American Journal of Epidemiology

Vol. 175, No. 12
DOI: 10.1093/aje/kwr464
Advance Access publication:
April 24, 2012

Original Contribution

Association Between Arsenic Exposure From Drinking Water and Plasma Levels of Cardiovascular Markers

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Initially submitted September 6, 2011; accepted for publication November 14, 2011.

The authors conducted a cross-sectional study to assess the relation between arsenic exposure from drinking water and plasma levels of markers of systemic inflammation and endothelial dysfunction (matrix metalloproteinase-9, myeloperoxidase, plasminogen activator inhibitor-1, soluble E-selectin, soluble intercellular adhesion molecule-1 (ICAM-1), and soluble vascular adhesion molecule-1 (VCAM-1)) using baseline data from 668 participants (age, >30 years) in the Health Effects of Arsenic Longitudinal Study in Bangladesh (2007–2008). Both well water arsenic and urinary arsenic were positively associated with plasma levels of soluble VCAM-1. For every 1-unit increase in log-transformed well water arsenic (ln μg/L) and urinary arsenic (ln μg/g creatinine), plasma soluble VCAM-1 was 1.02 (95% confidence interval: 1.01, 1.03) and 1.04 (95% confidence interval: 1.01, 1.07) times greater, respectively. There was a significant interaction between arsenic exposure and higher body mass index, such that the increased levels of plasminogen activator inhibitor-1 and soluble VCAM-1 associated with arsenic exposure were stronger among people with higher body mass index. The findings indicate an effect of chronic arsenic exposure from drinking water on vascular inflammation and endothelial dysfunction that could be modified by body mass index and also suggest a potential mechanism underlying the association between arsenic exposure and cardiovascular disease.

arsenic; Bangladesh; cardiovascular disease; cross-sectional studies; environmental exposure; inflammation

Abbreviations: CI, confidence interval; HEALS, Health Effects of Arsenic Longitudinal Study; ICAM-1, intercellular adhesion molecule-1; MMP-9, matrix metalloproteinase-9; PAI-1, plasminogen activator inhibitor-1; VCAM-1, vascular adhesion molecule-1.

Arsenic is an abundant element in the earth's crust and can enter drinking water supplies from natural deposits. An estimated 13 million Americans and 57 million people in Bangladesh have been exposed to drinking groundwater with arsenic concentrations exceeding the World Health Organization's standard of 10 μg/L (1). High levels of arsenic exposure (>500 μg/L) from drinking water have been related to elevated risks of cardiovascular disease, including hypertension (2, 3), cerebrovascular disease (4), peripheral vascular disease (5), ischemic heart disease (6, 7), and carotid atherosclerosis (8). Studies of low-to-moderate levels (<300 μg/L) were less consistent; some reported positive associations for mortality from coronary heart disease, hypertensive heart disease, ischemic heart disease, and diseases of arteries, arterioles, and capillaries (9–14), while others did not find an association or found association in subgroups (9, 15). However, epidemiologic studies that address underlying mechanisms are limited.

Experimental studies have suggested that arsenic increases the production of reactive oxygen species such as hydrogen peroxide (16, 17), hydroxyl radicals (18), and others (16). The induction of oxidative stress by arsenic may in turn mediate abnormal gene expression, inflammatory responses, or impaired nitric oxide homeostasis (19). These events may ultimately lead to endothelial dysfunction, which disrupts the balance in vasomotor tone between relaxation and contraction

Table 1. Plasma Levels of MMP-9, Myeloperoxidase, PAI-1, Soluble E-Selectin, Soluble ICAM-1, and Soluble VCAM-1 by Demographic, Lifestyle, and Arsenic Exposure Variables, Health Effects of Arsenic Longitudinal Study, Bangladesh, 2007–2008[a]

	No.	%	MMP-9, ng/mL		Myeloperoxidase, ng/mL		PAI-1, ng/mL		Soluble E-Selectin, ng/mL		Soluble ICAM-1, ng/mL		Soluble VCAM-1, ng/mL	
			Median	*P* Value[b]	Median	*P* Value[b]	Median	*P* Value[b]	Median	*P* Value[b]	Median	*P* Value[b]	Median	*P* Value[b]
Sex														
Women	390	58.6	78.9	<0.001	15.6	<0.001	63.5	0.006	31.8	<0.001	122.4	0.14	1,012.5	<0.001
Men	276	41.4	99.9		18.7		73.1		35.1		137.6		1,120.5	
Age, years														
31–39	249	37.4	92.5	0.09	17.1	0.45	63.5	0.73	31.0	0.002	123.5	0.48	1,004.0	0.001
40–49	246	36.9	89.0		16.3		70.7		34.2		127.6		1,044.0	
50–60	171	25.7	80.4		17.1		69.2		33.9		132.7		1,116.0	
BMI (tertiles)														
<17.86	223	33.5	81.8	0.005	16.3	0.06	63.0	<0.001	31.3	<0.001	137.3	0.01	1,116.0	<0.001
17.86–20.85	222	33.3	85.7		15.8		64.6		33.8		127.1		1,043.5	
>20.85	221	33.2	92.3		18.0		76.3		35.3		123.5		973.8	
Education, years														
No	374	56.2	81.2	0.04	16.3	0.21	63.5	0.005	32.7	0.05	130.2	0.14	1,043.0	0.14
<6	168	25.2	93.5		16.3		67.4		32.5		126.1		1,068.5	
6–9	65	9.8	92.5		18.6		72.5		36.5		118.7		1,017.0	
≥10	59	8.9	97.5		17.7		86.2		33.8		122.7		945.8	
Smoking status														
Never	413	62.0	78.0	<0.001	15.4	<0.001	64.2	0.001	32.5	0.03	118.7	<0.001	996.2	<0.001
Past	53	8.0	97.5		18.0		73.7		30.1		133.7		1,129.0	
Current														
Light	138	20.7	105.2		17.9		69.8		35.0		151.1		1,105.5	
Heavy	62	9.3	104.4		22.7		77.4		34.4		163.2		1,190.5	
SBP, mm Hg														
<140	578	86.8	86.5	0.15	16.4	0.05	67.0	0.22	33.0	0.16	127.1	0.46	1,044.5	0.27
≥140	88	13.2	97.5		18.7		75.7		32.7		128.9		1,039.0	

Table continues

and increases the risk for vascular diseases such as hypertension and atherosclerosis (20).

Circulating markers of vascular inflammation and endothelial dysfunction, such as matrix metalloproteinase-9 (MMP-9) (21, 22), myeloperoxidase (23), plasminogen activator inhibitor-1 (PAI-1) (24), soluble E-selectin, soluble intercellular adhesion molecule-1 (ICAM-1), and soluble vascular adhesion molecule-1 (VCAM-1) (25–29), have been shown to predict future cardiovascular disease in epidemiologic studies. In experimental studies, PAI-1 protein levels are elevated in human microvascular endothelial cells exposed to arsenic (30). Expression of ICAM-1 and VCAM-1 in human umbilical vein endothelial cells was higher in cells stimulated with arsenic than in those without arsenic (31). PAI-1 and MMP-9 expression were time-dependently increased in mice chronically exposed to arsenic (32). Treatment of mice with arsenic trioxide was associated with a clear increase in expression of E-selectin, ICAM-1, and VCAM-1 (33). However, epidemiologic studies are needed to examine these associations in human populations at doses relevant to environmental levels of arsenic exposure.

In a cross-sectional analysis of 115 individuals, we found a positive association between arsenic exposure and plasma levels of soluble ICAM-1 and soluble VCAM-1 (34). However, the arsenic exposure level was high (median level, 231 μg/L), and all the subjects had arsenic-related skin lesions, limiting the generalizability of the findings. In the present study, we conducted independent separate cross-sectional analyses to evaluate the effects of arsenic exposure at lower concentrations (median level, 23 μg/L) on plasma levels of MMP-9, myeloperoxidase, PAI-1, soluble E-selectin, soluble ICAM-1, and soluble VCAM-1 among 668 relatively healthy subjects randomly selected from participants >30 years of age in the Health Effects of Arsenic Longitudinal Study (HEALS) in Araihazar, Bangladesh (2007–2008).

MATERIALS AND METHODS

The Health Effects of Arsenic Longitudinal Study

The parent study, HEALS, is an ongoing prospective cohort study involving 20,033 participants in Araihazar, Bangladesh. Details of the study methods have been presented elsewhere (35, 36). Briefly, prior to subject recruitment, water samples and their geographic coordinates were collected for 10,971 contiguous wells in a well-defined geographic area of 25 km^2

Table 1. Continued

	No.	%	MMP-9, ng/mL		Myeloperoxidase, ng/mL		PAI-1, ng/mL		Soluble E-Selectin, ng/mL		Soluble ICAM-1, ng/mL		Soluble VCAM-1, ng/mL	
			Median	*P* Value[b]	Median	*P* Value[b]	Median	*P* Value[b]	Median	*P* Value[b]	Median	*P* Value[b]	Median	*P* Value[b]
DBP, mm Hg														
<90	587	88.1	86.5	0.17	16.4	0.08	66.6	0.11	32.9	0.34	128.8	0.15	1,044.0	0.004
≥90	79	11.9	98.9		19.1		77.5		32.7		123.6		1,046.0	
Betel nut use														
Never	355	53.4	89.6	0.97	17.3	0.96	67.0	0.64	32.6	0.24	125.0	0.19	1,020.0	0.12
Past	30	4.5	85.8		18.0		65.4		34.7		162.1		1,087.5	
Current	280	42.1	87.7		16.3		68.9		33.3		128.0		1,046.5	
Skin lesion														
Yes	23	3.5	71.7	0.22	14.3	0.05	59.6	0.12	28.3	0.30	129.1	0.04	1,079.0	0.88
No	643	96.5	89.0		17.0		68.6		33.0		116.1		1,043.0	
Well water arsenic, μg/L														
0.10–2.00	168	26.0	94.5	0.83	17.2	0.71	69.3	0.35	32.7	0.76	129.9	0.76	972.2	<0.001
2.01–23.13	156	24.1	80.0		16.5		65.1		33.6		119.3		1,031.0	
23.14–73.46	160	24.8	87.6		17.0		67.5		33.2		123.1		1,112.5	
73.47–500.62	162	25.1	90.9		16.3		72.2		33.0		133.8		1,068.5	
Urinary arsenic, μg/g creatinine[c]														
12.05–88.21	163	25.1	101.9	0.09	18.3	0.14	70.1	0.50	34.4	0.39	128.6	0.56	979.8	0.003
88.22–141.69	163	25.1	84.6		15.5		66.0		32.6		122.2		1,037.0	
141.7–275.63	161	24.8	79.1		17.0		68.7		31.9		129.4		1,096.0	
275.64–1,869.57	163	25.1	91.2		15.7		68.6		32.9		130.3		1,135.0	

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; ICAM-1, intercellular adhesion molecule-1; MMP-9, matrix metalloproteinase-9; PAI-1, plasminogen activator inhibitor-1; SBP, systolic blood pressure; VCAM-1, vascular adhesion molecule-1.

[a] Data were missing on serum levels of myeloperoxidase for 3 subjects and on levels of MMP-9, PAI-1, soluble E-selectin, soluble ICAM-1, and soluble VCAM-1 for 2 subjects. Data were also missing on betel nut use for 1 subject, on well water arsenic for 20 subjects, and on urinary arsenic for 16 subjects.

[b] Computed with log-transformed MMP-9, myeloperoxidase, PAI-1, soluble E-selectin, soluble ICAM-1, and soluble VCAM-1 in the linear regression models.

[c] Creatinine-adjusted urinary arsenic = (arsenic in μg/L × 1,000)/(creatinine in mg/dL × 10).

in Araihazar. Participants meeting the following eligibility criteria were recruited: 1) married (to reduce loss to follow-up) and aged 18–75 years, 2) residing in the study area for at least 5 years prior to recruitment, and 3) primary user of one of the tested wells, designated as the "index" well, for at least 3 years. Between October 2000 and May 2002, we recruited 11,746 men and women for the original HEALS cohort. HEALS was expanded to include an additional 8,287 participants in 2007–2008 (expansion cohort) following the same methodologies. The overall response rate was 97%. Demographic and lifestyle information was collected by using a standardized questionnaire. Trained clinicians measured blood pressure with an automatic sphygmomanometer (15). The study procedures were approved by the Bangladesh Medical Research Council Ethics Committee and the institutional review boards of Columbia University and the University of Chicago. Verbal consent was obtained from study participants.

The present study included a total of 668 participants randomly selected from the 5,136 participants older than 30 years of age in the expansion cohort. Because participants aged 30 years or less are not at appreciable risk of cardiovascular disease, we focused only on participants older than 30 years in our study to investigate intermediate phenotypes for cardiovascular disease. Web Table 1, the first of 3 Web tables posted on the *Journal*'s website (http://www.aje.oxfordjournals.org/), shows that distributions of demographic, lifestyle, and arsenic exposure variables were similar between the study population and the individuals in the expansion cohort who were older than 30 years of age at enrollment.

Measurements of exposure

At baseline, water samples were collected in 20-mL polyethylene scintillation vials after rinsing several times with groundwater. The samples were acidified to 1% with high-purity Optima hydrochloric acid (Fisher Scientific, Pittsburgh, Pennsylvania) for at least 48 hours before analysis. This process has been shown to ensure redissolution of any iron oxides that could have precipitated (37). Water samples were then diluted 1:10 in a solution spiked with germanium-73 and -74 (^{73}Ge and ^{74}Ge) for internal drift correction and were analyzed

Table 2. Associations Between Baseline Well Water Arsenic (μg/L) and Plasma Levels of MMP-9, Myeloperoxidase, PAI-1, Soluble E-Selectin, Soluble ICAM-1, and Soluble VCAM-1, Health Effects of Arsenic Longitudinal Study, Bangladesh, 2007–2008[a]

	Baseline Concentrations of Well Water Arsenic (μg/L) in Quartiles								Per Log-transformed Well Water Arsenic, μg/L[b]	
	0.10–2.00 (*n* = 168)		2.01–23.13 (*n* = 156)		23.14–73.46 (*n* = 160)		73.47–500.62 (*n* = 162)			
	Exp (β)	95% CI	Exp (β)	95% CI	Exp (β)	95% CI	Exp (β)	95% CI	Exp (β)	95% CI
Mean, μg/L[c]	1.20		9.04		47.15		156.75			
MMP-9, ng/mL										
Model 1[d]	1.00	Reference group	0.87	0.77, 0.99	0.93	0.82, 1.06	0.97	0.85, 1.09	0.99	0.97, 1.02
Model 2[e]	1.00	Reference group	0.88	0.77, 0.99	0.96	0.85, 1.08	0.99	0.88, 1.12	1.00	0.98, 1.02
Myeloperoxidase, ng/mL										
Model 1[d]	1.00	Reference group	0.94	0.83, 1.07	0.94	0.83, 1.07	0.98	0.86, 1.11	0.99	0.97, 1.02
Model 2[e]	1.00	Reference group	0.94	0.83, 1.07	0.96	0.84, 1.09	0.99	0.87, 1.13	1.00	0.97, 1.02
PAI-1, ng/mL										
Model 1[d]	1.00	Reference group	0.98	0.87, 1.11	0.92	0.81, 1.04	1.09	0.97, 1.24	1.01	0.99, 1.03
Model 2[e]	1.00	Reference group	0.98	0.87, 1.10	0.95	0.84, 1.07	1.13	1.00, 1.28	1.02	0.99, 1.04
Soluble E-selectin, ng/mL										
Model 1[d]	1.00	Reference group	1.01	0.91, 1.11	0.98	0.89, 1.07	0.98	0.89, 1.08	1.00	0.98, 1.01
Model 2[e]	1.00	Reference group	1.00	0.91, 1.09	0.99	0.90, 1.08	1.00	0.91, 1.10	1.00	0.98, 1.02
Soluble ICAM-1, ng/mL										
Model 1[d]	1.00	Reference group	0.85	0.73, 0.99	0.90	0.78, 1.04	1.00	0.86, 1.16	1.00	0.98, 1.03
Model 2[e]	1.00	Reference group	0.87	0.75, 1.00	0.90	0.78, 1.04	0.99	0.86, 1.15	1.00	0.97, 1.03
Soluble VCAM-1, ng/mL										
Model 1[d]	1.00	Reference group	1.04	0.97, 1.12	1.10	1.03, 1.18	1.10	1.02, 1.18	1.02	1.01, 1.04
Model 2[e]	1.00	Reference group	1.04	0.97, 1.12	1.09	1.02, 1.17	1.09	1.01, 1.16	1.02	1.01, 1.03

Abbreviations: CI, confidence interval; Exp (β), exponentiated regression coefficient; ICAM-1, intercellular adhesion molecule-1; MMP-9, matrix metalloproteinase-9; PAI-1, plasminogen activator inhibitor-1; VCAM-1, vascular adhesion molecule-1.

[a] Models were run with log-transformed MMP-9, myeloperoxidase, PAI-1, soluble E-selectin, soluble ICAM-1, and soluble VCAM-1.

[b] Computed with the log-transformed arsenic level entered as a continuous variable in the linear regression models.

[c] Category-specific mean values of well water arsenic.

[d] Adjusted for sex and age (years).

[e] Adjusted for sex, age, body mass index, education, and smoking status (never vs. ever).

for arsenic by high-resolution, inductively coupled plasma mass spectrometry. Further details on field sampling and laboratory analysis procedures are described elsewhere (38, 39). The detection limit of this method for arsenic is typically <0.2 μg/L. The long-term reproducibility determined from consistency standards included with each run averages 4% (1 − sigma) in the 40–500 μg/L range.

Spot urine samples were collected in 50-mL acid-washed tubes. The total urinary arsenic concentration was measured by graphite furnace atomic absorption, by using a Perkin-Elmer A Analyst 600 graphite furnace system (Waltham, Massachusetts) with a detection limit of 2 μg/L (40). Urinary creatinine was analyzed by a colorimetric Sigma Diagnostics Kit (Sigma, St. Louis, Missouri) for adjusting urinary total arsenic concentration (41). The average intersample and intrasample coefficients of variation were 7.8% and 4.9% for arsenic as well as 3.9% and 1.7% for creatinine, respectively. All the urine samples were detectable for total urinary arsenic.

Measurements of plasma levels of cardiovascular disease markers

Venous blood samples were collected in 10-mL Vacutainers (Becton, Dickinson and Company, Franklin Lakes, New Jersey) containing ethylenediaminetetraacetic acid, and they were spun in a table-top centrifuge to separate plasma. Plasma levels of MMP-9, myeloperoxidase, PAI-1, soluble E-selectin, soluble ICAM-1, and soluble VCAM-1 were analyzed by multiplex assays using MILLIPLEX MAP human cardiovascular disease panel 1 kits (Millipore, Billerica, Massachusetts). Briefly, plasma samples were votexed and diluted 1:25 in the assay buffer. Twenty-five microliters of the diluted plasma, quality controls, and standards were added to each well of a 96-well

Table 3. Associations Between Baseline Urinary Arsenic (μg/g Creatinine) and Plasma Levels of MMP-9, Myeloperoxidase, PAI-1, Soluble E-Selectin, Soluble ICAM-1, and Soluble VCAM-1, Health Effects of Arsenic Longitudinal Study, Bangladesh, 2007–2008[a]

	Baseline Concentrations of Urinary Arsenic (μg/g Creatinine) in Quartiles								Per Log-transformed Urinary Arsenic (μg/g Creatinine)[b]	
	12.05–88.21 (*n* = 163)		88.22–141.69 (*n* = 163)		141.70–275.63 (*n* = 161)		275.64–1,869.57 (*n* = 163)			
	Exp (β)	95% CI	Exp (β)	95% CI	Exp (β)	95% CI	Exp (β)	95% CI	Exp (β)	95% CI
Mean[c]	57.53		115.11		203.91		542.70			
MMP-9, ng/mL										
Model 1[d]	1.00	Reference group	0.87	0.77, 0.98	0.83	0.74, 0.94	0.91	0.80, 1.03	0.96	0.92, 1.01
Model 2[e]	1.00	Reference group	0.89	0.79, 1.01	0.86	0.76, 0.97	0.95	0.84, 1.08	0.98	0.93, 1.03
Myeloperoxidase, ng/mL										
Model 1[d]	1.00	Reference group	0.85	0.75, 0.97	0.89	0.78, 1.01	0.89	0.79, 1.02	0.97	0.92, 1.02
Model 2[e]	1.00	Reference group	0.87	0.76, 0.99	0.91	0.80, 1.03	0.92	0.81, 1.05	0.98	0.93, 1.03
PAI-1, ng/mL										
Model 1[d]	1.00	Reference group	0.92	0.81, 1.04	0.90	0.80, 1.02	1.04	0.92, 1.17	1.02	0.97, 1.07
Model 2[e]	1.00	Reference group	0.96	0.85, 1.09	0.95	0.84, 1.07	1.11	0.90, 1.26	1.05	1.00, 1.11
Soluble E-selectin, ng/mL										
Model 1[d]	1.00	Reference group	0.94	0.86, 1.04	0.89	0.81, 0.98	0.95	0.87, 1.05	0.99	0.95, 1.02
Model 2[e]	1.00	Reference group	0.96	0.88, 1.06	0.92	0.84, 1.01	0.99	0.90, 1.09	1.00	0.96, 1.04
Soluble ICAM-1, ng/mL										
Model 1[d]	1.00	Reference group	0.93	0.80, 1.08	0.98	0.85, 1.14	1.02	0.88, 1.19	1.02	0.96, 1.08
Model 2[e]	1.00	Reference group	0.92	0.79, 1.06	0.96	0.83, 1.11	1.00	0.86, 1.16	1.01	0.95, 1.07
Soluble VCAM-1, ng/mL										
Model 1[d]	1.00	Reference group	1.03	0.96, 1.11	1.10	1.03, 1.18	1.12	1.05, 1.20	1.05	1.02, 1.08
Model 2[e]	1.00	Reference group	1.02	0.95, 1.09	1.08	1.01, 1.16	1.09	1.02, 1.17	1.04	1.01, 1.07

Abbreviations: CI, confidence interval; Exp (β), exponentiated regression coefficient; ICAM-1, intercellular adhesion molecule-1; MMP-9, matrix metalloproteinase-9; PAI-1, plasminogen activator inhibitor-1; VCAM-1, vascular adhesion molecule-1.

[a] Models were run with log-transformed MMP-9, myeloperoxidase, PAI-1, soluble E-selectin, soluble ICAM-1, and soluble VCAM-1.

[b] Computed with the log-transformed arsenic level entered as a continuous variable in the linear regression models.

[c] Category-specific mean values of urinary arsenic.

[d] Adjusted for sex and age (years).

[e] Adjusted for sex, age, body mass index, education, and smoking status (never vs. ever).

plate with 25 μL of the bead solution. Detection antibodies and streptavidin-phycoerythrin solution were then added, and the plate was analyzed on a Luminex 200 instrument (Luminex Corporation, Austin, Texas). The data were saved and evaluated as median fluorescent intensity by using a weighted 5-parameter logistic method for calculating concentrations in the samples. Prior to the main study, a pilot study of 48 samples from randomly selected participants was conducted to evaluate the coefficient of variation and intraclass correlation of the assays. The ranges of the interplate, interindividual, and intraindividual coefficients of variation for the 6 markers were 0%–2.1%, 3.5%–11%, and 1.9%–5.6%, respectively, and the range for intraclass correlation was 0.75–0.90.

Statistical analysis

Descriptive analyses were conducted to compare demographic, lifestyle, and arsenic exposure variables of the study population and those in the overall expansion cohort who were older than 30 years of age. All plasma marker levels were log transformed to improve the approximation to the normal distribution. We first estimated median plasma levels of cardiovascular disease markers by demographic, lifestyle, and arsenic exposure variables. Linear regression was conducted to assess associations of demographic and lifestyle variables with plasma levels of cardiovascular disease makers.

Linear regression was conducted to evaluate the associations between arsenic exposure and plasma levels of cardiovascular disease markers. In addition to crude models, we first adjusted for sex and age (years) and, in a separate model, we additionally adjusted for body mass index (weight (kg)/height $(m)^2$), education (years), and smoking status (never vs. ever), known risk factors that may modify health effects of arsenic exposure (12, 35, 42). We estimated the ratio of the geometric means of cardiovascular disease markers, which was interpreted as exponentiated regression coefficients, comparing each of the

Table 4. Associations Between Baseline Arsenic Exposure Variables and Plasma Levels of MMP-9, Myeloperoxidase, PAI-1, Soluble E-Selectin, Soluble ICAM-1, and Soluble VCAM-1, by Body Mass Index, Health Effects of Arsenic Longitudinal Study, Bangladesh, 2007–2008[a]

	Body Mass Index, ≤19.1			Body Mass Index, >19.1			$P_{interaction}$
	No.	Exp (β)[b]	95% CI	No.	Exp (β)[b]	95% CI	
Baseline well water arsenic, μg/L	324			322			
MMP-9		1.00	0.97, 1.03		0.99	0.96, 1.02	0.33
Myeloperoxidase		0.97	0.94, 1.01		1.01	0.98, 1.05	0.45
PAI-1		0.99	0.96, 1.02		1.04	1.01, 1.08	0.03
Soluble E-selectin		0.98	0.96, 1.00		1.02	0.99, 1.05	0.09
Soluble ICAM-1		0.98	0.94, 1.01		1.03	0.99, 1.07	0.10
Soluble VCAM-1		1.00	0.99, 1.02		1.04	1.02, 1.06	0.009
Baseline urinary arsenic, μg/g creatinine	325			325			
MMP-9		0.96	0.90, 1.04		0.98	0.91, 1.05	0.93
Myeloperoxidase		0.94	0.87, 1.02		1.00	0.93, 1.08	0.51
PAI-1		0.98	0.91, 1.04		1.12	1.04, 1.20	0.008
Soluble E-selectin		0.94	0.90, 0.99		1.05	0.99, 1.12	0.05
Soluble ICAM-1		0.94	0.87, 1.03		1.08	0.99, 1.18	0.04
Soluble VCAM-1		0.99	0.95, 1.03		1.09	1.04, 1.13	0.002

Abbreviations: CI, confidence interval; Exp (β), exponentiated regression coefficient; ICAM-1, intercellular adhesion molecule-1; MMP-9, matrix metalloproteinase-9; PAI-1, plasminogen activator inhibitor-1; VCAM-1, vascular adhesion molecule-1.

[a] Models were run with log-transformed MMP-9, myeloperoxidase, PAI-1, soluble E-selectin, soluble ICAM-1, and soluble VCAM-1.

[b] Computed with the log-transformed arsenic level entered as a continuous variable in the linear regression models, adjusting for sex, age (years), education, and smoking status (never vs. ever).

higher 3 quartiles of well water arsenic or urinary arsenic concentration with the bottom quartile. We examined the assumption of nonlinear effect of arsenic exposure by including higher order polynomial terms for arsenic exposure variables in the models, and there was no indication of any nonlinear relation. Arsenic exposure variables were also log transformed and entered as continuous variables in the model to assess whether the association is proportional to a power of dose in exposure, a relation known to occur frequently from both human and animal studies (43).

Because the study population was lean (mean body mass index, 19.8), we conducted stratified analyses to evaluate to what extent the associations between arsenic exposure and cardiovascular disease markers differ by levels of body mass index. Additional exploratory stratified analyses by sex, age, smoking status, and educational level were also conducted. Age and body mass index were dichotomized by the median value in the overall population. The significance of multiplicative interaction was determined by the *P* values of the cross-product terms of the effect modifiers, and arsenic exposure was expressed as continuous variables in multivariate linear regression models. Sensitivity analyses were conducted excluding those with skin lesions ($n = 23$) and in the subpopulation ($n = 511$) with longer-term arsenic exposure defined as those with ≥5 years' duration of exposure with known arsenic concentrations. We also conducted sensitivity analyses by entering creatinine as a separate variable in the regression models, as suggested by Barr et al. (44). All statistical analyses were performed by using SPSS, version 19.0, software (SPSS, Inc., Chicago, Illinois).

RESULTS

Table 1 shows the associations of demographic, lifestyle, and arsenic exposure variables with plasma levels of cardiovascular disease markers. Men had higher levels of MMP-9, myeloperoxidase, PAI-1, soluble E-selectin, and soluble VCAM-1 than did women. Older participants had increased levels of soluble E-selectin and soluble VCAM-1. Higher body mass index was associated with increased levels of MMP-9, PAI-1, and soluble E-selectin. Interestingly, soluble ICAM-1 and soluble VCAM-1 levels were inversely correlated with body mass index. Higher educational attainment was related to increased levels of MMP-9, PAI-1, and soluble E-selectin. Higher diastolic blood pressure was related to decreased levels of soluble VCAM-1. Cigarette smokers were more likely to have higher plasma levels of all the markers investigated. Individuals with skin lesions (melanosis, leucomelanosis, or keratosis) had lower levels of soluble ICAM-1; however, the number of cases in the study population was limited, so the observations may be due to chance. In univariate analyses, well water arsenic and urinary arsenic were positively related to plasma levels of soluble VCAM-1. Plasma levels of soluble VCAM-1 in individuals exposed to 23.1–73.5 μg/L and >73.5 μg/L of well water arsenic were 1.09 (95% confidence interval (CI): 1.02, 1.17) and 1.08 (95% CI: 1.01, 1.16) times greater, respectively, compared with the levels in participants exposed to ≤2 μg/L (Table 2). A dose-response relation remained significant after adjustment for body mass index, education, and smoking status in addition to sex and age (model 2, Table 2, $P_{trend} = 0.003$). For every 1-unit increase

in log-transformed well water arsenic (ln μg/L), plasma soluble VCAM-1 was 1.02 (95% CI: 1.01, 1.03) times greater. The highest quartile of well water arsenic was related to an increased level of PAI-1 ($P = 0.04$); however, the linear trend was not significant. On the other hand, there was no association between well water arsenic and plasma levels of MMP-9, myeloperoxidase, soluble E-selectin, or soluble ICAM-1.

We observed similar patterns of associations when we used urinary arsenic as the exposure variable in the analyses (Table 3). Plasma levels of soluble VCAM-1 were 1.08 (95% CI: 1.01, 1.16) and 1.09 (95% CI: 1.02, 1.17) times greater, respectively, in individuals with 141.7–275.6 μg/g creatinine and >275.6 μg/g creatinine of urinary arsenic compared with the levels in participants with ≤88.2 μg/g creatinine (Table 3). For every 1-unit increase in log-transformed urinary arsenic (ln μg/g creatinine), plasma soluble VCAM-1 was 1.04 (95% CI: 1.01, 1.07) times greater (model 2, $P_{trend} = 0.02$). There were no apparent associations between urinary arsenic and the other markers. The effect estimates did not materially change when creatinine was entered as a separate variable. For instance, plasma soluble VCAM-1 was 1.03 (95% CI: 1.00, 1.06; $P_{trend} = 0.04$) times greater for every 1-unit increase in log-transformed urinary arsenic (μg/L), after adjustment for the confounding factors (data not shown). In the subpopulation with ≥5 years of arsenic exposure, well water arsenic and urinary arsenic were also positively associated with soluble VCAM-1 (Web Table 2). Finally, we explored the potential interaction of arsenic exposure with body mass index. There was a significant interaction between arsenic exposure and higher body mass index, such that the increased levels of PAI-1 and soluble VCAM-1 associated with arsenic exposure were stronger among individuals with higher body mass index (Table 4). The associations between well water arsenic and markers did not differ by sex, age, smoking status, or education (Web Table 3).

DISCUSSION

In this large cross-sectional study of arsenic exposure and multiple plasma markers of inflammation and endothelial dysfunction, we found a positive association between arsenic exposure, measured by using either well water arsenic or urinary arsenic concentration, and plasma levels of soluble VCAM-1. We also found an interaction between arsenic exposure and higher body mass index in PAI-1 and soluble VCAM-1.

Accumulating evidence suggests that systemic inflammation and endothelial activation underlie the development of cardiovascular disease. On the surface of activated endothelial cells, the expression of cell adhesion molecules, such as selectins, ICAM-1, and VCAM-1, is markedly increased. Soluble parts of these molecules are released into the bloodstream and can be measured in plasma (45). The adhesion molecules mediate the attachment of circulating leukocytes to the endothelium and their transmigration into the arterial wall, an early step of atherosclerosis (46). The selectins, including E-selectin and P-selectin, mediate transient rolling of the leukocytes along the endothelium (47), while stronger attachment is mediated by ICAM-1 and VCAM-1 (48). We observed a positive association between arsenic exposure and soluble VCAM-1 but no significant association between arsenic exposure and plasma levels of soluble E-selectin and soluble ICAM-1. Although the biologic explanation for the variability in results across markers is not clear, the findings support the link between arsenic exposure and elevated concentrations of certain markers of endothelial dysfunction and suggest a potential mechanism underlying the effect of long-term arsenic exposure on cardiovascular disease. Studies have reported positive associations between blood levels of soluble VCAM-1 and risk of nonfatal and fatal cardiovascular disease events (27, 49), as well as the presence and extent of coronary artery disease (50–52), independent of other inflammatory markers. These studies were mostly conducted in participants with some preexisting forms of cardiovascular disease. In contrast to ICAM-1 that is also expressed by fibroblasts and hematopoietic cells, VCAM-1 is mainly expressed on atherosclerotic plaques by activated endothelial cells and smooth muscle cells (53). Therefore, soluble VCAM-1 may be a late-stage marker for cardiovascular disease and a more specific mark for atherosclerosis (54) that is more relevant to the cardiovascular effect of arsenic exposure. Based on our estimates, the plasma level of soluble VCAM-1 was about 10% greater comparing moderate (>23.1 μg/L) with low levels of arsenic exposure (Table 2). It has been observed that the risk for cardiovascular mortality was significantly increased by 10% for every 100-ng/mL increase in soluble VCAM-1 (55), which is approximately equivalent to a 10% increase in soluble VCAM-1 as the median level of soluble VCAM-1 was 1,043 ng/mL in our population. Therefore, our findings suggest that the effect of arsenic exposure on soluble VCAM-1 may translate to an increased risk of clinical cardiovascular disease.

In a previous cross-sectional study of 115 cases of skin lesions with high levels of arsenic exposure (median level, 231 μg/L), we observed that well water arsenic was positively related to soluble VCAM-1 (34). Because 98% of the study population of the present study was exposed to a well water arsenic level of <300 μg/L (median level, 23 μg/L), our results confirm the association between low-to-moderate levels of arsenic exposure and soluble VCAM-1. In addition, only 23 (3.4%) participants in the present study had skin lesions, and the analysis results excluding these cases were similar (data not shown). Therefore, the findings of the study demonstrate the association in relatively healthy individuals, excluding the possibility that the observed association may be a consequence of skin lesions.

In addition to soluble VCAM-1, we also found that the PAI-1 level was increased in the highest quartile of well water arsenic, although the linear trend was not significant. PAI-1 is a member of the plasminogen activator inhibitors that interact with plasminogen activators to keep the balance between the coagulation and fibrinolytic systems of endothelial cells. Increased coagulation activity and lowered fibrinolytic activity might lead to thrombosis. Sodium arsenite has been shown to increase PAI-1 expression of human microvascular endothelial cells and thus lead to reduced fibrinolytic activity (30). The PAI-1 level was significantly increased in patients with blackfoot disease, a peripheral vascular occlusive disease closely related to arsenic exposure from drinking water (56). Increased concentrations of PAI-1 were predictive of acute myocardial infarction in individuals with a high prevalence

of coronary heart disease (57). Because our study population was exposed to low-to-moderate levels of arsenic, our findings do not preclude that high levels of arsenic exposure may be related to increased levels of PAI-1.

We did not find an association of either well water arsenic or urinary arsenic with plasma levels of MMP-9 and myeloperoxidase. MMP-9 is located at the shoulder region of plaques and areas of foam cell accumulation and plays an important role in several stages of atherosclerosis (58). Myeloperoxidase has been implicated as a catalyst for low-density lipoprotein oxidation, a critical step in atherogenesis (59). Positive associations of circulating levels of MMP-9 and myeloperoxidase with cardiovascular disease have been observed in both case-control (59, 60) and cohort (21–23) studies. A recent study in Bangladesh ($n = 91$) showed that individuals chronically exposed to arsenic had significantly higher myeloperoxidase activities in serum than the unexposed group; however, no further dose-response relation was found among the exposed group (61). The biologic importance of these markers in mediating the cardiovascular effects of arsenic exposure needs further investigation.

The positive associations of sex, age, and cigarette smoking with plasma levels of markers in our study support their roles as established risk factors for cardiovascular disease (62). Body mass index was not positively related to plasma levels of soluble ICAM-1 and soluble VCAM-1. Our study population was mostly lean, and only 7.3% of the population was overweight or obese (body mass index, $\geq$25). It is possible that the relation between body mass index and soluble ICAM-1 or soluble VCAM-1 differs at the lower end of the scale of body mass index. González-Ordóñez et al. (63) found a reverse relation between body mass index and soluble VCAM-1 among venous thromboembolism patients. We also found a highly significant interaction between arsenic exposure and higher body mass index, such that the increased levels of PAI-1 and soluble VCAM-1 associated with arsenic exposure were stronger in individuals with higher body mass index. Accumulating evidence has suggested a dose-response relation between body mass index and inflammation biomarkers in cardiovascular disease, such as C-reactive protein (64–68). It is possible that higher body mass index potentiates the inflammatory response to arsenic exposure, thus leading to elevated levels of inflammatory markers.

Other strengths of the study include the large sample size, the wide range of exposure level, and use of multiple measures for arsenic in both water and urine. Moreover, plasma levels of cardiovascular disease markers in the present study were consistent with those reported in studies using the same multiplex assays (69, 70) that have been reported to be valid alternatives to conventional enzyme-linked immunosorbent assays (71). There are several limitations of the study. First, the study results might not be generalizable to other populations with a different profile of risk factors for cardiovascular disease. However, the association did not differ by sex, age, education, or smoking status. Second, only a single baseline measurement of cardiovascular disease markers and urinary arsenic concentration was available. However, the study population had consumed water from their designated wells for more than 3 years, and analysis restricted to those with $\geq$5 years of use showed similar results, indicating long-term effects of arsenic exposure on levels of soluble VCAM-1. In our previous study, we found that levels of soluble ICAM-1 and soluble VCAM-1 were relatively stable over time despite the short-term treatments implemented in the study, and the intraclass correlations were 0.80 (95% CI: 0.71, 0.86) and 0.82 (95% CI: 0.73, 0.87) for soluble ICAM-1 and soluble VCAM-1, respectively (34). Therefore, the reproducibility of soluble VCAM-1 is excellent, and the levels of soluble VCAM-1 measured once are representative of its long-term levels.

In summary, we found a dose-response relation between arsenic exposure and plasma levels of soluble VCAM-1. The associations between arsenic exposure and plasma levels of PAI-1 and soluble VCAM-1 differ by body mass index, where individuals with higher body mass index had elevated levels of PAI-1 and soluble VCAM-1. These findings indicate an effect of chronic arsenic exposure at low-to-moderate levels on inflammation and endothelial dysfunction that could be modified by body mass index.

ACKNOWLEDGMENTS

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This work was supported by the National Institutes of Health (grants R01 ES017541, P42 ES010349, P30 ES000260, and R01 CA107431).

The authors thank the dedicated project staff and field workers in Bangladesh, without whom this work would not have been possible.

Conflict of interest: none declared.

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