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Research Article

Arsenic Exposure and Cancer Mortality in a US-Based Prospective Cohort: The Strong Heart Study

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Abstract

Background: Inorganic arsenic, a carcinogen at high exposure levels, is a major global health problem. Prospective studies on carcinogenic effects at low–moderate arsenic levels are lacking.

Methods: We evaluated the association between baseline arsenic exposure and cancer mortality in 3,932 American Indians, 45 to 74 years of age, from Arizona, Oklahoma, and North/South Dakota who participated in the Strong Heart Study from 1989 to 1991 and were followed through 2008. We estimated inorganic arsenic exposure as the sum of inorganic and methylated species in urine. Cancer deaths (386 overall, 78 lung, 34 liver, 18 prostate, 26 kidney, 24 esophagus/stomach, 25 pancreas, 32 colon/rectal, 26 breast, and 40 lymphatic/hematopoietic) were assessed by mortality surveillance reviews. We hypothesized an association with lung, liver, prostate, and kidney cancers.

Results: Median (interquartile range) urine concentration for inorganic plus methylated arsenic species was 9.7 (5.8–15.6) μg/g creatinine. The adjusted HRs [95% confidence interval (CI)] comparing the 80th versus 20th percentiles of arsenic were 1.14 (0.92–1.41) for overall cancer, 1.56 (1.02–2.39) for lung cancer, 1.34 (0.66, 2.72) for liver cancer, 3.30 (1.28–8.48) for prostate cancer, and 0.44 (0.14, 1.14) for kidney cancer. The corresponding hazard ratios were 2.46 (1.09–5.58) for pancreatic cancer, and 0.46 (0.22–0.96) for lymphatic and hematopoietic cancers. Arsenic was not associated with cancers of the esophagus and stomach, colon and rectum, and breast.

Conclusions: Low to moderate exposure to inorganic arsenic was prospectively associated with increased mortality for cancers of the lung, prostate, and pancreas.

Impact: These findings support the role of low–moderate arsenic exposure in development of lung, prostate, and pancreas cancer and can inform arsenic risk assessment. *Cancer Epidemiol Biomarkers Prev; 22(11); 1944–53.* ©2013 AACR.*Cancer Epidemiol Biomarkers Prev; 22(11); 1944–53. ©2013 AACR.*

Introduction

Inorganic arsenic is a major public health problem worldwide. The International Agency for Research on

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Cancer (IARC) has established a causal role for arsenic on skin, lung, and bladder cancers, and has shown suggestive but limited human evidence for liver, kidney, and prostate cancers (1). Key epidemiologic evidence came from populations chronically exposed to high arsenic levels in drinking water (>150 μg/L) in southwestern Taiwan (2), Bangladesh (3), northern Chile (4), and Argentina (5). Recent experimental studies have shown that inorganic arsenic and its methylated metabolites can induce lung (6, 7), liver (8), and bladder (7, 9) cancers in animal models and malignant transformation of human prostate epithelium in cellular model systems (10).

Main sources of arsenic exposure include drinking water and certain foods (e.g., rice, grains or juices; refs. 11, 12). The current safety standard for arsenic in drinking water established by the World Health Organization, the U.S. Environmental Protection Agency (USEPA), and the European Union is 10 μg/L (13, 14). There are concerns that this limit is too high to protect human populations from excessive cancer risk (15). For food, there are no current safety standards, despite widespread low–moderate inorganic arsenic exposure through the diet. Few epidemiologic studies have evaluated the carcinogenic

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effects of arsenic at low–moderate arsenic levels in drinking water and food, most of them ecologic or retrospective (16). We evaluated the association of low–moderate exposure to inorganic arsenic with overall and site-specific cancer mortality in American Indian men and women from Arizona, Oklahoma, and North/South Dakota who participated in the Strong Heart Study (SHS) from 1989 to 91 and were followed through 2008. For cancers of the lung, liver, prostate, and kidney, we hypothesized a positive association on the basis of human studies at high arsenic levels (1). For other cancers, we had no *a priori* hypothesis, although arsenic has been associated with pancreas cancer in an ecologic study from Japan (17) and a case–control study from Spain (18). Our prospective cohort study can inform about cancer mortality risk at low–moderate arsenic exposure in U.S. populations.

Materials and Methods

Study population

The SHS recruited 4,549 men and women, 45 to 75 years of age, from 13 tribes and communities during 1989 to 1991. In Arizona and Oklahoma, every eligible person was invited; in North/South Dakota a cluster sampling technique was used (19). The baseline participation rate was 62%. We excluded 576 participants due to insufficient urine for arsenic analysis, and 38 participants because of missing information in other variables, leaving 3,935 participants for this study. The SHS protocol was approved by local institutional review boards, the Indian Health Service and the study communities. All participants provided oral and written informed consent.

Baseline data collection

Study visits were conducted by trained and certified examiners following a standard protocol (19), and included a questionnaire (sociodemographic factors, smoking status, alcohol use, and medical history), a physical examination (height, weight, and blood pressure), and biospecimen collection (blood and urine). Hypertension was defined as mean systolic blood pressure 140 mm Hg or more, mean diastolic blood pressure 90 mm Hg or more, or antihypertensive medication. Diabetes was defined as fasting plasma glucose 126 mg/dL or more, 2-hour postload plasma glucose 200 mg/dL or more, hemoglobin A1c 6.5% or more, or use of insulin or oral hypoglycemic agents. Plasma creatinine was measured by an alkaline–picrate rate method to estimate glomerular filtration rate (eGFR). Urine creatinine was measured by an automated alkaline–picrate methodology (19).

Urinary arsenic and creatinine determinations

The analytic methods to determine arsenic species for this study have been previously described in detail (20). Inorganic arsenic (arsenite, arsenate), monomethylarsonate (MMA), dimethylarsinate (DMA), and arsenobetaine plus other arsenic cations were measured using anion-exchange high-performance liquid chromatography (HPLC; Agilent 1100 HPLC, Agilent Technologies) cou-

pled with inductively coupled plasma mass spectrometry (Agilent 7700x ICPMS). The limits of detection were 0.1 μg/L for inorganic arsenic, MMA, DMA, and arsenobetaine plus other cationic arsenic species. The percentages of participants with concentrations below the limit of detection were 5.3% for inorganic arsenic, 0.7% for MMA, 0.03% for DMA, and 2.1% for arsenobetaine plus other cations. For undetectable concentrations, we divided the limit of detection by $\sqrt{2}$. The interassay coefficients of variation were 6.0% for inorganic arsenic, 6.5% for MMA, 5.9% for DMA, and 6.5% for arsenobetaine plus other cations. Arsenobetaine concentrations were low [median, 0.76 μg/L, interquartile range (IQR) 0.47–1.70 μg/L], confirming that seafood intake is rare in the SHS population.

Cancer mortality follow-up

Follow-up for mortality was complete for 99.8% of the study population. Death certificates were obtained from the State Departments of Health. If the death certificate indicated that an autopsy had been conducted, the medical examiner's report was obtained. Death certificate codes were recorded according to the International Classification of Diseases, 9th Revision (ICD-9) by a single nosologist. In addition to overall cancer deaths, we evaluated the following cancers: esophagus and stomach (ICD-9 150-151); colon and rectum (ICD-9 153-154); liver and intrahepatic bile ducts (ICD-9 155); gallbladder and extrahepatic bile ducts (ICD-9 156; ICD-9 155 and 156 were combined due to small sample size and similar results in separate analyses); pancreas (ICD-9 157); trachea, bronchus, and lung (ICD-9 162; referred from now on as lung cancer); breast (ICD-9 174); prostate (ICD-9 185); kidney (ICD-9 189.0); and lymphatic and hematopoietic tissue (ICD-9 200–208). Skin and bladder cancers were not evaluated as the corresponding number of deaths was very small (2 and 1, respectively).

Time to event was calculated from the date of baseline examination to the date of death or to December 31, 2008 whichever occurred first. The mean follow-up time among participants who did not have a cancer death was 14.6 years.

Statistical methods

We used the sum of inorganic (arsenite and arsenate) and methylated (MMA and DMA) arsenic as the biomarker of exposure to inorganic arsenic. To account for urine dilution in spot urine samples, we divided urinary arsenic by urinary creatinine. Urinary arsenic concentrations were markedly right-skewed and log-transformed for statistical analyses. Statistical analyses were conducted using Stata 11.2 (StataCorp).

The prospective association between the creatinine-corrected sum of inorganic and methylated arsenic and cancer mortality (overall and site-specific) was assessed using Cox-proportional hazards models with age as time scale and individual starting follow-up times (age at baseline) treated

as staggered entries. The assumption of hazards proportionality was evaluated on the basis of the smoothed association between age and scaled Schoenfeld residuals, with no major departures from proportionality. The nonparametric underlying baseline hazards were allowed to differ by study region. We modeled arsenic in three ways: (i) tertiles; (ii) 80th versus 20th percentiles of log-transformed arsenic; and (iii) restricted cubic splines with knots at the 10th (3.80 μg/g creatinine), 50th (9.68 μg/g), and 90th (24.0 μg/g) percentiles to evaluate potential nonlinear relationships. P values for linear trend were obtained from Wald tests by introducing log-arsenic concentrations as a continuous variable. Departures from linearity in the restricted cubic spline models were evaluated using the Wald test.

All Cox-proportional hazards models initially accounted for region (stratification factor) and age (model 1). In addition, we further adjusted for sex, education, smoking status, drinking status, and body mass index (BMI; model 2). Model 2 was further adjusted for menopausal status and parity for breast cancer, for hypertension and reduced eGFR for kidney cancer, and for diabetes for prostate cancer. We conducted exploratory interactive analyses (multiplicative scale) for overall cancer mortality including interaction terms for log-transformed arsenic concentrations with indicator variables for subgroups defined by age, sex, smoking status, diabetes, %DMA, and %MMA in separate models. Interactive analyses were not conducted for specific cancers because the number of deaths across subgroups was too small.

We conducted several sensitivity analyses. First, we further adjusted for cigarette pack-years ($n = 3,789$). Second, we estimated proportional hazard regression models accounting for competing risks according to the method of Fine and Gray (21). Third, to minimize the possibility that prevalent cancers at baseline modified urinary arsenic excretion, we repeated the analyses excluding participants

during the first 2 or 5 years of follow-up. Fourth, to evaluate potential differences in latency, we conducted separate analyses for the first and second decades of follow-up. Fifth, we repeated the analyses for overall cancer excluding cancer deaths coded as "malignant neoplasms of other and unspecified sites". The results of these sensitivity analyses were similar to the main analyses (not shown). Finally, we used two alternative strategies to account for urine dilution: (i) adjusting for log-transformed urinary creatinine in the regression models instead of dividing urinary arsenic by urinary creatinine concentrations (22); and (ii) adjusting urine arsenic concentrations to the overall mean specific gravity (SG) in the study population (1.019 g/mL) (arsenic (μg/L) × [mean SG (g/mL) − 1]/[SG (g/mL) − 1]) (23). This last analysis was restricted to participants without albuminuria and diabetes because specific gravity is inadequate to adjust for dilution if albumin or glucose are present in the urine (24).

Results

A total of 225 women and 160 men died from cancer during the studied period. The most common cancer sites were lung ($n = 34$) and breast ($n = 26$) in women, and lung ($n = 44$) and prostate ($n = 18$) in men. A total of 46 cancer deaths were unspecified (ICD-9 190–199 and 239). Older participants, participants living in North and South Dakota, participants with lower education, current smokers, and never drinkers at baseline had higher cancer mortality (Table 1).

The median (IQR) concentration for inorganic plus methylated arsenic at baseline was 9.7 (5.8–15.6) μg/g creatinine [10.4 (6.12–18.4) μg/L]. Participants living in Arizona and North and South Dakota, participants who had a lower education, current drinkers, and those with lower BMI and diabetes had higher urinary arsenic concentrations (Table 2).

Table 1. Baseline characteristics of study participants overall and by cancer mortality status

| | All participants | Cancer death | |
	($n = 3,935$)	Yes ($n = 386$)	No ($n = 3,549$)
Age, y	56.2 (8.0)	59.8 (8.1)	55.9 (7.9)
Men,%	40.7	41.5	40.7
Postmenopausal women, %[a]	77.0	87.6	75.8
Arizona,%	33.4	28.2	34.0
Oklahoma,%	33.5	32.4	33.6
Dakota,%	33.1	39.4	32.4
<High school,%	47.3	53.4	46.6
Current smoking,%	33.3	42.8	32.4
Former smoking,%	34.0	31.1	34.3
Current drinking,%	41.9	35.5	42.6
BMI, kg/m^2	30.9 (6.3)	30.4 (6.6)	30.9 (6.3)
Diabetes,%	49.4	50.4	49.3

NOTE: Data in the table are percentages for categorical variables or means (SD) for continuous variables.
[a]Subsample of women ($n = 2,332$).

Table 2. Median (IQR) for the sum of inorganic and methylated arsenic concentrations by participant characteristics

		All participants, arsenic (μg/g creatinine)			Participants without diabetes and albuminuria, arsenic (μg/L)[a]		
		n	Median (IQR)	P value[b]	*n*	Median (IQR)	P value[b]
Overall		3,935	9.7 (5.8–15.6)		1,765	11.1 (6.9–17.7)	
Age in years	<55	1,947	9.7 (5.9–15.7)		959	11.1 (7.1–17.9)	
	55–64	1,292	9.6 (5.7–15.4)		548	11.3 (6.7–17.6)	
	>65	696	9.7 (5.6–15.8)	0.28	258	10.9 (6.4–18.0)	0.53
Sex	Male	1,603	8.8 (5.1–14.3)		784	11.3 (6.8–17.6)	
	Female	2,332	10.4 (6.2–16.6)	<0.001	981	11.0 (6.9–17.9)	0.57
Region	Arizona	1,314	14.3 (9.9–20.8)		329	15.6 (10.3–23.6)	
	Oklahoma	1,318	5.6 (3.8–8.2)		702	7.5 (5.1–11.7)	
	Dakota	1,303	10.6 (6.9–15.8)	<0.001	734	13.0 (8.7–20.9)	<0.001
Education	No high school	868	13.1 (8.9–20.3)		302	15.5 (10.3–22.5)	
	Some high school	992	10.1 (5.9–16.6)		398	11.2 (6.8–18.6)	
	Completed high school	2,075	8.0 (5.0–13.2)	<0.001	1,065	10.0 (6.3–16.1)	<0.001
Smoking status	Never	1,284	10.1 (6.0–16.5)		511	10.5 (6.6–17.5)	
	Former	1,338	9.2 (5.6–15.0)		553	10.4 (6.5–16.1)	
	Current	1,313	9.7 (5.8–15.4)	0.01	791	12.4 (7.5–19.8)	0.008
Alcohol status	Never	636	9.2 (5.5–15.5)		241	9.7 (6.2–16.0)	
	Former	1,650	8.7 (5.3–14.4)		699	10.0 (6.5–15.4)	
	Current	1,649	10.8 (6.4–17.1)	<0.001	825	12.5 (7.9–21.2)	<0.001
BMI	<25 kg/m^2	610	10.7 (5.8–17.6)		364	12.3 (7.2–21.0)	
	25–30	1,324	9.6 (5.6–15.7)		683	10.8 (6.7–17.4)	
	>30	2,001	9.5 (5.9–15.0)	0.02	718	10.9 (6.9–16.8)	0.002
Diabetes	No	1,986	8.4 (5.1–13.6)		1,765	11.1 (6.9–17.7)	
	Yes	1,939	11.0 (6.6–18.0)	<0.001		-	
Glomerular filtration rate	≤60 mL/min/1.73 m^2	395	8.9 (5.3–14.6)		105	11.1 (6.9–17.9)	
	>60	3,468	9.8 (5.8–15.7)	0.03	1,628	11.5 (6.9–18.3)	0.67

[a]Adjusted to mean specific gravity in the study population of 1.019 using the following formula: arsenic (μg/L)*[1.019 (g/mL) −1]/ [SG (g/mL) − 1].
[b]P value from Kruskall–Wallis exact test.

After multivariate adjustment, the HR [95% confidence interval (CI)] for overall cancer mortality comparing the 80th versus 20th percentile of urinary arsenic concentrations was 1.14 (0.92–1.41; Table 3). The corresponding HRs for cancers of the lung, liver, prostate, and kidney were 1.56 (1.02–2.39), 1.34 (0.66–2.72), 3.30 (1.28–8.48), and 0.44 (0.14–1.14), respectively. For pancreatic cancer, the corresponding HR was 2.46 (1.09–5.58) and, for lymphatic and hematopoietic cancers, it was 0.46 (0.22–0.96). Arsenic was not associated with other cancers. The linear trend for increased mortality with increasing arsenic levels was statistically significant for lung ($P = 0.04$), prostate ($P = 0.01$), and pancreatic ($P = 0.03$) cancers. When modeling the dose–response relationship using restricted cubic splines, we observed no significant departures from linearity for those cancers (Fig. 1).

In subgroup analyses by participant characteristics (Fig. 2), the fully adjusted HR for overall cancer mortality

comparing the 80th versus 20th percentile of inorganic plus methylated arsenic was consistent for most participant subgroups except diabetes at baseline (P for interaction = 0.07).

In sensitivity analyses adjusting for log-transformed urinary creatinine, the results were similar compared with analyses dividing by urinary creatinine [HR (95% CI)] for overall cancer mortality comparing the 80th versus 20th percentile of arsenic was 1.12 (0.88–1.42) and, for lung cancer mortality, it was 1.53 (0.94–2.48)). Among participants without diabetes or albuminuria ($n = 1,765$; 166 overall cancers and 48 lung cancers), the corresponding HRs adjusting for overall specific gravity were also consistent, although not significant, for overall [1.29 (0.96–1.73)] and lung cancer mortality [1.35 (0.80–2.27)]. Finally, when excluding deaths coded as "malignant neoplasms of other and unspecified sites" ($n = 26$) from the analysis for overall cancer mortality, the results

Table 3. HRs (95% CI) for cancer mortality by urinary arsenic concentrations

	Sum inorganic and methylated arsenic tertiles			80th vs. 20th	
	<6.91 µg/g	6.91–13.32 µg/g	>13.32 µg/g	Percentiles[c]	P-trend[d]
Overall cancer (ICD-9 140 to 208)					
Cases/non-cases	121/1,198	135/1,181	130/1,170	386/3,549	
Model 1	1 (Referent)	1.17 (0.90–1.52)	1.27 (0.96–1.70)	1.16 (0.94–1.42)	0.16
Model 2	1 (Referent)	1.17 (0.90–1.53)	1.23 (0.92–1.65)	1.14 (0.92–1.41)	0.24
Trachea, bronchus, and lung (ICD-9 162)					
Cases/non-cases	27/1,292	20/1,296	31/1,269	78/3,857	
Model 1	1 (Referent)	0.89 (0.49–1.62)	1.95 (1.09–3.49)	1.59 (1.05–2.42)	0.03
Model 2	1 (Referent)	0.94 (0.51–1.72)	1.82 (1.00–3.32)	1.56 (1.02–2.39)	0.04
Liver, gallbladder, and bile ducts (ICD-9 155–156)					
Cases/non-cases	8/1,311	13/1,303	13/1,287	34/3,901	
Model 1	1 (Referent)	1.51 (0.59–3.88)	1.56 (0.56–4.32)	1.50 (0.76–2.97)	0.24
Model 2	1 (Referent)	1.38 (0.53–3.62)	1.36 (0.47–3.95)	1.34 (0.66–2.72)	0.41
Prostate (ICD-9 185)					
Cases/non-cases	6/605	5/526	7/454	18/1,585	
Model 1	1 (Referent)	1.25 (0.37–4.26)	2.90 (0.85–9.92)	1.91 (0.82–4.41)	0.13
Model 2	1 (Referent)	1.55 (0.45–5.33)	4.58 (1.31–16.6)	3.30 (1.28–8.48)	0.01
Kidney (ICD-9 189.0)[b]					
Cases/non-cases	9/1,310	9/1,307	8/1,292	26/3,909	
Model 1	1 (Referent)	0.68 (0.25–1.86)	0.50 (0.15–1.42)	0.69 (0.25–1.90)	0.28
Model 2	1 (Referent)	0.69 (0.25–1.90)	0.44 (0.14–1.40)	0.44 (0.14–1.40)	0.36
Esophagus and stomach cancer (ICD-9 150–151)					
Cases/non-cases	8/1,311	8/1,308	8/1,292	24/3,911	
Model 1	1 (Referent)	1.19 (0.42–3.39)	1.33 (0.41–4.29)	0.94 (0.40–2.24)	0.89
Model 2	1 (Referent)	1.33 (0.46–3.84)	1.57 (0.47–5.26)	1.09 (0.45–2.66)	0.85
Pancreas (ICD-9 157)					
Cases/non-cases	7/1,312	7/1,309	11/1,289	25/3,910	
Model 1	1 (Referent)	1.04 (0.34–3.19)	2.14 (0.67–6.82)	2.26 (1.04–4.88)	0.04
Model 2	1 (Referent)	1.04 (0.33–3.29)	2.14 (0.67–6.82)	2.46 (1.09–5.58)	0.03
Colon and rectal cancer (ICD-9 153–154)					
Cases/non-cases	12/1,307	14/1,302	6/1,294	32/3,903	
Model 1	1 (Referent)	1.46 (0.65–3.29)	0.87 (0.30–2.57)	0.83 (0.40–2.24)	0.62
Model 2	1 (Referent)	1.41 (0.62–3.21)	0.82 (0.27–2.48)	0.78 (0.36–1.67)	0.52
Breast (ICD-9 174)[a]					
Cases/non-cases	7/701	13/772	6/833	26/2,306	
Model 1	1 (Referent)	1.79 (0.67–4.74)	0.90 (0.27–3.07)	0.84 (0.37–1.93)	0.99
Model 2	1 (Referent)	1.92 (0.71–5.15)	0.96 (0.28–3.22)	1.00 (0.44–2.28)	0.69
Lymphatic and hematopoietic tissue (ICD-9 200–208)					
Cases/non-cases	11/1,308	20/1,296	9/1,291	40/3,895	
Model 1	1 (Referent)	1.63 (0.74–3.61)	0.69 (0.26–1.87)	0.59 (0.29–1.17)	0.13
Model 2	1 (Referent)	1.44 (0.64–3.25)	0.57 (0.21–1.58)	0.46 (0.22–0.96)	0.04

NOTE: Model 1: Unadjusted, stratified by center.
Model 2: Adjusted for sex, age, education (no high school/some high school/completed high school), smoking status (never, former, current), drinking status (never, former, current), and BMI (kg/m^2).
[a]Model 2 for breast cancer was further adjusted for menopausal status and parity.
[b]Model 2 for kidney cancer was further adjusted for estimated glomerular filtration rate (<60, $\geq$60 mL/min/1.72m^2) and hypertension status (yes/no).
[c]Models comparing the 80th vs. 20th percentiles of urine arsenic distributions and associated P trend were obtained from Cox proportional hazards models with log-transformed arsenic as a continuous variable.
[d]P trend calculated modeling log-arsenic as continuous.

Figure 1. HRs for cancer mortality by urinary arsenic concentrations. Lines represent the HR (thick line) and 95% CIs (thin line) for overall and specific cancer mortality based on restricted cubic splines for log-transformed sum of inorganic and methylated species with knots at the 10th (3.8 µg/g creatinine), 50th (9.7 µg/g), and 90th (24.0 µg/g) percentiles. The reference was set at the 10th percentile of arsenic distribution. Models were adjusted for age, sex, education (no high school, some high school, or completed high school), smoking status (never, former, or current), drinking status (never, former, or current), and BMI (kg/m^2). Vertical bars represent the histogram of arsenic distribution in the study population.
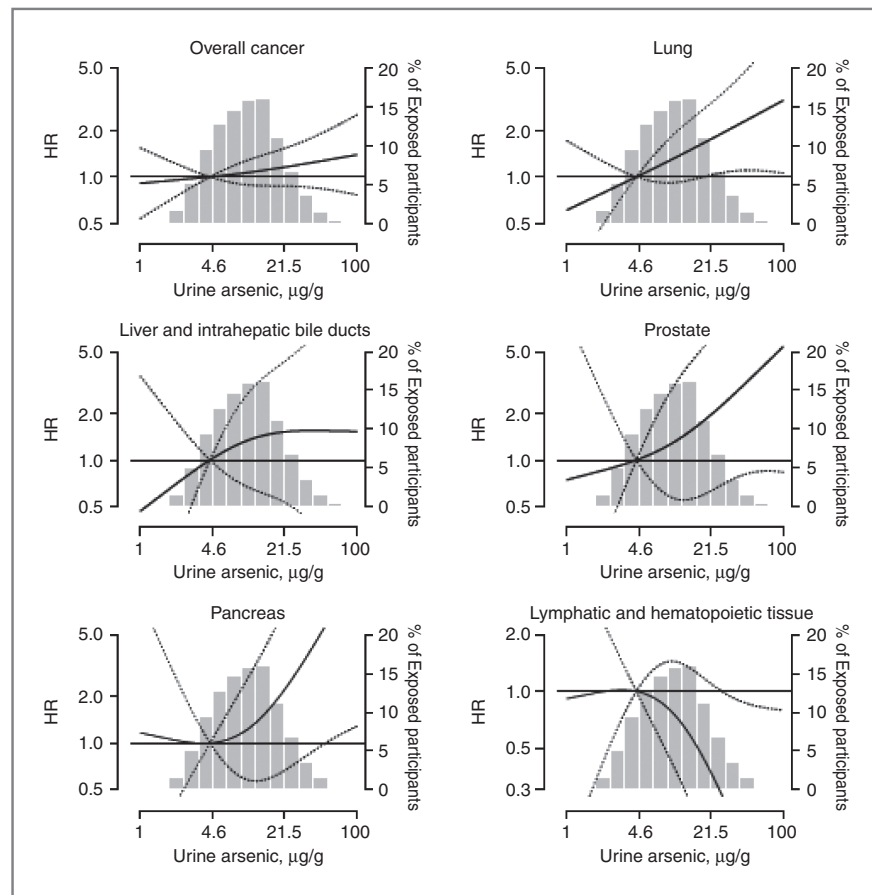
remained similar, and the observed HR (95%CI) comparing the 80th versus 20th percentiles of arsenic was 1.16 (0.93–1.44).

Discussion

Summary of the findings

In this U.S.-based prospective cohort study, low to moderate inorganic arsenic exposure, as measured in urine, was associated with increased mortality from lung, prostate, and pancreatic cancers over almost 20 years of follow-up. The associations persisted after adjustment for sociodemographic and behavioral cancer risk factors. Unexpectedly, arsenic exposure was associated with decreased mortality from lymphatic and hematopoietic cancers. Arsenic was not associated with kidney cancer, and for liver cancer the increased risk was small and statistically nonsignificant. Both tumors have been associated with high arsenic exposure in other populations (17, 25). Overall, increasing urinary arsenic concentrations showed a positive but nonsignificant association with total cancer mortality. Our results extend the associations of arsenic with lung and prostate cancer, observed previously only at high levels of exposure (17, 26–28). In

addition, we found supportive evidence for pancreatic cancer, a cancer with limited epidemiologic evidence available.

Sources of arsenic exposure in the SHS

Participants in the SHS rely either on small public water systems or on private wells. At the time of the study, arsenic concentrations in public drinking water systems for the study communities ranged from less than 10 µg/L up to 61 µg/L in Arizona and from less than 10 µg/L up to 21 µg/L in North and South Dakota. Levels in private wells are not known but, given arsenic concentrations in groundwater in those regions (29), it is likely that arsenic levels exceeded 10 and even 50 µg/L. In Oklahoma, arsenic levels in public water systems were less than 10 µg/L, and similar to other populations with low arsenic levels in drinking water (30), we expect diet to be the main source of arsenic exposure in Oklahoma.

Epidemiologic evidence at high levels of exposure

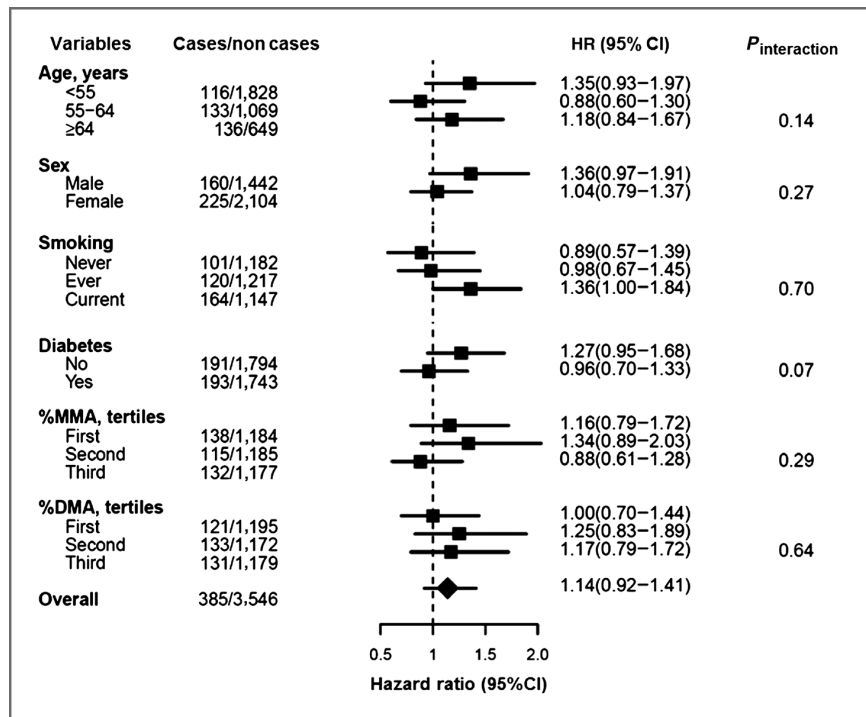Several studies have evaluated the association between lung cancer and high levels of arsenic exposure. In 10,591

Figure 2. HRs (95% confidence interval) for overall cancer mortality comparing the 80th to the 20th percentiles of the sum of inorganic and methylated arsenic species (μg/g creatinine) by participant characteristics at baseline.

participants from Southwestern Taiwan (27), the HRs for lung cancer incidence were 1.09, 2.28, 3.03, and 3.29 for arsenic concentrations in drinking water of 10 to 99, 100 to 299, 300 to 699, and 700 μg/L or more, respectively, compared with less than 10 μg/L. In a case–control study from Antofagasta (Chile; ref. 31), the odds ratio for lung cancer comparing the highest (200–400 μg/L) to the lowest (<10 μg/L) categories of arsenic in drinking water was 8.9 (95%CI: 4.0–19.6). Lung cancer mortality was also increased among young adults in northern Chile who were exposed to high doses of arsenic *in utero* (32).

Regarding prostate cancer, ecologic evidence from Southwestern Taiwan showed increased prostate cancer mortality compared with the overall Taiwanese population (2). Based on the Taiwanese evidence, the IARC concluded there was evidence for a dose–response relationship (1). In a population from Utah exposed to moderate arsenic levels in drinking water, the SMR for prostate cancer compared to the overall U.S. population was 1.48 (95%CI: 1.07–1.91; ref. 33).

Epidemiologic evidence for an association between arsenic and pancreas cancer is scarce. Japanese individuals exposed during infancy to high arsenic levels through contaminated milk showed an SMR for pancreatic cancer of 1.79 (95%CI: 1.23–2.61) compared with unexposed (17). In a case–control study from Spain (18), at low–moderate arsenic in drinking water (34), the odds ratio for exocrine pancreatic cancer comparing the highest (≥0.11 μg/g) to the lowest (≤0.05 μg/g) toenail arsenic quartiles was 2.02 (1.08–3.78).

Few studies have evaluated the association between arsenic and overall cancer mortality. In a prospective cohort including 115,903 participants in Bangladesh, the HRs (95%CI) for cancer mortality were 1.10 (0.77–1.59), 1.44 (1.06–1.95), 1.75 (1.28–2.40), and 1.56 (1.06–2.30) comparing arsenic concentrations in drinking water of 10 to 49, 50 to 149, 150 to 299, and 300 or more to less than 10 μg/L, respectively (35).

Epidemiologic evidence at low–moderate levels of exposure

Evidence at low–moderate arsenic levels remains limited for most cancers. Bladder cancer has been the most frequently studied cancer, with inconsistent findings (33, 36, 37). For lung cancer, a case–control study conducted in New Hampshire and Vermont counties found an odds ratio for small-cell and squamous cell carcinoma of 2.75 (95%CI: 1.39–5.91) comparing toenail arsenic concentrations 0.114 or more versus less than 0.05 μg/g (38). In our study, histologic information was not available, and we cannot evaluate if the association found with lung cancer mortality was also related to those cancer types. At very low levels of exposure, a Danish cohort of 57,053 participants found no association between arsenic in drinking water (mean, 1.2 μg/L) with incidence of lung, liver, bladder, kidney, or prostate cancers over 10 years of follow-up (36). Our study, conducted in a population exposed to low–moderate arsenic levels, extends previous evidence for mortality associated with several cancer types, but included too few cases to

evaluate some cancers of interest, including bladder and skin cancers.

Mechanistic evidence

Despite the extensive epidemiologic evidence, the mechanisms for arsenic carcinogenicity remain unclear. A major challenge has been to develop animal models, with transplacental models being essential to advance our understanding of arsenic carcinogenesis (39). Some of the accepted mechanisms involve genetic and epigenetic changes, oxidative stress, enhanced cell proliferation, mitochondrial damage, and modulation of gene expression (40–42). For prostate cancer, arsenic accelerates prostate cancer cell progression by inducing androgen independence (10). Little is known about mechanisms for arsenic-related pancreatic cancer, although arsenic induces oxidative stress and apoptosis of pancreatic β-cells (43) and acinar cells (44), as well as changes in the pancreatic vasculature (45).

Lymphatic and hematopoietic cancers

In occupational settings (46, 47), and in infants exposed through milk powder (17), arsenic exposure has been associated with increased blood cancer mortality. Arsenic trioxide is used in the treatment of some leukemias such as acute promyelocytic leukemia (48), and is under investigation for multiple myeloma (49). Arsenic trioxide pharmacotherapy has been related to cytotoxicity and apoptosis in cancer cells (50). As we could not specifically evaluate the association between arsenic and specific lymphatic or hematopoietic cancers due to the small number of cases, this finding requires replication in other populations and needs to be interpreted with caution.

Strengths and limitations

The prospective design and long follow-up, the low rate of follow-up losses, the careful standardization and quality control of data collection and laboratory analyses, and the high sensitivity of our assay for urinary arsenic species are major strengths of this study (19, 20). Urinary arsenic, an integrated biomarker of ongoing exposure, has a relatively short half-life. Previous evidence in the SHS, however, showed relatively constant concentrations over a 10-year period (51), indicating that a single measure reflects long-term arsenic exposure in this population. Moreover, the SHS is a very stable population, and most of the participants were born on the communities and have lived there all their lives. The estimated migration rates during the study period are 0.5% in Arizona, 1.5% in Oklahoma, and 2.7% in North and South Dakota. Finally, this study provides information on determination of environmental cancer in American Indian communities, a population understudied for cancer risk that is disproportionately exposed to arsenic in drinking water compared with the general U.S. population (51).

Our study has some limitations. First, cancer mortality is an imperfect outcome to study tumors with relatively good prognosis, such as bladder and skin cancer. Second, we relied on death certificates, which may lack accuracy compared with clinical or pathology records (52). Cancer deaths, however, are considered to be better coded than other causes of death. Third, the number of deaths and statistical power was limited for some cancers, for subgroup analyses, and to detect a potential small increase in overall cancer mortality. For instance, we could not evaluate the association with some cancer deaths separately such as colon and rectal cancer, or esophagus and stomach cancer. Fourth, we did not account for multiple comparisons, although the number of statistically significant associations expected to happen by chance for a total of nine cancer-specific associations is less than one. Fifth, we could not account for family history of cancer or for clustering of arsenic exposure. Finally, arsenic levels in drinking water were only available at the community level. Arsenic in urine, however, correlates well with levels in drinking water (53) and integrates multiple sources of exposure including food (54).

Conclusions

Low to moderate inorganic arsenic exposure, as measured in urine, was prospectively associated with increased mortality for lung, prostate, and pancreatic cancers in the SHS. This study provides novel evidence regarding cancer mortality at low–moderate arsenic exposure in drinking water in rural communities from the United States. Future studies at low–moderate arsenic levels need to evaluate the association with cancer incidence and the role of arsenic metabolism in cancer development. The current U.S. EPA maximum contaminant level of 10 μg/L was established on the basis of lung and bladder cancer risk in highly exposed populations from southwestern Taiwan, and the risk of arsenic exposure at lower doses was derived using linear low-dose extrapolation. Our findings provide direct evidence at low–moderate arsenic levels and support a linear dose–response relationship with lung, prostate, and pancreatic cancers with no evidence of a threshold.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

A. Navas-Acien had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Authors' Contributions

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): E. García-Esquinas, M. Pollan, J.G. Umans, W. Goessler, E. Guallar, J. Farley, A. Navas-Acien
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): L.G. Best
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