A Dose–Response Study of Arsenic Exposure and Markers of Oxidative Damage in Bangladesh

Kristin N. Harper, PhD, Xinhua Liu, PhD, Megan N. Hall, ScD, Vesna Ilievski, MS, Julie Oka, MBA, Larissa Calancie, BS, Vesna Slavkovich, MS, Diane Levy, MS, Abu Siddique, MBBS, Shafiul Alam, MSc, Jacob L. Mey, PhD, Alexander van Geen, PhD, Joseph H. Graziano, PhD, and Mary V. Gamble, PhD

Objective: To evaluate the dose–response relationship between arsenic (As) exposure and markers of oxidative damage in Bangladeshi adults. **Methods:** We recruited 378 participants drinking water from wells assigned to five water As exposure categories; the distribution of subjects was as follows: (1) less than 10 μg/L ($n = 76$); (2) 10 to 100 μg/L ($n = 104$); (3) 101 to 200 μg/L ($n = 86$); (4) 201 to 300 μg/L ($n = 67$); and (5) more than 300 μg/L ($n = 45$). Arsenic concentrations were measured in well water, as well as in urine and blood. Urinary 8-oxo-2′-deoxyguanosine and plasma protein carbonyls were measured to assess oxidative damage. **Results:** None of our measures of As exposure were significantly associated with protein carbonyl or 8-oxo-2′-deoxyguanosine levels. **Conclusions:** We found no evidence to support a significant relationship between long-term exposure to As-contaminated drinking water and biomarkers of oxidative damage among Bangladeshi adults.

Long-term exposure to arsenic (As)-contaminated drinking water is associated with increased risk for cancers of the skin, lungs, kidney, bladder, and liver.[1] In addition, evidence suggests As exposure increases the risk of developing cardiovascular disease.[2–5] Some research also supports a link between As and type 2 diabetes,[6–8] although this subject is controversial.[9,10] In Bangladesh alone, tens of millions of people are exposed to As-contaminated drinking water, putting them at increased risk for the attendant health problems.[11]

Although the modes of action by which As causes disease are not completely understood, an increased level of oxidative stress is widely believed to be one possible mechanism.[12] Additional potential mechanisms, many of which are not mutually exclusive, include altered DNA repair capacity,[13,14] epigenetic dysregulation,[15–17] enzyme inhibition,[18] endocrine disruption,[19,20] and chromosomal instability.[21–23] Reactive oxygen species have the potential to damage critical molecules in the cell, including DNA and proteins. Increased levels of free radicals and oxidative damage have been observed in various types of cancers,[24,25] as well as chronic conditions such as cardiovascular disease[26,27] and diabetes.[28] Thus, oxidative stress has been implicated in many of the conditions linked to As exposure, though the causal relationship between reactive oxygen species and these health outcomes remains unclear.

In vitro and in vivo studies indicate that As induces oxidative damage. For example, treatment of human–hamster hybrid fibroblast cells, rat lung epithelial cells, and breast-cancer cells with sodium arsenite at doses ranging from 2 to 15 μM has been shown to result in higher levels of reactive oxygen species and 8-oxo-2′-deoxyguanosine (8-oxo-dG), a biomarker of DNA damage.[29–31] Similarly, rats exposed to either 100 mg/L sodium arsenite or 200 to 400 mg/L dimethylarsenic acid (DMA) in drinking water display increased levels of various markers of oxidative stress, including glutathione disulfide, malondialdehyde, a marker of lipid oxidation,[32] and 8-oxo-dG.[33,34] It should be noted that the levels of As exposure used in these studies are orders of magnitude higher than those humans are exposed to via naturally contaminated drinking water.

Some, but not all, studies in humans have yielded evidence indicating that As exposure is associated with oxidative damage. Three population-based studies with sample sizes greater than 100 and detailed information about potential confounders have been performed to assess the relationship between As and DNA damage, as measured by urinary 8-oxo-dG. One reported a significant positive association between urinary As and 8-oxo-dG in 212 pregnant women in Matlab, Bangladesh.[35] The other two studies found no such association. One of these null studies was performed in Arizona and Sonora, where levels of drinking-water As were relatively low (less than 40 μg/L; $n = 124$),[36] and the other took place in the Andes of northern Argentina, where exposure was higher (median level of water As, approximately 200 μg/L; $n = 108$).[37] Finally, a recent study on the same Bangladeshi sample investigated in the present article reported that water, urinary, and blood As levels were all inversely associated with levels of blood glutathione, an important endogenous antioxidant.[38] Nevertheless, As exposure was not linked to levels of blood glutathione disulfide, the oxidized form of glutathione that increases under conditions of oxidative stress.[38] In summary, evidence supporting a causal relationship between long-term As exposure and measures of oxidative damage in humans has not yet been firmly established.

In this study, we set out to evaluate the dose–response relationship between As exposure and oxidative damage. We conducted a cross-sectional study of 378 individuals with long-term drinking water As exposure in Araihazar, Bangladesh. A wide range of water As concentrations is present in this area, and detailed information about exposure and potential confounders is available. We collected As measurements for drinking water, as well as for blood and urine from each individual in the study. Finally, we measured two biomarkers of oxidative damage: (1) plasma levels of protein carbonyls, a measure of protein damage; and (2) urinary 8-oxo-dG, to investigate damage to DNA.

From the Departments of Environmental Health Sciences (Dr Harper, Ms Ilievski, Ms Oka, Ms Calancie, Ms Slavkovich, Dr Graziano, and Dr Gamble), Biostatistics (Dr Liu and Ms Levy), and Epidemiology (Dr Hall), Mailman School of Public Health, Columbia University, New York, NY; Columbia University Arsenic Project in Bangladesh (Mr Siddique and Mr Alam), Dhaka, Bangladesh; Lamont-Doherty Earth Observatory (Dr Mey and Dr van Geen), Columbia University, Palisades, NY; and Kingsbridge Community College (Dr Mey), New York, NY.

This work was supported by grants 1 P42 ES10349, P30 ES009089, RO1 CA133595, RO1 ES017875, and K99 ES018890 from the National Institutes of Health. The Cancer Epidemiology Training Program at the Mailman School of Public Health (5-R25-CA 094061) and the Robert Wood Johnson Health & Society Scholars program also provided financial support.

The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute, the National Institute of Environmental Health Sciences, or the National Institutes of Health.

The authors declare that they have no conflicts of interest.

Address correspondence to: Mary V. Gamble, PhD, Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, 722 W 168th St, Room 1107E, New York, NY 10032 (mvg7@columbia.edu).

DOI: 10.1097/JOM.0000000000000166

METHODS

Study Overview

The study site, Araihazar, is located roughly 30 km east of Dhaka, Bangladesh. Araihazar is 1 of 509 thanas, or administrative units, in Bangladesh. In the year 2000, the Health Effects of Arsenic Longitudinal Study cohort recruitment began, eventually enrolling roughly 30,000 adults.[39] Every two years, both As exposure and the development of various health outcomes are assessed in study participants. A subset of the Health Effects of Arsenic Longitudinal Study cohort was recruited into the Folate and Oxidative Stress study between February and August of 2008, forming the basis of this investigation.

Study Sample

Study participants between the ages of 30 and 65 years were selected on the basis of their well water As concentrations, so that the final study sample represented the full range of water As values in the region. We set out to recruit 75 individuals in each of five water As exposure categories: (1) less than 10 μg/L (corresponding to the World Health Organization's drinking water standard); (2) 10 to 100 μg/L; (3) 101 to 200 μg/L; (4) 201 to 300 μg/L; and (5) more than 300 μg/L. This approach was chosen to maximize the ability to study dose–response relationships. Exclusion criteria included (1) women currently pregnant; (2) individuals taking nutritional supplements; (3) individuals who had not been drinking water from their current well for at least 3 months; and (4) individuals with diseases known to be associated with oxidative stress, such as diabetes, cardiovascular disease, or renal disease. As a result of water remediation and well-switching interventions between the year 2000 and the time of recruitment for the Folate and Oxidative Stress study, some water As categories included more or less than the 75 participants originally planned. The final study sample totaled 378 participants. Well water As concentrations were remeasured at the time of recruitment into the Folate and Oxidative Stress study. The distribution of subjects within water As categories was as follows: (1) less than 10 μg/L ($n = 76$); (2) 10 to 100 μg/L ($n = 104$); (3) 101 to 200 μg/L ($n = 86$); (4) 201 to 300 μg/L ($n = 67$); and (5) more than 300 μg/L ($n = 45$).

At each recruitment visit, a trained staff member described the purpose of the study to the participant. A Bangladeshi field staff physician read an approved assent form to each participant, after which oral informed consent was obtained and a subsequent visit to the field clinic was scheduled. At the field clinic, a trained interviewer administered a detailed questionnaire to each participant and a physician collected a venous blood sample.

This study was approved by both the Bangladesh Medical Research Council and the institutional review board at Columbia University Medical Center.

Collection of Biological Samples

Each participant came to our field clinic in Araihazar where blood and urine samples were collected and processed. Spot-collected urine samples were stored in 50-mL acid-washed tubes and frozen at -20°C. Blood was collected in EDTA-containing tubes; not all participants were fasting at the time of collection. Plasma samples were immediately separated at our field clinic, and blood and plasma samples were frozen in -80°C freezers in Araihazar. Samples were later transported in batches to Dhaka on dry ice, and stored in our Dhaka laboratory at -80°C. All samples were transported in coolers with dry ice to Columbia University, where they were analyzed.

Laboratory Assays

Water As

Our field sample collection and laboratory analysis procedures have been described elsewhere in detail.[40,41] Briefly, water samples were collected in 20-mL polyethylene scintillation vials. At least 48 hours before analysis, high-purity Optima HCl (Fisher Scientific, Pittsburg, PA) was used to acidify the samples to 1%.[42] After being diluted 1:10, water samples were analyzed using high-resolution inductively coupled plasma mass spectrometry (ICP-MS). A germanium (Ge) spike was added to correct fluctuations in instrument sensitivity. The detection limit of the method is typically less than 0.2 μg/L. The intra- and interassay coefficients of variation (CVs) for the assay were 6.0% and 3.8%, respectively.

Blood As

Whole blood As was measured using ICP-MS with a dynamic reaction cell,[43,44] with the modifications to the blood sample preparation process suggested by the Laboratory for ICP-MS Comparison Program, Institut National de Sante Publique du Quebec. Briefly, whole blood samples were thawed, mixed, and then diluted 50× with a solution containing 1% HNO_3, 1% methanol, 0.2% Triton-X-100, and 0.5% NH_4OH. Diluted samples were centrifuged for 10 minutes at 3500 revolutions per minute, and the analysis was performed on the supernatant. Matrix-induced interference was corrected using an iridium (Ir) internal standard and the instrument's dynamic reaction cell technology feature. Quality-control samples with known As concentration were obtained from the Quebec Institute and run each time the instrument was calibrated, as well as after every 10 samples. For study samples, the intra- and interprecision coefficients were 2.1% and 4.9%, respectively. Total blood As was obtained by adding inorganic As, monomethylarsonic acid, and DMA concentrations.

Urinary As

Urinary As was measured using the Aanalyst 600 graphite furnace system (PerkinElmer, Shelton, CT), as described previously.[45] The intraclass correlation coefficient between the samples calibrated by the Quebec laboratory and our laboratory's values was 0.99. Specific gravity was measured using a handheld refractometer (TS 400; Reichert, Depew, NY). In addition, urinary As metabolites were measured as previously described.[46] The method involves the separation of arsenobetaine, arsenocholine (AsC), arsenate, arsenite, monomethylarsonic acid, and DMA, using high-performance liquid chromatography, followed by detection using ICP-MS. The intra- and interassay CVs for urinary As were 3.9% and 5.6%, respectively, for quality-control samples. For study samples, the intra- and interassay CVs were 3.8% and 5.1%, respectively. Total urinary As was obtained by adding inorganic As, monomethylarsonic acid, and DMA concentrations.

8-oxo-2′-deoxyguanosine

8-oxo-2′-deoxyguanosine was measured in urine by using the "New 8-OHdG Check" enzyme-linked immunosorbent assay kit at the Genox Corporation's laboratory (Baltimore, MD). Before analysis, samples were centrifuged at 6000*g* for 5 minutes to remove particulate matter. Samples were measured in triplicate. The detection level for this assay is 0.64 ng/mL urine. The average intra- and interassay CVs were calculated using control samples run in triplicate on 21 plates; they were 7.5% and 7.7%, respectively.

Protein Carbonyls

Plasma protein carbonyls were measured using a noncompetitive enzyme-linked immunosorbent assay on the basis of the method described by Winterbourn and Buss,[47] as explained in detail in Zipprich et al.[48] Briefly, the protein concentration of each sample was

measured using a Bicinchoninic Acid Kit (Sigma-Aldrich, St Louis, MO). Proteins were derivatized using 2,4-dinitrophenylhydrazine. This was followed by the addition of a primary antibody (anti-dinitrophynyl-KLH Rabbit + immunoglobulin G 1:1500) and then a secondary antibody (streptavidin-biotinylated horseradish peroxidase complex). 3,3′,5,5′-tetramethylbenzidine was used as a visualizing reagent, after which the reaction was stopped using sulfuric acid and plates were read at 450 nm. Samples were measured in duplicate. The average intra- and interassay CVs were calculated using controls run in duplicate on 12 plates; they were 2.0% and 13.8%, respectively.

Statistical Analysis

Descriptive statistics were calculated to describe the sample characteristics, overall and by sex. To examine bivariate associations between variables, Spearman correlations were used for two continuous variables, and *t* tests were used to detect mean differences in continuous variables between two levels of binary variables, including sex. Before performing *t* tests, the appropriate transformations were applied to variables with skewed distributions.

Linear regression models were used to examine the relationship between each As exposure variable and protein carbonyls, with and without adjustment for potential confounding factors. Because 8-oxo-dG had a skewed distribution, with 18% ($n = 68$) of values lower than the detection limit of 0.64 ng/mL urine, we set lower-than-the-detection-limit values to half the detection limit, or 0.32 ng/mL urine; dichotomized the variable at its median, adjusted for urine concentration (10.9 ng/mL urine); and used logistic models to assess the association between 8-oxo-dG levels and each of the As variables, adjusting for covariates. We derived odds ratios with 95% confidence intervals from the model parameters. Candidate control variables included those suggested in the literature or our previous studies or those that showed associations with both As exposure and an outcome variable at a significance level of 0.10 in the bivariate analysis. Control variables were included in the final models if adding them resulted in a change to *B* of at least 10%. Urinary 8-oxo-dG and urinary As levels were adjusted for urine concentration by applying the following equation: [(sample mean for specific gravity − 1)/(individual specific gravity − 1)] × (individual urinary analyte value).[49] Models with and without adjustment for urine concentration were run. As the main predictors in our models, markers of As exposure were investigated both as continuous and categorical variables, divided into water groups or quintiles. Variables with skewed distributions were transformed to reduce the impact of extreme values or improve the fit of models. Blood and urinary As were transformed using the natural logarithm, and water As was square root transformed.

RESULTS

Subject Demographics

Demographic information on the subjects is presented in Table 1. The sample included a similar number of men ($n = 184$) and women ($n = 194$). The median age of our participants was 42 years, and most had only a few years of education (median duration: 4 years for men, 1 year for women). Median water As levels were 114.0 μg/L, but ranged from 0.4 to 700 μg/L. Smoking was common among men (55.6%) but not among women (2.6%), and betel nut was used equally by both sexes (approximately 35%). Fifty-eight percent of individuals in this study owned a television.

No potential confounders were associated with markers of oxidative stress at the $P < 0.05$ level. Nevertheless, we observed a trend toward an inverse correlation between years of education and plasma protein carbonyl levels (Spearman $r = -0.09$; $P = 0.07$). There was also a trend toward television owners being more likely to have urinary 8-oxo-dG values higher than the median (53.6% of owners vs 44.9% of nonowners; $\chi^2 = 2.78$; $P = 0.10$).

Plasma Protein Carbonyls

No significant associations between water, blood, or urinary As and plasma protein carbonyl levels were detected, either in bivariate analysis or in linear regression analysis, with As variables included as continuous and categorical variables. The results of linear regression analyses with As exposure variables included as continuous variables are presented in Table 2. No potential confounders were associated with both protein carbonyl and As levels. We fit the models adjusted for current smoking status and found no appreciable changes in the results. Mean protein carbonyl values for each water As exposure group are shown in Table 3.

Urinary 8-oxo-dG

No significant positive associations between As exposure and urinary 8-oxo-dG levels higher than the median were detected, whether As variables were included in the models as continuous or categorical variables. The results of logistic regression analyses with As exposure variables included as continuous variables are presented in Table 4. Among the potential confounders, only television

TABLE 1. Descriptive Statistics for Folate and Oxidative Stress Study Participants

	Total Sample ($N = 378$)*	Men ($n = 184$)*	Women ($n = 194$)*	P†
Age, yr	42 (30–63)	44 (31–63)	40 (30–62)	0.006
Education, yr	3 (0–16)	4 (0–14)	1 (0–16)	0.11
BMI, kg/m^2	19.7 (13.8–35.3)	19.0 (14.2–32.3)	20.7 (13.8–35.3)	0.003
Water As, μg/L	114.0 (0.4–700.0)	114.1 (0.4–700.0)	113.1 (0.4–492.8)	0.67
Blood As, μg/L	12.5 (2.5–53.1)	14.1 (3.7–53.1)	11.5 (2.5–37.9)	<0.001
Urinary As, μg/L‡	170.4 (12.9–1088.3)	171.2 (15.2–1015.0)	170.1 (12.9–1088.3)	<0.001
8-oxo-dG, ng/mL urine‡	10.9 (0.5–301.3)	10.9 (0.6–71.9)	11.0 (0.5–301.3)	<0.001
Protein carbonyls, ng/g proteins	243.0 (75.1–383.0)	249.2 (75.1–362.9)	235.1 (165.5–383.0)	0.39
Current smoker	28.2	55.6	2.6	<0.0001
Current betel nut user	35.8	35.0	36.5	0.77
Television owner	58.2	59.2	57.2	0.69

*Values have been given as median (range) or percentage.
†*P* values were obtained from testing for differences between the sexes, using *t* tests for continuous variables and chi-squared tests for binary variables.
‡Adjusted for specific gravity.
BMI, body mass index; 8-oxo-dG, 8-oxo-2′-deoxyguanosine.

TABLE 2. Associations Between Arsenic Exposure and Plasma Protein Carbonyls, Using Multiple Linear Regression Models*

	Full Sample ($N = 378$)	Men ($n = 184$)	Women ($n = 194$)
	B (95% CI)		
Water As, μg/L	0.17 (−0.56 to 0.90)	0.41 (−0.59 to 1.41)	−0.12 (−1.16 to 0.92)
Blood As, μg/L	3.44 (−5.85 to 12.7)	0.86 (−12.3 to 14.0)	5.22 (−8.25 to 18.7)
Urinary As,† μg/L	− 0.94 (−4.65 to 2.77)	− 2.57 (−8.37 to 3.22)	0.14 (−4.69 to 4.96)
Urinary As,‡ μg/L	− 0.37 (−4.94 to 4.20)	− 0.40 (−7.44 to 6.64)	−0.45 (−6.47 to 5.57)

*Blood As and urinary As were log transformed before inclusion in models. Water As was square root transformed.
†Not adjusted for specific gravity.
‡Adjusted for specific gravity.
CI, confidence interval.

TABLE 3. Mean Levels of Plasma Protein Carbonyls and Urinary 8-oxo-dG for Each of Five Water As Exposure Groups*

Water As Exposure Group	Plasma Protein Carbonyls, ng/g	Urinary 8-oxo-dG, ng/mL Urine
Group 1: <10 μg/L As ($n = 76$)	253.14 ± 40.43	13.09 ± 34.16
Group 2: 10–100 μg/L As ($n = 104$)	233.96 ± 42.24	10.47 ± 7.30
Group 3: 101–200 μg/L As ($n = 86$)	238.10 ± 42.66	12.80 ± 8.37
Group 4: 201–300 μg/L As ($n = 67$)	245.48 ± 43.62	11.96 ± 10.35
Group 5: >300 μg/L As ($n = 45$)	253.99 ± 44.41	10.10 ± 5.52

*8-oxo-2′-deoxyguanosine levels were adjusted for urine concentration, using specific gravity. Values are given as mean ± SD.
8-oxo-dG, 8-oxo-2′-deoxyguanosine.

TABLE 4. Odds Ratios for the Association Between Each Arsenic Exposure Measure and 8-oxo-2′-deoxyguanosine Levels Higher Than the Median, Derived From Logistic Regression Models[a]

	Full Sample ($N = 377$)	Men ($n = 184$)	Women ($n = 193$)
Urinary analytes unadjusted for specific gravity, μg/L			
Water As	1.02 (0.99–1.06)	1.02 (0.97–1.06)	1.03 (0.98–1.09)
Blood As	0.79 (0.51–1.21)	0.94 (0.52–1.71)	0.65 (0.34–1.23)
Urinary As	3.58 (2.70–4.76)*	4.04 (2.62–6.23)*	3.26 (2.25–4.75)*
Urinary analytes adjusted for specific gravity, μg/L			
Water As	1.03 (1.00–1.07)	1.02 (0.97–1.07)	1.05 (1.00–1.10)
Blood As	0.89 (0.58–1.36)	0.96 (0.53–1.74)	0.87 (0.46–1.63)
Urinary As	1.18 (0.95–1.47)	1.06 (0.77–1.47)	1.29 (0.97–1.73)

*$P < 0.0001$.
[a]Water As was transformed using the square root. Blood As and urinary As were transformed using the natural logarithm. All models were adjusted for television ownership. Values are given as odds ratio (95% confidence interval).

ownership was associated with both 8-oxo-dG and As levels and thus included in the models. When current smoking status was included in the models, no appreciable differences in the results were found. Models with and without adjustment for urine concentration gave similar results, except for the unadjusted model that included urinary As as a predictor of urinary 8-oxo-dG (Table 4). A strong association was found between these two variables ($P < 0.0001$), as would be expected because they are both strongly influenced by urine concentration. Mean 8-oxo-dG level values for each water As exposure group are shown in Table 3.

DISCUSSION

Given the premise that oxidative damage to the cell's structural molecules lies on a causal pathway leading from As exposure to associated diseases, one would expect to observe elevated levels of oxidative damage in individuals with long-term As exposure who have not yet become ill. In this cross-sectional, dose–response study of generally healthy individuals, we found no evidence of an association between water, blood, and urinary As levels and plasma protein carbonyl or 8-oxo-dG levels.

This study has a number of strengths. First, a wide range of water As levels, from 0.4 to 700 μg/L, was examined. Second, subjects were selected by exposure level to yield a relatively even distribution across a wide range of water As concentrations, an asset when studying potential dose–response relationships between exposure and markers of oxidative damage. Third, three measures of As exposure were examined (well water, blood, and urinary As levels), to optimize our assessment of exposure status. Fourth, extensive information on possible confounding variables, including age, sex, body mass index, education level, cigarette and betel nut use,

and television ownership (an indicator of socioeconomic status), was collected. Fifth, two different measures of oxidative damage were examined, one targeting damage to proteins (plasma protein carbonyls) and the other to DNA (urinary 8-oxo-dG). These biomarkers measure oxidative damage over different periods of time: 8-oxo-dG has a circulating half-life of hours[50] and protein carbonyls reflect exposure over a period of weeks.[51] Interestingly, we found no significant association between the two biomarkers themselves (Spearman $r = -0.006$; $P = 0.91$), adding support to previous reports of a lack of association between levels of oxidative damage to different types of biomolecules in humans.[52,53] Our data suggest that a single oxidative damage biomarker may be insufficient for drawing conclusions about oxidative damage in general. Finally, with 378 participants, this study was considerably larger than previous population-based studies of As exposure and oxidative damage.

There are also several potential limitations of this study. First, we measured oxidative damage in plasma and urine samples, but damage leading to As-related skin lesions and cancers may be tissue specific. It is possible that oxidative damage is occurring in tissues not sampled in this study. Nevertheless, previous positive reports of As-associated oxidative damage have also examined biomarkers in plasma[54,55] and urine,[35,56] as was done here. In addition, we note that peripheral blood mononuclear cells are a target for As toxicity,[57] so if chronic As toxicity to these cells occurs via oxidative damage, one might expect to observe evidence of it in plasma. Moreover, urinary 8-oxo-dG is viewed as a biomarker of "whole body" damage,[58] so if oxidative damage to DNA is significantly increased in other target tissues, one would expect this to be reflected in levels of this marker in urine.

The second limitation is that this study focused on whether drinking water As affected biomarkers of oxidative damage, but it has been shown that exposure to As can also occur through food items; this contribution is, relatively speaking, greater when water As levels are low.[59] In this study, data on dietary As exposure were not available. Nevertheless, we did measure As levels in both blood and urine, which reflect exposure from all sources, including the diet.

A third potential limitation is that the two markers of oxidative damage examined, plasma protein carbonyls and urinary 8-oxo-dG, were not associated with variables that might be expected to affect oxidative stress levels, such as current smoking status.[60–62] An association between smoking and these biomarkers has not been detected in many other studies, however.[63–68] In a low-income setting such as Araihazar, it is likely that many sources of oxidative damage, such as air pollution,[69,70] are present. Under these conditions, the level of damage associated with smoking may be relatively insignificant. Like us, other researchers have also failed to find an association between age and plasma protein carbonyls,[71,72] as well as urinary 8-oxo-dG.[73] Similarly, past studies have failed to find an association between sex and plasma protein carbonyls[74] and urinary 8-oxo-dG, after controlling for urine concentration.[75]

The mixed data from human population studies reported here and elsewhere contrast with the stronger and more-consistent evidence that As exposure results in oxidative damage obtained from in vitro[29–31] and animal experiments.[32–34] Although one important difference that may explain this discrepancy is the ready availability of target tissues in cell culture and animal experiments, it has also been shown that As exposure raises the levels of oxidative damage in blood[32] and urine[34] samples from rats. Thus, on the basis of laboratory experiments, one might expect that oxidative damage markers in blood and urine in human studies would also rise with As exposure. The doses of drinking water As used in animal experiments are often orders of magnitude higher and of shorter duration than those encountered by humans in natural settings.[29,32–34] It is possible that humans can better adapt to lower levels of As exposure over time and, to some extent, compensate for As-induced metabolic changes, thereby limiting oxidative damage.[76] In addition, species-specific differences in As metabolism and related health effects have been well documented,[77–79] and it is possible that such differences also apply to As-induced oxidative damage.

In summary, in this study, designed to detect dose–response effects of As on two biomarkers of oxidative damage in humans, we found no evidence that exposure to As increases levels of oxidative damage. No association was found between As and plasma protein carbonyl or 8-oxo-dG levels. A previous study on this sample has shown that As exposure is linked to a more-oxidized redox state, as measured by Eh values based on blood glutathione and glutathione disulfide.[38] It is possible that rather than manifesting in oxidative damage, As-induced changes in the redox state could influence the activity of redox-sensitive enzymes and thereby have downstream effects such as altered DNA methylation[80] or changes in gene expression.[81] Future studies should clarify the downstream effects of the more-oxidized cellular environment associated with As exposure.

ACKNOWLEDGMENTS

The authors thank Regina Santella and Qiao Wang for their help with the assays.

REFERENCES

1. Chen C, Chuang Y, Lin T, Wu S. Malignant neoplasms among residents of a blackfoot disease–endemic area in Taiwan: high arsenic artesian well water and cancers. *Cancer Res*. 1985;45:5895–5899.
2. Chiou H, Huang W, Su C, Chang S, Hsu Y, Chen C. Dose–response relationship between prevalence of cerebrovascular disease and ingested inorganic arsenic. *Stroke*. 1997;28:1717–1723.
3. Chen C, Chiou H, Chiang M, Lin L, Tai T. Dose–response relationship between ischemic heart disease mortality and long-term arsenic exposure. *Arterioscler Thromb Vasc Biol*. 1996;16:504–510.
4. Navas-Acien A, Sharrett A, Silbergeld E, et al. Arsenic exposure and cardiovascular disease: a systematic review of the epidemiologic evidence. *Am J Epidemiol*. 2005;162:1037–1049.
5. Chen Y, Graziano J, Parvez F, et al. Arsenic exposure from drinking water and mortality from cardiovascular disease in Bangladesh: prospective cohort study. *BMJ*. 2011;342:d2431.
6. Navas-Acien A, Silbergeld E, Pastor-Barriuso R, Guallar E. Arsenic exposure and prevalence of type 2 diabetes in US adults. *J Am Med Assoc*. 2008;300:814–822.
7. Navas-Acien A, Silbergeld E, Pastor-Barriuso R, Guallar E. Arsenic exposure and prevalence of type 2 diabetes: updated findings from the National Health Nutrition and Examination Survey, 2003–2006. *Epidemiology*. 2009;20:816–820.
8. Del Razo L, García-Vargas G, Valenzuela O, et al. Exposure to arsenic in drinking water is associated with increased prevalence of diabetes: a cross-sectional study in the Zimapán and Lagunera regions in Mexico. *Environ Health*. 2011;10:73.
9. Steinmaus C, Yuan Y, Liaw J, Smith A. Low-level population exposure to inorganic arsenic in the United States and diabetes mellitus: a reanalysis. *Epidemiology*. 2009;20:807–815.
10. Chen Y, Ahsan H, Slavkovich V, et al. No association between arsenic exposure from drinking water and diabetes mellitus: a cross-sectional study in Bangladesh. *Environ Health Perspect*. 2010;118:1299–1305.
11. Smith A, Lingas E, Rahman M. Contamination of drinking-water by arsenic in Bangladesh: a public health emergency. *Bull World Health Organ*. 2000;78:1093–1103.
12. Kitchin K, Ahmad S. Oxidative stress as a possible mode of action for arsenic carcinogenesis. *Toxicol Lett*. 2003;137:3–13.
13. Andrew A, Burgess J, Meza M, et al. Arsenic exposure is associated with decreased DNA repair in vitro and in individuals exposed to drinking water arsenic. *Environ Health Perspect*. 2006;114:1193–1198.
14. Hamadeh H, Trouba K, Amin R, Afshari C, Germolec D. Coordination of altered DNA repair and damage pathways in arsenite-exposed keratinocytes. *Toxicol Sci*. 2002;69:306–316.
15. Davis C, Uthus E, Finley J. Dietary selenium and arsenic affect DNA methylation in vitro in Caco-2 cells and in vivo in rat liver and colon. *J Nutr*. 2000;130:2903–2909.

16. Pilsner J, Liu X, Ahsan H, et al. Genomic methylation of peripheral blood leukocyte DNA: influences of arsenic and folate in Bangladeshi adults. *Am J Clin Nutr*. 2007;86:1179–1186.
17. Ren X, McHale C, Skibola C, Smith A, Smith M, Zhang L. An emerging role for epigenetic dysregulation in arsenic toxicity and carcinogenesis. *Environ Health Perspect*. 2011;119:11–19.
18. Petrick J, Jagadish B, Mash E, Aposhian H. Monomethylarsonous acid (MMAIII) and arsenite: LD50 in hamsters and in vitro inhibition of pyruvate dehydrogenase. *Chem Res Toxicol*. 2001;14:651–656.
19. Davey J, Bodwell J, Gosse J, Hamilton J. Arsenic as an endocrine disruptor: effects of arsenic on estrogen receptor–mediated gene expression in vivo and in cell culture. *Toxicol Sci*. 2007;98:75–86.
20. Davey J, Nomikos A, Wungjiranirun M, et al. Arsenic as an endocrine disruptor: arsenic disrupts retinoic acid receptor– and thyroid hormone receptor–mediated gene regulation and thyroid hormone–mediated amphibian tail metamorphosis. *Environ Health Perspect*. 2008;116:165–172.
21. Sciandrello G, Caradonna F, Mauro M, Barbata G. Arsenic-induced DNA hypermethylation affects chromosomal instability in mammalian cells. *Carcinogenesis*. 2004;25:413–417.
22. Moore L, Smith A, Eng C, et al. Arsenic-related chromosomal alterations in bladder cancer. *N Natl Cancer Inst*. 2002;94:1688–1696.
23. Chou W, Hawkins A, Barrett J, Griffin C, Dang D. Arsenic inhibition of telomerase transcription leads to genetic instability. *J Clin Invest*. 2001;108:1541–1547.
24. Toyokuni S, Okamoto K, Yodoi J, Hiai H. Persistent oxidative stress in cancer. *FEBS Lett*. 1995;358:1–3.
25. Valko M, Rhodes C, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress–induced cancer. *Chem Biol Interact*. 2006;160:1–40.
26. Dhalla N, Temsah R, Netticadan T. Role of oxidative stress in cardiovascular diseases. *J Hypertens*. 2000;18:655–673.
27. Hoeschen R. Oxidative stress and cardiovascular disease. *Can J Cardiol*. 1997;13:1021–1025.
28. West I. Radicals and oxidative stress in diabetes. *Diabet Med*. 2000;17:171–180.
29. Kessel M, Liu S, Xu A, Santella R, Hei T. Arsenic induces oxidative DNA damage in mammalian cells. *Mol Cell Biochem*. 2002;234/235:301–308.
30. Li M, Cai J, Chiu J. Arsenic induces oxidative stress and activates stress gene expressions in cultured lung epithelial cells. *J Cell Biochem*. 2002;87:29–38.
31. Ruiz-Ramos R, Lopez-Carrillo L, Rios-Perez A, Vizcaya-Ruíz AD, Cebrian M. Sodium arsenite induces ROS generation, DNA oxidative damage, HO-1 and c-MYC proteins, NF-kB activation and cell proliferation in human breast cancer MCF-7 cells. *Mutat Res*. 2009;674:109–115.
32. Flora S. Arsenic-induced oxidative stress and its reversibility following combined administration of N-acetylcysteine and *meso* 2,3-dimercaptosuccinic acid in rats. *Clin Exp Pharmacol Physiol*. 1999;26:865–869.
33. Wei M, Wanibuchi H, Morimura K, et al. Carcinogenicity of dimethylarsinic acid in male F344 rats and genetic alterations in induced urinary bladder tumors. *Carcinogenesis*. 2002;23:1387–1397.
34. Yamanaka K, Takabayashi F, Mizoi M, An Y, Hasegawa A, Okada S. Oral exposure of dimethylarsenic acid, a main metabolite of inorganic arsenics, in mice leads to an increase in 8-oxo-2′-deoxyguanosine level, specifically in the target organs for arsenic carcinogenesis. *Biochem Biophys Res Commun*. 2001;287:66–70.
35. Engström K, Vahter M, Johansson G, et al. Chronic exposure to cadmium and arsenic strongly influences concentrations of 8-oxo-7,9-dihydro-2′-deoxyguanosine in urine. *Free Radic Biol Med*. 2010;48:1211–1217.
36. Burgess J, Meza M, Josyula A, et al. Environmental arsenic exposure and urinary 8-OHdG in Arizona and Sonora. *Clin Toxicol*. 2007;45:490–498.
37. Engström K, Vahter M, Lindh C, et al. Low 8-oxo-7,8-dihydro-2′-deoxyguanosine levels and influence of genetic background in an Andean population exposed to high levels of arsenic. *Mutat Res*. 2010;683:98–105.
38. Hall M, Niedzwiecki M, Harper K, et al. Association between chronic arsenic exposure and blood glutathione and glutathione disulfide concentration in Bangladeshi adults. *Environ Health Perspect*. 2013;121:1068–1074.
39. Ahsan H, Chen Y, Parvez F, et al. Health Effects of Arsenic Longitudinal Study (HEALS): description of a multidisciplinary epidemiologic investigation. *J Expo Sci Environ Epidemiol*. 2006;16:191–205.
40. Cheng Z, Zheng Y, Mortlock R, Van Geen A. Rapid multi-element analysis of groundwater by high-resolution inductively coupled plasma mass spectrometry. *Anal Bioanal Chem*. 2004;379:512–518.
41. Van Geen A, Cheng Z, Seddique AA, et al. Reliability of a commercial kit to test groundwater for arsenic in Bangladesh. *Environ Sci Technol*. 2005;39:299–303.
42. van Geen A, Cheng Z, Jia Q, et al. Monitoring 51 community wells in Araihazar, Bangladesh, for up to 5 years: implications for arsenic mitigation. *J Environ Sci Health A Tox Hazard Subst Environ Eng*. 2007;42:1729–1740.
43. Stroh A. Determination of Pb and Cd in whole blood using isotope dilution ICP-MS. *Atom Spectrosc*. 1993;37:1575–1579.
44. Pruszkowski E, Neubauer K, Thomas R. An overview of clinical applications by inductively coupled plasma mass spectrometry. *Atom Spectrosc*. 1998;19:111–115.
45. Nixon D, Mussmann G, Eckdahl S, Moyer T. Total arsenic in urine: palladium-persulfate vs nickel as a matrix modifier for graphite furnace atomic absorption spectrophotometry. *Clin Chem*. 1991;37:1575–1579.
46. Heitkemper D, Vela N, Stewart K, Westphal C. Determination of total and speciated arsenic in rice by ion chromatography and inductively coupled plasma mass spectrometry. *J Anal At Spectrom*. 2001;16:299–306.
47. Winterbourn C, Buss I. Protein carbonyl measurement by enzyme-linked immunosorbent assay. *Methods Enzymol*. 1999;300:106–111.
48. Zipprich J, Terry M, Liao Y, et al. Plasma protein carbonyls and breast cancer risk in sisters discordant for breast cancer from the New York site of the Breast Cancer Family Registry. *Cancer Res*. 2009;69:2966–2972.
49. Cone E, Caplan Y, Moser F, Robert T, Shelby M. Normalization of urinary drug concentrations with specific gravity and creatinine. *J Anal Toxicol*. 2009;33:1–7.
50. Loft S, Larsen P, Rasmussen A, et al. Oxidative DNA damage after transplantation of the liver and small intestine in pigs. *Transplantation*. 1995;59:16–20.
51. Griffiths H. The influence of diet on protein oxidation. *Nutr Res Rev*. 2002;15:3–17.
52. Drury J, Jeffers G, Cooke R. Urinary 8-hydroxydeoxyguanosine in infants and children. *Free Radic Res*. 1998;28:423–428.
53. England T, Beatty E, Rehman A, et al. The steady-state levels of oxidative DNA damage and of lipid peroxidation (F2-isoprostanes) are not correlated in healthy human subjects. *Free Radic Res*. 2000;32:355–362.
54. Pi J, Yamauchi H, Kumagai Y, et al. Evidence for induction of oxidative stress caused by chronic exposure of Chinese residents to arsenic contained in drinking water. *Environ Health Perspect*. 2002;110:331–336.
55. Wu M, Chiou H, Wang T, et al. Association of blood arsenic levels with increased reactive oxidants and decreased antioxidant capacity in a human population of northeastern Taiwan. *Environ Health Perspect*. 2001;109:1011–1017.
56. Yamauchi H, Aminaka Y, Yoshida K, Sun G, Pi J, Waalkes M. Evaluation of DNA damage in patients with arsenic poisoning: urinary 8-hydroxydeoxyguanine. *Toxicol Appl Pharmacol*. 2004;198:291–296.
57. Soignet S, Maslak P, Wang Z, et al. Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide. *New Eng J Med*. 1998;339:1341–1348.
58. Olinski R, Rozalksi R, Gackowski D, Foksinski M, Siomek A, Cooke M. Urinary measurement of 8-oxodg, 8-oxogua, and 5HMUra: a noninvasive assessment of oxidative damage to DNA. *Antioxid Redox Signal*. 2006;8:1011–1019.
59. Kile M, Houseman E, Breton C, et al. Dietary arsenic exposure in Bangladesh. *Environ Health Perspect*. 2007;115:889–893.
60. Priemé H, Loft S, Klarlund M, Grønbaek K, Tønnesen P, Poulsen H. Effect of smoking cessation on oxidative DNA modification estimated by 8-oxo-7,8-dihydro-2′-deoxyguanosine excretion. *Carcinogenesis*. 1998;19:347–351.
61. Loft S, Vistisen K, Ewertz M, Tjønneland A, Overvad K, Poulsen H. Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. *Carcinogenesis*. 1992;13:2241–2247.
62. Marangon K, Devaraj S, Jialal I. Measurement of protein carbonyls in plasma of smokers and in oxidized LDL by an ELISA. *Clin Chem*. 1999;45:577–578.
63. Wu H, Wang Q, Wang L, et al. Urinary 8-oxodeoxyguanosine, aflatoxin B1 exposure and hepatitis B virus infection and hepatocellular carcinoma in Taiwan. *Carcinogenesis*. 2007;28:995–999.
64. Nilsson R, Nordlinder R, Tagesson C, Walles S, Järvholm B. Genotoxic effects in workers exposed to low levels of benzene from gasoline. *Am J Ind Med*. 1996;30:317–324.
65. Singh R, Kaur B, Kalina I, et al. Effects of environmental air pollution on endogenous oxidative DNA damage in humans. *Mutat Res*. 2007;620:71–82.
66. Wu L, Chiou C, Chang P, Wu J. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. *Clin Chim Acta*. 2004;339:1–9.

67. Honda M, Yamada Y, Tomonaga M, Ichinose H, Kamihira S. Correlation of urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG), a biomarker of oxidative DNA damage, and clinical features of hematological disorders: a pilot study. *Leuk Res*. 2000;24:461–468.
68. Rossner P, Terry M, Gammon M, et al. Plasma protein carbonyl levels and breast cancer risk. *J Cell Mol Med*. 2007;11:1138–1148.
69. Svecova V, Rossner P, Dostal M, Topinka J, Solansky I, Sram R. Urinary 8-oxodeoxyguanosine levels in children exposed to air pollutants. *Mutat Res*. 2009;662:37–43.
70. Chuang K, Chan C, Su T, Lee C, Tang C. The effect of urban air pollution on inflammation, oxidative stress, coagulation, and autonomic dysfunction in young adults. *Am J Respir Crit Care Med*. 2007;176:370–376.
71. Garibaldi S, Aragno I, Odetti P, Marinari U. Relationships between protein carbonyls, retinol and tocopherols level in human plasma. *Biochem Mol Biol Int*. 1994;34:729–736.
72. Garibaldi S, Valentini S, Aragno I, Pronzato M, Tavers N, Odetti P. Plasma protein oxidation and antioxidant defense during aging. *Int J Vitam Nutr Res*. 2001;71:332–338.
73. Poulsen H, Loft S, Prieme H, et al. Oxidative DNA damage in vivo: relationship to age, plasma antioxidants, drug metabolism, glutathione-S-transferase activity and urinary creatinine excretion. *Free Radic Res*. 1998;29:565–571.
74. Bloomer R, Fisher-Wellman K. Blood oxidative stress biomarkers: influence of sex, exercise training status, and dietary intake. *Gend Med*. 2008;5:218–228.
75. Pilger A, Germadnik D, Riedel K, Meger-Kossien I, Scherer G, Rüdiger H. Longitudinal study of urinary 8-hydroxy-2′-deoxyguanosine excretion in healthy adults. *Free Radic Res*. 2001;35:273–280.
76. Coppin J, Qu W, Waalkes M. Interplay between cellular methyl metabolism and adaptive efflux during oncogenic transformation from chronic arsenic exposure in human cells. *J Biol Chem*. 2008;283:19342–19350.
77. Vahter M. Species differences in the metabolism of arsenic compounds. *App Organomet Chem*. 1994;8:175–182.
78. Basu A, Mahata J, Gupta S, Giri A. Genetic toxicology of a paradoxical human carcinogen, arsenic: a review. *Mutat Res*. 2001;488:171–194.
79. Drobná Z, Walton F, Harmon A, Thomas D, Styblo M. Interspecies differences in metabolism or arsenic by cultured primary hepatocytes. *Toxicol Appl Pharmacol*. 2010;245:47–56.
80. Niedzwiecki M, Hall M, Liu X, et al. Blood glutathione redox status and global methylation of peripheral blood mononuclear cell DNA in Bangladeshi adults. *Epigenetics*. 2013;8:7.
81. Allen R, Tresini M. Oxidative stress and gene regulation. *Free Radic Biol Med*. 2000;28:463–499.

