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A pilot study of low-moderate drinking water arsenic contamination and chronic diseases among reproductive age women in Timi County, Romania

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Abstract

We conducted a pilot study of associations between drinking water contaminated by inorganic arsenic (iAs), mostly <10 µg/L, and self-reported chronic diseases in 297 pregnant women. Adjusted for confounding variables, we identified a positive association between iAs and heart disease (OR=1.63, 95% CI 0.81-3.04, p=0.094), which was stronger for women living at their current residence 10 years (OR=2.47, 95% CI 0.87-10.43, p=0.058). Confounder-adjusted associations were also suggested for iAs with kidney disease (OR=1.32, 95% CI 0.77-2.21, p=0.265) and with high blood pressure (OR=1.36, 95% CI 0.68-2.39, p=0.300). A post-hoc power analysis indicated the need for a larger study with more statistical power.

Keywords

Arsenic; blood pressure; drinking water; heart disease; kidney disease; thyroid disorder

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1. Introduction

In recent years, exposure to high levels of inorganic arsenic (iAs) through consumption of contaminated drinking water, concentrations above 10 µg/L and frequently greater than 50 µg/L, has been associated with the development of a number of chronic human health effects (Kwok, 2007; Tseng, 2008; Zheng et al., 2014). Yet, few data are available to evaluate the impact of more widespread low-moderate levels of drinking water iAs contamination, those less than 10 µg/L (van Halem et al., 2009), in particular among women of reproductive age. To begin to address the pending data gap, we conducted a hypothesis-generating study in a population of pregnant women to guide the design of a future, more definitive investigation.

2. Materials and Methods

2.1 Study sample

Our sample was recruited as part of a larger case-control study of 297 women, with 300 pregnancies (i.e., n=3 women participated as both a control and a case), designed to explore relations between drinking water iAs exposure and spontaneous pregnancy loss in Timi County, Romania. This area is recognized for low-moderate iAs groundwater contamination. A detailed description of participant recruitment and data collection was previously published (Bloom et al., 2014). Briefly, 150 cases of spontaneous clinical pregnancy loss were enrolled along with 150 gestational age-matched controls (± 1 week) undergoing routine prenatal care at the Emergency County Hospital (“Bega Hospital”) in Timis County, Romania. Participation rates were high among cases (88%) and among controls (83%). Prenatal care is provided at no cost using centralized county medical facilities (i.e., the Bega Hospital in Timis County) and is compulsory in Romania. All new pregnancies are registered by family physicians, and referred to a gynecologist for evaluation and clinical examination by one month gestation. Follow-up prenatal care visits are required in the second and third trimesters. Approximately 85% of pregnant women comply with the policy and receive compulsory pregnancy services; more than 99% of women do so at no cost using county medical facilities. Demographic data, medical and reproductive histories, and residential and occupational drinking water consumption histories were captured via physician-administered questionnaire. Women were asked if they had ever received the case diagnoses, including heart disease, kidney disease, high blood pressure (HBP), low blood pressure (LBP), or thyroid disorders, and if so at what age. Clinical data were collected by a research nurse at intake. We collected water samples from up to two residential drinking sources for each participant, including municipal sources comprised primarily of surface waters or mixed surface and groundwater, and public or private groundwater wells, as previously described in detail (Neamtiu et al., 2015).

2.2 Laboratory analysis

Within a few weeks of study enrollment, drinking water samples were collected from taps or wells into decontaminated containers and transferred to the Environmental Health Center laboratory in Cluj-Napoca, Romania. Specimens were analyzed by hydride generation-atomic absorption spectrometry, using a Zeenit 700p atomic absorption spectrometer (Analytikjena, Jena, Germany), as previously described in detail (Bloom et al., 2014). The

method detection limit (MDL) was 0.5 µg/L and values were reported without imputation below the MDL. To preclude the introduction of bias that has been demonstrated using substitution in regression models (Schisterman et al., 2006), we entered the “machine-read” values from the instrument into regression models. We defined average iAs concentration (µg/L) as the arithmetic mean of iAs levels measured in up to two residential sources. We defined the peak iAs concentration (µg/L) as the higher of iAs levels in up to two residential sources. Finally, we defined daily iAs consumption (µg/day) as the reported average daily water intake from non-bottled sources, including mixed beverages and soups, multiplied by the average iAs concentration.

2.3 Statistical analysis

Cases were defined as self-reported diagnosis of heart disease, kidney disease, HBP, LBP, or thyroid disorders. Women without a study outcome diagnosis constituted the reference group for each endpoint. We variously excluded n=30 who did not reside at the study address at the time of case diagnosis, and n=39 who did not report an age of diagnosis or had a missing value for a diagnosis.

We natural log transformed iAs exposure metrics (adding a constant to accommodate zeros) to achieve a normal distribution prior to analysis. We compared women living at their current residence for at least 10 years to women residing at their current address for less than 10 years, using bivariate analysis. We used individual Firth logistic regression models to explore the effects of iAs exposure on heart disease, kidney disease, HBP, LBP, and thyroid disorder. Firth regression employs penalized maximum likelihood estimation rather than traditional maximum likelihood estimation to accommodate separation resulting from limited sample sizes (Firth, 1993). Artificial data, which have no influence on model inferences, are “added” to generate stable estimates in the context of a large number of empty cells. We constructed unadjusted and adjusted regression models for age, education, and cigarette smoking, and calculated profile-likelihood based 95% confidence intervals (Heinze and Schemper, 2002). In sensitivity analyses, we incorporated “lag times,” by restricting the sample to women residing at the study address for 5 years or 10 years.

We used SAS v.9.3 (SAS Institute Inc., Cary, NC) for all analysis and defined statistical significance as $p < 0.05$ for a two-tailed test. We also conducted *post hoc* analysis using PASS v.13 (NCSS, LLC. Kaysville, UT), to estimate sample sizes required for 80% power to detect effect estimates at $\alpha = 0.05$.

3. Results

Table 1 describes our study participants. The women were 29.2 years of age on average and more than one-third (38.7%) reported having smoked over 100 cigarettes over their lifetime. The prevalences of heart disease, kidney disease, HBP, LBP, and thyroid disorder were 2.4% (n=7), 11.8% (n=35), 5.4% (n=16), 10.4% (n=31), and 4.0% (n=12), respectively. None of the case diagnoses were received during the study pregnancy. Geometric mean average iAs concentration (2.9 vs. 1.1 µg/L; $p = 0.098$) and daily iAs exposure (4.3 vs. 1.3 µg/day; $p = 0.056$) were higher for kidney disease cases than for referents, by Student T-test. Women residing at the study address for 10 years reported higher daily water consumption

(1.4 vs. 1.2 L; $p=0.003$), and had been less likely to complete university (33.6% vs. 48.6%; $p=0.012$) than those residing at the study address for <10 years.

Table 2 describes unadjusted and covariate-adjusted Firth logistic regression models for case diagnosis on iAs exposure. We detected a borderline-significant 63% higher adjusted odds for heart disease per 2.72-fold (i.e., natural log increase) higher average drinking water iAs concentration (OR=1.63; 95%CI 0.81-3.04; $p=0.094$). Additionally, we identified a 32% higher adjusted odds for kidney disease in association with a 2.72-fold higher average iAs concentration (OR=1.32; 95%CI 0.77-2.21; $p=0.265$), though not significant. We found analogous raised, but non-significant, adjusted odds for average iAs concentration in association with HBP (OR=1.36; 95%CI 0.68-2.39; $p=0.300$) and thyroid disorder (OR=1.30; 95%CI 0.65-2.27; $p=0.354$). The results were similar when using peak iAs concentration and daily iAs consumption as exposure metrics (data not shown).

We also conducted sensitivity analyses by excluding women with any case diagnosis of interest from the referent groups, and by incorporating 5 and 10 year time “lags” to evaluate the impact of exposure duration on associations. Our results were comparable when we excluded all case women from the referent groups (data not shown). Effects were similar to overall estimates when the sample was restricted to women living at the study residence for 5 years (data not shown). However, when restricted to women residing at the study residence for 10 years (Table 2), a 2.72-fold higher average drinking water iAs concentration was associated with more than double the covariate-adjusted odds for heart disease, with borderline significance (OR=2.47; 95%CI 0.87-10.43; $p=0.058$), although imprecise. We found analogously borderline significant elevated adjusted ORs for peak iAs and heart disease (OR=2.26; 95%CI 0.80-8.96; $p=0.076$), and for daily iAs and heart disease (OR=2.05; 95%CI 0.79-8.80; $p=0.091$), when restricted to women residing at the study address for 10 years. In contrast, no effect was indicated for average iAs concentration and HBP using 5 year (OR=1.06; 95%CI 0.44-2.00; $p=0.859$) or 10 year (OR=0.88; 95%CI 0.31-2.18; $p=0.782$) lag times, or when using peak and daily iAs as exposures. The lag analyses had negligible impacts on kidney and thyroid disorder estimates.

According to *post hoc* power analysis (Table 2), a five to six-fold increase in sample size is required for 80% power to detect most effect estimates at $\alpha=0.05$. More specifically, $n=1520$ to detect OR=1.63 between average drinking water iAs concentration and heart disease, $n=1059$ to detect OR=1.32 for kidney disease, and $n=1760$ to detect OR=1.36 for HBP.

4. Discussion

The overall results of this pilot study suggest a higher prevalence of heart disease in association with higher drinking water iAs exposure, at low-moderate levels. In particular, longer iAs exposure may be associated with a higher risk. While effects were more modest, our results also raise the possibility for associations between low-moderate level drinking water iAs exposure and kidney disease and HBP among women in Timi County, Romania. Given the widespread nature of drinking water iAs exposure and the reproductive age of our study participants, these results should raise concern.

Our results are similar to the few prior studies that evaluated associations between low-moderate drinking water iAs exposure and chronic health outcomes. In a Texas cohort exposed to a mean of 6.2 µg/L drinking water iAs (Gong and O'Bryant, 2012), higher odds of heart disease was detected in association with higher exposure (OR=1.10; 95%CI 1.00-1.21; $p<0.05$), as was an elevation for HBP (OR=1.10; 95%CI 1.03-1.17; $p<0.05$). A recent case-control study in China (Wade et al., 2015) also reported higher odds for cardiovascular disease in association with a 10 µg/L increase in drinking water iAs concentrations (OR=1.19; 95%CI 1.03-1.38; $p=0.021$). A large cross-sectional study conducted in an aging U.S. population (mean=62 years), reported no significant associations between participant reported heart disease or HBP and exposure to drinking water iAs 10 µg/L (Zierold et al., 2004). Yet, participants exposed to iAs >10 µg/L in that study experienced higher odds for HBP (OR=1.68; 95%CI 1.13-2.49; $p<0.05$). A recent U.S. study, reported an increased probability for incident chronic kidney disease in association with higher quartiles of urine total iAs ($p<0.01$), among Native Americans with low-moderate level drinking water iAs exposure (Zheng et al., 2015). Another recent U.S. study reported higher odds for hypothyroidism among Hispanics with exposure to 8-22 µg/L drinking water iAs in Texas (OR=2.00, 95%CI 1.06-3.76; $p=0.032$), which the authors attributed in part to residential location (Gong et al., 2015). A study of low to moderate drinking water iAs exposures in nearby Arad County, Romania demonstrated a positive association with male blood pressure reactivity, an early indicator for hypertension and cardiovascular disease (Kunrath et al., 2013).

In our study, we evaluated the impact of exposure duration in sensitivity analyses. We detected higher odds for heart disease in association with higher average, peak, and daily iAs concentrations for women living at the study residence for 10 years, relative to the overall analysis. However, the overall suggested effects for HBP dissipated when we incorporated the time lag, indicating an acute impact for iAs on blood pressure regulation, or that the suggested effect occurred by chance. In contrast, the time lags appeared to have no meaningful impact for the iAs effects on kidney disease, LBP, and thyroid disorder. We detected differences in water consumption and education between women living at the study residence for 10 years and those residing at the study address for <10 years. Given the higher reported rate of water consumption, women residing longer may have had higher cumulative iAs exposures, accounting in part for the stronger heart disease effect in the 10 years group.

Our study population comprised women previously recruited to a case-control study of incident spontaneous pregnancy loss and drinking water iAs exposure, for which we found no main iAs effect (Bloom et al., 2014). In the context of Romanian prenatal care, our study population was likely to comprise a representative sample of all pregnant Timis County women between December, 2011 and January, 2013 and with representative drinking water iAs exposures. To maximize the available statistical power for the current cross-sectional analysis, we included all women in referent groups for each outcome diagnosis, including women with other diagnoses under study. This might bias the results if iAs exposure was associated with selection to the referent group. Still, our results were nearly identical in a sensitivity analysis excluding case women from referent groups and so such bias was unlikely. Prevalences for heart (1.5%) and kidney diseases (9.7%) were similar in previous

reports for Romanian women of reproductive age (Bunescu et al., 2008; Cepoi et al., 2012). However, HBP was reported for 13.9% of Romanian women 18-44 years of age (Dorobantu et al., 2010). Our sample of pregnant women may be distinct from groups considered in these prior surveys, as only pregnant Timis County women were enrolled. Still, we do not believe that outcome misclassification would be related to exposure and so any bias is likely to be towards the null hypothesis.

Our study has several limitations, stemming primarily from the secondary nature of the study hypotheses and the limited sample size. Given our limited sample size we may also have missed subtle differences in odds between cases and referents. A five to six-fold increase in sample size, suggested by our *post hoc* power analysis, is beyond the scope of this pilot study, but indicates the need for a larger investigation with more statistical power. Furthermore, we had no biomarker for iAs exposure, although we generated individual exposure estimates utilizing questionnaire data weighted by environmental sampling, which took into account water use patterns. Finally, the cross-sectional nature of our design precludes assessment of temporality between the iAs exposure and study outcomes, and so we cannot rule out reverse causation.

In summary, our results raise the possibility for elevated odds for a history of heart disease, and modestly elevated odds for a history of kidney disease and HBP associated with long-term exposure to low-moderate drinking water iAs contamination in pregnant Timi County women. A more definitive study will require an increase in sample size. Given the widespread nature of drinking water iAs contamination, the young age of our study population, and the few data available to assess the risks at low to moderate levels of exposure, these preliminary results warrant further examination in a larger, more comprehensive investigation.

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Table 1
Distribution of demographic and clinical variables among pregnant women from Timi County, Romania.

Factors	n	Mean	SD	Minimum	25 th %tile	Median	75 th %tile	Maximum
Demographics								
Age (years)	297	29.2	5.9	18.1	24.9	28.8	33.5	44.3
Body mass index (kg/m ²)	295 ^a	22.5	3.9	15.1	20.2	21.6	24.1	46.6
Education level ^b	-	-	-	-	-	-	-	-
University degree (%)	127	42.7	-	-	-	-	-	-
High school (%)	96	32.3	-	-	-	-	-	-
Less than high school (%)	74	25.0	-	-	-	-	-	-
Smoked 100 cigarettes (%)	115	38.7	-	-	-	-	-	-
Exposure metrics								
Average iAs (µg/L)	297	4.2	14.4	-0.6	-0.2	0.4	3.6	175.1
Peak iAs (µg/L)	297	4.8	14.7	-0.6	-0.2	0.5	3.8	175.1
Daily iAs (µg/day)	297	6.1	23.1	-0.9	-0.2	0.6	5.0	350.2
Daily water consumption (L) ^b	297	1.4	0.6	0	1.0	1.3	1.8	5.0
Study endpoints ^c								
Heart disease Dx. (%)	7	(2.4)	-	-	-	-	-	-
Kidney disease Dx. (%)	35	(11.8)	-	-	-	-	-	-
High blood pressure Dx. (%)	16	(5.4)	-	-	-	-	-	-
Low blood pressure Dx. (%)	31	(10.4)	-	-	-	-	-	-
Thyroid disorder Dx. (%)	12	(4.0)	-	-	-	-	-	-

Dx., self-reported diagnosis; SD, standard deviation.

^a n=2 missing;

^b p<0.05 for difference between women living at the study residence for 10 years and women living at the study residence for <10 years;

^c excluding women that did not provide age at diagnosis.

Logistic regression analysis of self-reported case diagnoses on average drinking water iAs concentration ($\mu\text{g/L}$) among pregnant women from Timi County, Romania.

Table 2

Study endpoints	n Cases	n Referents	OR ^a	95% CI ^{a,b}		P-value ^a	aOR ^c	95% CI ^{b,c}		P-values ^c	n aOR ^d
				Low	High			Low	High		
All women:											
Heart disease Dx.	6	289	1.40	0.71	2.44	0.262	1.63	0.81	3.04	0.094	1520
Kidney disease Dx.	9	261	1.55	0.91	2.52	0.081	1.32	0.77	2.21	0.265	1059
High blood pressure Dx.	8	281	1.06	0.53	1.83	0.849	1.36	0.68	2.39	0.300	1760
Low blood pressure Dx.	6	265	0.88	0.35	1.71	0.732	1.07	0.42	2.15	0.832	19,928
Thyroid disorder Dx.	7	284	1.18	0.60	2.05	0.577	1.30	0.65	2.27	0.354	3216
10 year lag women ^e :											
Heart disease Dx.	2	114	2.35	0.85	7.78	0.090	2.47	0.87	10.43	0.058	-
Kidney disease Dx.	6	105	1.30	0.63	2.49	0.435	1.24	0.60	2.45	0.523	-
High blood pressure Dx.	7	107	0.77	0.32	1.51	0.484	0.88	0.31	2.18	0.782	-
Low blood pressure Dx.	2	106	0.90	0.19	2.53	0.840	0.96	0.17	3.21	0.930	-
Thyroid disorder Dx.	4	112	1.16	0.47	2.52	0.707	1.21	0.47	2.85	0.611	-

aOR, adjusted odds ratio; CI, confidence intervals; OR, odds ratio; Dx., self-reported case diagnosis.

^a Unadjusted;

^b profile-likelihood CIs;

^c adjusted for age, education, and cigarette smoking, using Firth penalized maximum likelihood estimation;

^d sample size required for 80% statistical power to detect aOR at $\alpha=0.05$;

^e women living at the study residence for 10 years.