinetics difference between women) is higher when %DMA is lower, meaning a reduced metabolic capability.

For both models, the adjusted regression coefficient (95% Confidence Interval) is showed.

We then evaluated if arsenic or the interaction term with arsenic toxicokinetic differences were associated with both outcomes, stratifying it by pregnancy trimester. As seen in **Table 5**, there was no association between urinary arsenic exposure and the interaction term with birth weight and gestational age at birth.

Table 5. Association between urinary arsenic and interaction with arsenic metabolism with birth weight and gestational age at birth stratified by visit.

Trimester	**Regression term**	**Birth weight**		**Gestational age at birth**	
		Adjusted	**p-value**	**Adjusted**	**p-value**
Second	Urinary Arsenic	1.61 (-1.44 ; 4.67)	0.298	-5.11 (-14.43 ; 4.20)	0.28
	Urinary Arsenic * Score 1[α]	-1.36 (-3.32 ; 0.59)	0.170	-5.13 (-12.00 ; 1.75)	0.142
Third	Urinary Arsenic	-1.91 (-6.09 ; 2.27)	0.368	7.88 (-5.81 ; 21.57)	0.257
	Urinary Arsenic * Score 1	1.60 (-0.84 ; 4.05)	0.197	-0.81 (-8.19 ; 6.57)	0.828

Regressions were adjusted for mother's age, pregestational body mass index and education.

Coefficients for gestational age at birth are scaled (Urinary arsenic/1000).

[α]PCA Score 1 (arsenic toxicokinetics difference between women) is higher when %DMA is lower, meaning a reduced metabolic capability.

For both models, the adjusted regression coefficient (95% Confidence Interval) is showed.

Discussion

The present study aimed to evaluate the association between urinary arsenic and metabolism with birth weight and gestational age at birth. It was found no association with these outcomes, and this null relationship is unaffected by arsenic toxicokinetic differences reflected in urine.

No association may have been found because exposure levels might not be high enough to exert an effect. Previous studies have found a decrease in birth weight with increasing levels of urinary arsenic, at exposure levels ≥100 µg/L.[3] In this study, the median level of urinary arsenic for the cohort across pregnancy was 33.34 µg/L with a range of 2.50 – 167.48 µg/L. A total of 25 and 36 women showed urinary tAs levels ≥100 µg/L in the second and third trimester of pregnancy, respectively, but no difference in birth weight was found (**Supplementary material 3**). In some previous studies, low levels of arsenic in urine (1.8 – 27.7 µg/L) have not been found to be associated with a decrease in birth weight.[17] However, other studies with similar exposure levels in urine have found a significant association with birth weight or estimated foetal weight.[18, 19] A Wuhan cohort study that showed median urinary arsenic levels of 31.22 µg/L for the first, 25.23 µg/L for the second, and 24.98 µg/L for the third trimester found a significant decrease of 24.27

g in birth weight only for the third trimester.[12] This suggests that even low exposure levels might be harmful for foetal development. Additionally, it is important to remark that no arsenic exposure level is considered to be safe since even water arsenic exposure levels between 1 – 10 µg/L has been associated with increased cardiovascular mortality compared to concentrations <1 µg/L.[20].

In a cohort study from Bangladesh, it was found that water and toenail arsenic association with birth weight was mediated by gestational age.[21, 22] In the present study, pregnancy duration, seen as gestational age at birth, was not associated with arsenic exposure. This difference might be attributed to the level of arsenic exposure in drinking water observed in the Bangladeshi cohort. Although the median arsenic concentration was 2.3 µg/L at the time of enrolment, 33.3% of pregnant women were exposed to levels ranging from 18.4 to 1400 µg/L. [21] On the other hand, it has been found that low arsenic levels in biological samples such as umbilical cord (3.82 ± 3.81 µg/L) and whole blood (4.13 ± 3.21 µg/L) were associated with a decrease in gestational age by 0.342 weeks.[23] On the contrary, in a study that included a total of 212 mother-infant pairs, no association was found between total urinary arsenic (median 7.77 µg/L) and urinary DMA (3.44 µg/L) with gestational age.[24] The lack of association with birth weight and gestational age at birth could be due to an exposure below harmful levels, or to unmeasured nutritional, genetical and other factors.

When analysing the impact of arsenic exposure on birth outcomes by newborn sex, we found no significant relationship between arsenic levels and birth weight. However, for male infants, there was a notable increase in gestational age—specifically, an increase of 0.0746 weeks for every 10 units rise in urinary arsenic concentration. In contrast, a previous study involving 113 mother-child pairs reported no significant associations between arsenic exposure and gestational age across both sexes. [25] This discrepancy may stem from different exposure levels, particularly if Tacna has higher arsenic concentrations. Despite the modest effect size observed in our study, it remains unclear why urinary arsenic correlates positively with gestational age.

Arsenic can be metabolized, and a higher arsenic methylation capability of the body can reduce this metalloid toxicity.[26] Higher concentration of urinary MMA and urinary iAs are shown to have the biggest impact in decreasing birth weight and birth length, respectively [13]; evidence is less clear for DMA; Nonetheless, a higher proportion of DMA, which means a better arsenic metabolism, is associated with better health outcomes compared to those with lower DMA, such as general health status of children [27] and neurodevelopment in low birth weight preterm children.[28] We have observed in pregnant women from Tacna, Peru that DMA at 84.78% (total urinary arsenic minus arsenobetaine) represents the main arsenic component present in urine. This may explain the low negative impact of arsenic on birthweight and gestational age at birth; and suggests that the difference in arsenic toxicokinetics might modify the association.

The effect modification of arsenic toxicokinetics was also assessed in the study by including the interaction term of arsenic with the PCA Score 1. For both birth weight and gestational age at birth, differences in arsenic metabolism seemed to modify the association by reducing these outcomes, although it was non-significant. Despite not finding an association, there might be an interaction between arsenic exposure and

metabolism, as suggested in a Romanian longitudinal pilot study, where women who had low birth weight children showed a higher percentage of inorganic arsenic and MMA [29], suggesting a slower or reduced metabolism.

Consideration of arsenic species and speciation is essential for a better understanding of exposure, not only in research studies but also in nationwide screenings such as the one done in the NHANES survey.[30, 31] Currently, the Peruvian Demographic and Health Survey does not consider water or urinary arsenic evaluation.

It is possible that birth weight was not affected due to the variation in arsenic exposure between pregnancy trimesters. Other studies showed that there are seasonal variations in water and urinary arsenic concentration [32-34], although depending on the area, the change can be very small (3.3 µg/L in well water between the dry and rainy season).[35] The first study visit was conducted in summer and autumn, while the second visit occurred during winter and spring. At the second visit, median tAs was 28.32 µg/L, compared with 41.57 µg/L found in the first study visit. In the stratified analysis, no association was found with arsenic exposure, nor with toxicokinetic differences.

The foetus experiences the fastest weight gain during the third trimester [36], and different arsenic exposures in this developmental window have been found to reduce birth weight [37], although some authors have found that early pregnancy arsenic exposure might be the critical window for birth weight and other pregnancy outcomes.[38] Nonetheless, trimester-based analysis might not reflect an adequate association.[39] Daily exposure assessment is difficult for exposures that need biological samples such as urinary arsenic. Arsenic has been found to be associated with a decrease in birth weight and gestational age at birth, possibly through lowering thyroid hormones ratio during early pregnancy.[18] Seasonal variation in exposure, along with the analysis of pregnancy-relevant hormones should be considered for a better evaluation and interpretation.

It is notable that pregnant women from Tacna, , despite living in the highest arsenic-exposed region in Peru, have one of the highest mean birth weights.[10] One contributing factor may be the considerable proportion of individuals from the Aymara ethnicity in Tacna.[8, 14] This is an indigenous group, predominantly located in high altitude settings, that is known for higher birth weight compared to other high-altitude populations.[40] In our sample, neonates of pregnant women who self-identified as Aymara had a mean birth weight of 3711 g, higher compared to the other ethnic groups (3536 g for mestizo and 3466 g for Quechua) (**Supplementary material 4)**. These findings suggests that the Aymara population may possess genetic traits that supports foetal weight gain, even in the context of arsenic exposure.

When considering arsenic metabolism, polymorphisms in the *AS3MT* gene related increased arsenic metabolic capability [41-44], were found in Aymara populations of Argentina.[45] However, while 55.41% of our sample self-identified as Aymara, %DMA was not different between ethnic groups in our study (**Supplementary material 5**). These hypotheses should be explored in further studies.

The study has some limitations. There were unmeasured confounders such as the consumption of folates, which are part of the one-carbon metabolism and methyl donors for arsenic metabolism, which could modify the association between arsenic metabolism

and birth weight.[46] Based on the Peruvian national program on pregnancy, it is mandatory to supplement women with folic acid; therefore, the folate deficiency in our population is reduced, however it should be considered in further studies. Covariates such as gestational weight gain should also be evaluated since it is strongly associated with birth weight, especially during the first half of gestation.[47] The exposure assessment at the beginning of pregnancy (first trimester) is encouraged, since it would also allow testing arsenic effects on placenta formation, as has been suggested in both human [48] and animal studies.[49] This would also allow for a better evaluation of seasonal variation in arsenic exposure. This study used specific gravity to adjust arsenic concentration in urine, which may have different sources of measurement error than creatinine adjustment.[50]

Conclusions

Arsenic was not associated with birth weight or gestational age at birth in this study, and this null relationship was unaffected by arsenic toxicokinetic differences reflected in the analysis. This should not be interpreted as if the Tacna population is protected against arsenic toxicity. Further studies should include other variables to better understand this phenomenon and the mechanism(s) behind it, including the evaluation of other clinical outcomes. Additionally, the inclusion of arsenic exposure assessment and its speciation in national programs should be encouraged for better monitoring, along with the elimination of arsenic contamination in drinking water.

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CRediT authorship contribution statement

DFS: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing-Original Draft **MOG:** Conceptualization, Methodology, Formal Analysis, Writing – Review & Editing, Visualization, Supervision **CVV:** Investigation, Writing –

Review & Editing **CRA:** Conceptualization, Resources, Writing – Review & Editing **JA:** Conceptualization, Resources, Writing – Review & Editing **JKW:** Conceptualization, Writing – Review & Editing, Supervision **MYL:** Conceptualization, Writing – Review & Editing, Supervision **DBB:** Validation, Investigation, Resources, Writing – Review & Editing **GFG:** Conceptualization, Resources, Writing – Review & Editing, Visualization, Supervision, Project administration, Funding acquisition.

Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declaration of competing interests

The authors declare that they have no competing interests.

Ethics statement

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Universidad Peruana Cayetano Heredia (Project identification code R-121-12-23).

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Table 1. Urinary arsenic species concentration and relative content across pregnancy.

Arsenic specie (µg/L)	Total		Second trimester		Third trimester		p-value[β]
	Median	IQR	Median	IQR	Median	IQR	
tAs	33.34	30.58	41.57	33.95	28.32	20.67	<0.001
AsIII	1.57	1.57	2.08	1.9	1.24	1.03	<0.001
AsV	1.36	1.3	1.36	1.36	1.36	1.21	0.553
iAs	2.99	2.8	3.54	2.99	2.68	2.03	0.001
MMA	2.1	1.79	2.17	2.07	2.07	1.35	0.165
DMA	28.36	26.86	35.55	29.06	23.36	16.75	<0.001
Asb	2.37	2.55	2.64	3.05	2.09	2.24	0.002
%iAs*	8.85	2.72	8.3	2.59	9.49	2.73	<0.001
%MMA*	6.37	2.21	5.41	1.87	7.47	2.06	<0.001
%DMA*	84.78	4.05	86.28	3.56	83.03	3.89	<0.001

tAs: Total urinary arsenic.

Asb: Arsenobetaine.

MMA: Monomethylarsonic acid.

DMA: Dimethylarsinic acid.

[β] Wilcoxon's sign-rank for total arsenic and arsenic species concentration; and paired Student's t-test for arsenic species relative content (%).

*Values are shown as mean and standard deviation.

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Table 2. Association between urinary arsenic and interaction with arsenic metabolism with birth weight.

Variable	Unadjusted	95% CI	Adjusted	95% CI
Urinary Arsenic	0.04	-1.27 ; 1.36	0.16	-1.07 ; 1.39
Score 1[α]	0.62	-16.09 ; 17.33	1.27	-14.11 ; 16.65
Urinary arsenic*Score 1	-0.10	-0.89 ; 0.69	-0.05	-0.76 ; 0.65
Mother's age	4.40	-7.81 ; 16.60	3.63	-7.97 ; 15.23
Pregestational BMI	**23.76**	**9.59 ; 37.92**	**20.65**	**6.94 ; 34.35**
Education				
Elementary		Ref.		Ref.
Secondary	305.65	-1.68 ; 612.97	**371.28**	**72.67 ; 669.93**
Tertiary	212.99	-101.37 ; 527.36	**312.73**	**8.70 ; 616.77**

Residuals were calculated from the model $tAs \sim \beta_1(Arsenobetaine)+\beta_2(Arsenobetaine)^2$

Models were adjusted for mother's age, mother's education level, pre-gestational body mass index.

95% CI: 95% Confidence Interval.

[α]Score 1 (arsenic toxicokinetics difference between women), obtained from principal components analysis, is higher when %DMA is lower, meaning a reduced metabolic capability.

BMI: Body mass index

Bold letters indicate a $p<0.05$.

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Table 3. Association between urinary arsenic and interaction with arsenic metabolism with gestational age at birth

Variable	Adjusted	95% CI	Adjusted	95% CI
Urinary Arsenic	-0.08	-2.48 ; 2.32	0.02	-2.37 ; 2.40
Score 1[α]	0.01	-0.02 ; 0.04	0.01	-0.02 ; 0.04
Urinary Arsenic*Score 1	-0.19	-1.63 ; 1.24	-0.17	-1.53 ; 1.19
Mother's age	**-0.03**	**-0.06 ; -0.004**	**-0.03**	**-0.06 ; -0.001**
Pregestational BMI	**-0.04**	**-0.07 ; -0.01**	-0.03	-0.07 ; 0.003
Education				
Elementary		Ref.		Ref.
Secondary	0.62	-0.12 ; 1.36	0.48	-0.27 ; 1.23
Tertiary	0.55	-0.21 ; 1.31	0.32	-0.45 ; 1.08

Residuals were calculated from the model $tAs \sim \beta_1(Arsenobetaine)+\beta_2(Arsenobetaine)^2$
Models were adjusted for mother's age, mother's education level, pre-gestational body mass index.

95% CI: 95% Confidence Interval.
BMI: Body mass index.
Bold letters indicate a $p<0.05$.
[α]Score 1 (arsenic toxicokinetics difference between women), obtained from principal components analysis, is higher when %DMA is lower, meaning a reduced metabolic capability.

Table 4. Association between urinary arsenic and interaction with arsenic metabolism with birth weight and gestational age at birth stratified by newborn sex.

Newborn sex	Regression term	Birth weight		Gestational age at birth	
		Adjusted	p-value	Adjusted	p-valu
Male	Urinary Arsenic	2.79 (-0.02 ; 5.60)	0.052	7.36 (0.30 ; 14.42)	0.041
	Urinary Arsenic * Score 1[α]	0.36 (-1.41 ; 2.13)	0.689	-2.79 (-8.84 ; 3.26)	0.364
Female	Urinary Arsenic	-0.47 (-4.27 ; 3.32)	0.806	-6.92 (-16.62 ; 2.77)	0.160
	Urinary Arsenic * Score 1	-1.41 (-3.79 ; 0.98)	0.245	0.95 (-4.83 ; 6.72)	0.745

Regressions were adjusted for mother's age, pregestational body mass index and education.

Coefficients for gestational age at birth are scaled (Urinary arsenic/1000).

[α]PCA Score 1 (arsenic toxicokinetics difference between women) is higher when %DMA is lower, meaning a reduced metabol capability.

For both models, the adjusted regression coefficient (95% Confidence Interval) is showed.

Table 5. Association between urinary arsenic and interaction with arsenic metabolism with birth weight and gestational age at birth stratified by visit.

Trimester	Regression term	Birth weight		Gestational age at birth	
		Adjusted	p-value	Adjusted	p-value
Second	Urinary Arsenic	1.61 (-1.44 ; 4.67)	0.298	-5.11 (-14.43 ; 4.20)	0.28
	Urinary Arsenic * Score 1[α]	-1.36 (-3.32 ; 0.59)	0.170	-5.13 (-12.00 ; 1.75)	0.142
Third	Urinary Arsenic	-1.91 (-6.09 ; 2.27)	0.368	7.88 (-5.81 ; 21.57)	0.257
	Urinary Arsenic * Score 1	1.60 (-0.84 ; 4.05)	0.197	-0.81 (-8.19 ; 6.57)	0.828

Regressions were adjusted for mother's age, pregestational body mass index and education.

Coefficients for gestational age at birth are scaled (Urinary arsenic/1000).

[α]PCA Score 1 (arsenic toxicokinetics difference between women) is higher when %DMA is lower, meaning a reduced metabolic capability.

For both models, the adjusted regression coefficient (95% Confidence Interval) is showed.

Supplementary Material 1. Arsenic Principal Component Analysis, correlation and eigenvectors.

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. pca ias_ mma_ dma_
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Principal components/correlation — Number of obs = 274; Number of comp. = 3; Trace = 3; Rotation: (unrotated = principal); Rho = 1.0000

Component	Eigenvalue	Difference	Proportion	Cumulative
Comp1	2.53069	2.27071	0.8436	0.8436
Comp2	.259982	.0506548	0.0867	0.9302
Comp3	.209328	.	0.0698	1.0000

Principal components (eigenvectors)

Variable	Comp1	Comp2	Comp3	Unexplained
ias_	0.5834	-0.0977	-0.8063	0
mma_	0.5729	0.7531	0.3233	0
dma_	0.5756	-0.6506	0.4954	0

Supplementary Material 2. Water arsenic concentrations distribution of pregnant women in the second and third trimester, and its correlation with urinary DMA.

Water arsenic level category (µg/L)[Ω]	Second trimester			Third trimester		
	#Pregnant women	%	DMA correlation[£]	#Pregnant women	%	DMA correlation[£]
5	15	9.43	0.345**	24	17.52	0.279*
10	32	20.13		47	34.31	
25	55	34.59		39	28.47	
50	33	20.75		19	13.87	
100	22	13.84		5	3.65	
250	2	1.26		3	2.19	

[Ω] Water arsenic concentrations were obtained by analyzing household drinking water samples, using a semi-quantitative method described in Fano et al., 2019.
[£] Spearman correlation analysis (Spearman's rho).
*p<0.01, **p<0.001

Fano D, Vásquez-Velásquez C, Aguilar J, Gribble MO, Wickliffe JK, Lichtveld MY, Steenland K, Gonzales GF. Arsenic Concentrations in Household Drinking Water: A Cross-Sectional Survey of Pregnant Women in Tacna, Peru, 2019. Expo Health. 2020 Dec;12(4):555-560. doi: 10.1007/s12403-019-00337-5. Epub 2019 Dec 7. PMID: 33210017; PMCID: PMC7668403.

Supplementary material 3. Mean birth weight comparison between women with total urinary arsenic exposure levels ≥100 µg/L and <100 µg/L by trimester of pregnancy.

Trimester	tAs exposure (µg/L)	#Participants	Birth weight		p-value*
			Mean	SD	
Second	<100	122	3623.82	489.99	0.749
	≥100	25	3589.79	412.38	
Third	<100	91	3622.06	430.73	0.865
	≥100	36	3606.32	631.28	

tAs: Total urinary arsenic
SD: Standard deviation
*p-value for Student's t-test

Supplementary material 4. Mean birth weight according to the mother's self-reported ethnic group, and one-way ANOVA analysis.

Ethnic group (n)	Birt weight		p-value*
	Mean	SD	
Mestizo (52)	3536.06	486	
Quechua (18)	3466.11	391.47	0.037
Aymara (87)	3711.38	480.2	

SD: Standard deviation

The group size for each ethnic group is displayed in parenthesis.

*p-value for One-way ANOVA test

Supplementary material 5. Percentage of dimethylarsonic acid (%DMA) in different ethnic groups.

Ethnic group (n)	%DMA		p-value*
	Mean	SD	
Mestizo (43)	84.88	4.1	
Quechua (14)	84.99	4.23	0.463
Aymara (65)	84.66	4.02	

DMA: Dimethylarsonic acid

The group size for each ethnic group is displayed in parenthesis

*p-value for one-way ANOVA test