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Arsenic exposure through drinking Water and oxidative stress Status: A cross-sectional study in the Ayeyarwady region, Myanmar

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ABSTRACT

Arsenic is a well-known toxic heavy metal that is naturally dispersed in groundwater. Whereas arsenic is widely accepted to be involved in oxidative stress damage, little is known about arsenic-induced oxidative damage in relationship to contaminated drinking water as a source. The aim of this study was to determine the association between arsenic exposure through drinking water and oxidative stress status by measuring levels of urinary 8-hydroxydeoxyguanosine (8 – OHdG) as a biomarker of oxidative stress damage in a Myanmar population. A questionnaire-based survey and drinking water and urine sampling (n = 198) were performed to assess the association between arsenic exposure and urinary 8 – OHdG concentration in the Ayeyarwady Region, Myanmar. Urinary arsenic concentrations were significantly correlated with drinking water arsenic concentrations (Spearman's rho = 0.32, $p < 0.001$). Multivariate linear regression analysis showed that higher urinary arsenic concentrations were significantly associated with higher 8 – OHdG concentrations (coefficient = 0.09, 95% confidence interval, 0.03 – 0.15; p = 0.002). The present study identified that exposure to arsenic through drinking water could induce an increase in the urinary 8 – OHdG concentration, reflecting increased oxidative DNA damage. These findings provide evidence that may explain the role of arsenic-induced oxidative stress in the pathophysiology of arsenic-induced diseases including cancers.

1. Introduction

Arsenic is a well-known toxic heavy metal that is naturally dispersed in groundwater [1,2]. Arsenic contamination in groundwater has become a significant public health concern, especially when groundwater is the primary source of drinking water for people living in contaminated areas [1,3]. Approximately 200 million people worldwide suffer from chronic arsenic toxicity acquired via arsenic-contaminated drinking water [1,3,4]. Arsenic toxicity may result in acute and chronic poisoning, and occurs primarily through ingestion [5]. Many studies have demonstrated that long-term arsenic exposure has adverse impacts on human health, causing skin lesions, cardiovascular diseases, neurological complications, reproductive and respiratory disorders, and malignant and non-malignant diseases [6–11]. Furthermore, arsenic crosses the placental barrier from mother to fetus, further extending the health consequences [12].

Oxidative DNA damage is one mechanism explaining the pathophysiology of arsenic-related clinical manifestations [13,14]. Arsenic promotes the generation of reactive oxygen species (ROS) during the methylation process [15]. In addition, it influences mitochondrial enzyme activities by altering the transfer of electrons from the respiratory chain [16]. ROS groups, particularly, hydroxyl radicals add to guanine at the fourth or eighth position in the purine base, resulting in oxidative DNA modification in the form of 8-hydroxydeoxyguanosine (8 – OHdG) [17]. Increased urinary excretion of DNA base modification, the 8 – OHdG concentration, could be an indicator of the average rate of arsenic-induced DNA damage in the body [18]. The urinary 8 – OHdG concentration is frequently considered to be a marker of oxidative stress-induced DNA damage; 8 – OHdG is mainly excreted in the urine and is technically non-invasive to collect [18,19]. In many previous studies, higher urinary 8 – OHdG concentrations were found in association with many human diseases, including diabetes mellitus, renal failure, atherosclerosis and various types of cancer [17,18,20,21].

Elevated arsenic levels in drinking water comprise an emerging

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public health issue in Myanmar. As reported by the World Bank in 2005, about 3.4 million of people in Myanmar were at risk of arsenic contamination [1]. Myanmar first gained attention due to arsenic contamination of surface water and groundwater in 2000 [22]. Since then, emerging data have confirmed the high levels of arsenic in groundwater in several areas of Myanmar [22–26]. As an example, a total of 123,964 drinking water sources were tested for arsenic in the Ayeyarwaddy Region; arsenic concentrations in 29% of these sources were higher than the World Health Organization (WHO) standard of 10 μg/L, and those in 8% of the sources exceeded 50 μg/L [27].

Arsenic is widely accepted to be involved in oxidative stress damage, but little is known about the oxidative damage associated with arsenic exposure from drinking water contamination, particularly at the household level. Heavy arsenic contamination of groundwater has been demonstrated in certain areas of Myanmar [22,27]. However, the actual concentrations in drinking water are not clear, and the information regarding the treatment of drinking water at the household level is lacking. Moreover, no research has been conducted to estimate the degree of oxidative DNA damage caused by arsenic in a Myanmar population. Therefore, this study aims to evaluate the effect of arsenic exposure through drinking water on oxidative stress status by measuring urinary 8−OHdG concentrations in a Myanmar population.

2. Material and methods

2.1. Study area and setting

This community-based cross-sectional study was conducted in Kyaunggone, Kyonpyaw and Ahtaung townships in the Ayeyarwady Region of Myanmar in 2016. Geographically, the region is bounded on the north by the Bago Region, on the east by the Yangon Region, and on the southwest by the Bay of Bengal. The population density in this region was 171/km^2 according to Census 2014. A sub-sample of 248 subjects from an existing birth-cohort study were asked for household-drinking water samples. A total of 198 drinking water samples were included for analysis in this study (Fig. 1). The detail information of the existing birth cohort study has been described previously [28]. The study subjects were pregnant women of 18 years old or older who were in the third trimester of pregnancy and had resided in the study area for at least 6 months. Pregnant women with severe medical conditions were excluded from the study. Eligible pregnant women were selected from a list of antenatal care attendance at the local health centers. Then, local health staff of the respective health centers assisted the research team to trace the study subjects. Each study subject underwent a face-to-face interview that was conducted using a pretested structured questionnaire. The questionnaire covered subjects' sociodemographic characteristics, including age, education, occupation, drinking water status, smoking status, pregnancy and birth history. After the interview, a spot urine sample was collected from each subject. Subjects were also asked to provide a household drinking water sample from their homes. The water samples were properly labelled and acidified with 60% nitric acid at a ratio of 4.5 mL of water and 75 μL of 60% nitric acid. The water and urine samples were first stored at – 20 °C at the corresponding health centers, and then transported to the University of Tokyo, Japan. The samples were stored at – 80 °C until analysis.

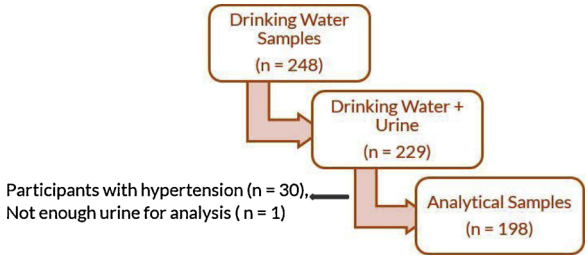


Fig. 1. Analytical Samples in the Study: In the final analysis, 50 drinking water samples were excluded due to the lack of corresponding urine samples (n = 20) and expected high urinary 8-hydroxydeoxyguanosine (8−OHdG) concentrations due to underlying hypertension (n = 30).

2.2. Measurement of arsenic concentrations

Urinary and drinking water arsenic concentrations were measured using inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7500ce ICP-MS, Agilent Technologies, Santa Clara, CA, USA). The procedure has been described in detail previously [28]. The limit of detection (LOD) was calculated as three times the standard deviation of the blank measurements. The LODs of arsenic for urine and water were 0.239 μg/L and 0.015 μg/L, respectively. All the measurements of the samples were above the LODs. For quality control, we used the National Institute of Standards and Technology (NIST) Standard Reference Material 1643f Trace Elements in Water (NIST, Gaithersburg, MD, USA) as a certified reference material (CRM) for water samples. For urine samples, we used the National Institute for Environmental Studies (NIES) CRM No.18 Human Urine (NIES, Tsukuba, Japan). To correct for variations in urine dilution, urinary arsenic concentrations were adjusted for creatinine. Urine creatinine concentrations were measured using Jaffe method with a creatinine measurement kit (LabAssay Creatinine Kit, Wako Pure Chemical, Osaka, Japan).

2.3. Measurement of urinary 8−OHdG concentrations

Corresponding urine samples (n = 198) were transported to the laboratory of Healthcare Systems Inc. (Aichi, Japan) under a cold chain, and urinary 8−OHdG concentrations were measured using an enzyme-linked immunosorbent assay (ELISA) (on-chip ELISA, Healthcare Systems Inc., Aichi, Japan), following the manufacturers' protocol [29]. In brief, the marker of 8−OHdG in urine was applied to an azopolymer surface that had been precoated with a specific monoclonal antibody against 8−OHdG. It was then treated with alkaline phosphatase-labelled streptavidin solution. The antibody binding capacity was measured by chemiluminescent detection. The amount of 8−OHdG was then interpolated from a standard curve of authentic 8−OHdG.

2.4. Statistical analysis

Data are described as medians, interquartile ranges (IQRs) and percentages. The urinary arsenic and 8−OHdG concentrations were log-transformed due to skewed distributions. Differences in urinary 8−OHdG concentrations according to demographic and other characteristics were evaluated by analysis of variance. Drinking water arsenic concentrations were dichotomized at 10 μg/L according to the WHO guidelines [27]. Bivariate and multivariate linear regression analyses were used to determine the associations between urinary arsenic exposure and oxidative stress. In this study, the urinary 8OHdG concentration was considered to be a biomarker of oxidative stress. The outcome variable was the log-transformed urinary 8−OHdG concentration, and the independent variables were the drinking water arsenic concertation as a categorical variable and the urinary arsenic concentration as a log-transformed continuous variable. First, a bivariate analysis was performed to identify associations between the variables. Variables that were significant in the bivariate analysis were included in the multivariate regression models. Adjusted variables were age, education, occupation, primary source of drinking water, rice cooking method, water treatment, and smoking status. The statistical analysis was performed using Stata 13 (StataCrop LP, Colledge Station, TX, USA), and significant level was considered at p-value < 0.05.

2.5. Ethics

This study was conducted under the approval of the Ethical Review

Table 1
Distribution of urinary arsenic and 8-hydroxydeoxyguanosine (8 − OHdG) concentrations (n = 198).

Variables	Median (IQR)	n (%)	Spearman's rho
Water arsenic (μg/L)	2.2 (0.4 – 8.7)		
Water arsenic > 10 μg/L		43 (21.7)	
Urinary arsenic concentrations			
Creatinine-adjusted (μg/g creatinine)	66.3 (41.3 – 107)		
Unadjusted (μg/L)	53.1 (30.5 – 93.4)		
Urinary 8-OHdG concentrations			
Creatinine-adjusted (ng/mg creatinine)	10.1 (8.1 – 12.0)		
Unadjusted (ng/mL)	8.6 (4.7 – 11.5)		
Correlations between water and urine arsenic			0.323*

IQR: Interquartile range; * p-value < 0.001.

Committee of the Graduate School of Medicine, the University of Tokyo (no. 11186) and the Department of Medical Research, Myanmar (ERC no. 009316). Written informed consent was obtained from each subject prior to her participation in the study.

3. Results

The arsenic concentrations in household drinking water and urine are presented in Table 1. Of the total 198 drinking water samples, arsenic concentrations ranged from 0.02–198 μg/L (median = 2.2 μg/L, IQR; 0.4–8.7 μg/L); 21.7% of drinking water samples were higher than the WHO standard of 10 μg/L. The median urinary arsenic concentration was 53.1 μg/L (IQR, 30.5–93.4 μg/L), and the creatinine-adjusted median value was 66.3 μg/g creatinine (IQR, 41.3–107 μg/g creatinine). A significant positive correlation was found between the drinking water arsenic concentration and the creatinine adjusted urinary arsenic concentration (Spearman's rho = 0.32, p < 0.001).

Of the198 subjects, more than 80% had at least primary school education levels (46% had completed primary school and 36.8% had completed middle school or above) (Table 2). Almost half (46.5%) of the subjects were unemployed or were housewives. Exposure to tobacco smoke (active or passive smoking) was reported by 68.7% of the subjects. Regarding household drinking water, overall, well or household pumping of groundwater was the most common primary source of drinking water (89.4%) in this study area (Table 2). Only 19.7% of participants reported that their water had been previously tested for arsenic. The majority (81.8%) of participants applied some kinds of water treatment method before drinking. As shown in Fig. 2, the most frequently used water treatment method was traditional cloth filtration (65.6%), followed by boiling (15.6%), settling (11.3%), use of filtering equipment/machine (3.8%), chlorination (2.8%) and others (1.0%).

The median urinary 8 − OHdG concentration among the study subjects was 10.1 ng/mg creatinine (IQR, 8.1–12.0 ng/mg creatinine) (Table 1). Urinary 8 − OHdG concentration was higher in the older age groups (Table 2). However, it did not significantly differ by educational level, occupation, rice cooking method, or smoking status. No significant difference in urinary 8 − OHdG concentrations was observed based on the water sources or treatment method. Subjects who did not pay attention to arsenic contamination in their drinking water had significantly higher 8 − OHdG concentrations than did those who were aware of the arsenic concentrations in their drinking water (Table 2).

On Pearson's correlation analysis, there was no significant association between arsenic concentrations in drinking water and urinary 8 − OHdG concentrations (r = 0.075, p = 0.292) (Fig. 3a), and the results did not differ with categorization of the arsenic level according to the WHO standard of 10 μg/L (Fig. 3b). However, urinary 8 − OHdG concentrations were significantly, and positively associated with urinary arsenic concentration (r = 0.209, p = 0.003) (Fig. 3c). Multiple linear regression model was performed with the urinary 8 − OHdG concentration serving as an outcome and the inclusion of age, education, occupation, smoking status, treatment of drinking water, primary source of drinking water, rice cooking method, arsenic concentration in drinking water and, creatinine-adjusted urinary arsenic concentration (Table 3). Urinary 8 − OHdG concentrations were significantly higher in the older age groups of 25–30 years (coefficient = 0.06, 95% CI 0.01–0.12, p = 0.031) and more than 30 years (coefficient = 0.06, 95% CI 0.01 – 0.12, p = 0.032), compared with the younger age group of 20 years and below. Interestingly, a 10% increase in the urinary arsenic concentration was significantly associated with a 0.9% increase in the urinary 8 − OHdG concentration (coefficient = 0.09, 95% CI 0.03 – 0.15, p = 0.002). However, the 8 − OHdG concentration appeared to have no significant association with individual characteristics, such as education, occupation, rice cooking methods, water treatment, or smoking status. To investigate individual variations in the response to arsenic exposure, different interaction terms were applied in the regression models. However, no significant interaction effect was detected between arsenic exposure and these individual characteristics (data not shown).

4. Discussion

In the present study, we assessed arsenic-induced oxidative stress status by investigating the association between arsenic exposure and urinary 8 − OHdG concentrations in a Myanmar population. As a biomarker of arsenic exposure, the urinary arsenic concentration was assessed because ingested arsenic is mainly excreted in the urine [30]. To trace the source of arsenic exposure, the present study assessed arsenic concentrations in household drinking water. This study showed that there was a significant positive correlation between the drinking water arsenic concentration and the urinary arsenic concentration. This study also identified that higher urinary arsenic concentrations were significantly associated with higher 8 − OHdG concentrations in a Myanmar population. However, no significant correlation was found between the drinking water arsenic concentration and the urinary 8 − OHdG concentration.

In this study, the median arsenic concentration in household drinking water was 2.2 μg/L, and 21.7% of the drinking water samples had arsenic concentrations exceeded the WHO standard of 10 μg/L. This finding was slightly lower than the previous national report stating that arsenic concentration in 29.2% of water samples were higher than the WHO standards of 10 μg/L [27]. The slight variations in the prevalence of contamination in this study could reflect the geographical variations in arsenic contamination in the groundwater.

Awareness of arsenic contamination may influence on the mitigation options, such as changing the drinking water source and/or water treatment method compared with doing nothing [31]. Thereafter, arsenic contamination in groundwater was discovered in Myanmar, in 2000, extensive arsenic mitigation programs have been initiated by governmental and non-governmental organizations [27,32]. However, in the present study, only 39 subjects (19.7%) reported that their drinking water sources had been previously tested for arsenic, and 60 subjects (30.3%) did not know whether the water had been tested for

Table 2
Urinary 8-hydroxydeoxyguanosine (8 – OHdG) concentrations stratified by descriptive variables (n = 198).

Variables	n (%)	Median[a](IQR)	p-value
Age (years)			0.045
20 and below	29 (14.7)	9.2 (7.2 – 11.6)	
> 20 and ≤ 25	66 (33.3)	9.6 (7.6 – 11.5)	
> 25 and ≤ 30	44 (22.2)	10.1 (8.2 – 12.0)	
> 30	59 (29.8)	10.4 (8.9 – 13.0)	
Education			0.696
Illiterate	4 (2.0)	9.2 (7.3 – 10.1)	
Able to read and write	30 (15.2)	9.6 (8.1 – 11.6)	
Primary school completed	91 (46.0)	10.1 (7.7 – 12.8)	
Middle school completed	40 (20.2)	10.4 (8.9 – 11.9)	
High school completed	26 (13.1)	9.8 (8.3 – 10.4)	
Graduate or above	7 (3.5)	10.2 (8.6 – 14.0)	
Occupation			0.634
Unemployed or housewives	92 (46.5)	10.1 (8.3 – 11.8)	
Farmers	66 (33.3)	9.4 (7.2 – 11.7)	
Private sectors	2 (1.0)	11.4 (9.3 – 13.6)	
Government officers	6 (3.0)	8.7 (7.4 – 11.3)	
Own business	17 (8.6)	10.2 (9.4 – 12.3)	
Others	15 (7.6)	11.4 (8.8 – 14.3)	
Smoking status			0.221
No exposure at all	68 (34.3)	9.8 (7.6 – 11.7)	
Have ever smoked or was exposed to passive smoking	130 (68.7)	10.1 (8.2 – 12.0)	
Rice cooking method			0.337
Traditional boiling	171 (86.4)	10.0 (8.0 – 11.9)	
Rice cocker	27 (13.6)	10.1 (8.7 – 12.3)	
Primary source of drinking water			0.956
Ground water (well/ hand pump/ motor pump)	177 (89.4)	10.1 (8.9 – 12.0)	
River/lake/ household water supply	2 (1.0)	10.1 (8.0 – 12.0)	
Others	19 (9.6)	10.5 (9.7 – 11.3)	
Any treatment before drinking			0.374
No	36 (18.2)	10.1 (8.4 – 11.7)	
Yes	162 (81.8)	10.1 (8.1 – 12.0)	
If yes, how often			0.738
Always	152 (76.8)	10.1 (8.0 – 12.1)	
Usually	6 (3.0)	8.8 (8.6 – 11.3)	
Sometimes or few	4 (2.0)	10.6 (7.9 – 13.6)	
Never	36 (18.2)	10.1 (8.4 – 11.7)	
Most recent water testing			0.384
Within 1 month	8 (4.0)	8.3 (7.2 – 11.1)	
Within 1 – 3 months	13 (6.6)	10.1 (9.2 – 11.8)	
Within 3 – 6 months	29 (14.7)	10.4 (8.0 – 12.2)	
More than 6 months	68 (34.3)	10.2 (8.7 – 11.8)	
Never	79 (39.9)	9.5 (7.6 – 11.9)	
Missing	1 (0.5)	10.0 (10.0 – 10.0)	
If tested, purpose of testing			0.456
Microbial	102 (51.5)	10.2 (8.5 – 12.3)	
Chemical	10 (5.0)	10.9 (7.7 – 11.7)	
Both	3 (1.5)	11.2 (10.5 – 11.5)	
Others	3 (1.5)	8.7 (7.6 – 10.2)	
Ever been tested for arsenic			0.582
No	99 (50.0)	10.2 (7.8 – 12.9)	
Yes	39 (19.7)	9.9 (7.7 – 11.5)	
Don't know	60 (30.3)	10.1 (8.8 – 11.7)	
If yes, aware that			0.009
Above standard (> 10 μg/L)	3 (7.7)	10.0 (8.6 – 14.1)	
Below standard (≤ 10 μg/L)	25 (64.1)	9.3 (7.1 – 11.2)	
Don't know	11 (28.2)	11.5 (8.9 – 14.2)	

[a] ng/mg creatinine; p-value is derived from ANOVA.

arsenic. Only 3.8% of the subjects used scientifically sound arsenic removal methods, such as the use of filtering machine and equipment, suggesting that knowledge of the correct treatment method for arsenic was scarce in the study population. People tend to be more concerned about visible physical particles or suspensions in drinking water than they are about hazardous invisible pollutants. People living in contaminated areas may still have limited health knowledge specifically arsenic-related health outcomes and proper mitigation methods. The findings of this study indicate that extensive health policy and education programs should be implemented to enhance awareness and to inspire behavioral modifications.

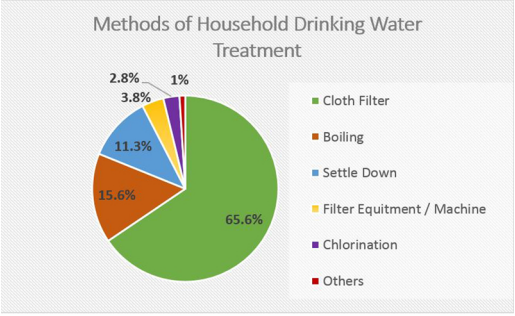


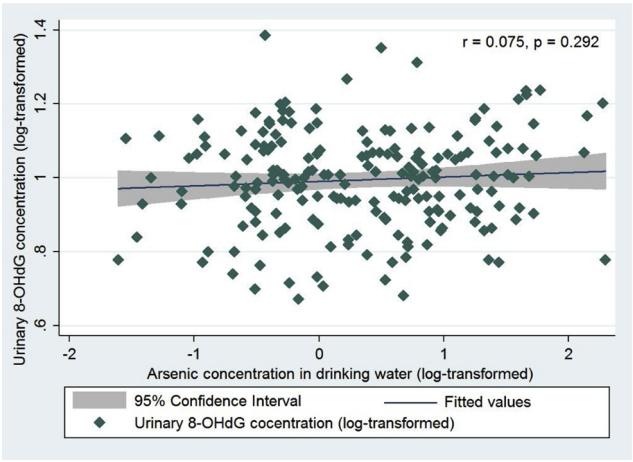
Fig. 2. Methods of Household Drinking Water Treatment (n = 162): Multiple responses were allowed, and percentages were calculated from a total of 212 responses.

The current study revealed a significant positive correlation between arsenic concentrations in drinking water and urine (Spearman's rho = 0.32, $p <$ 0.001) (Table 1). This finding suggests that the probable source of arsenic exposure is contaminated drinking water, and that toxicity was acquired through its ingestion. Although the possibility of exposure through food consumption could not be ruled out, the use of arsenic contaminated water and arsenic-containing compounds in the soil appear to be prevailing factors of arsenic concentrations detected in food in the contaminated area [33]. A previous study also showed that arsenic content in the cooked rice is 10–35% greater than that in raw rice, and thus that cooking processes may influence the amount retained in rice [34]. Myanmar people consume rice as a major component of daily meals, and rice plants accumulate high levels of arsenic [33]. Arsenic content in rice should also be estimated for mitigation in the contaminated areas of Myanmar.

In this study, the urinary 8 – OHdG concentration, a biomarker of oxidative DNA damage, was significantly increased in response to urinary arsenic concentration. Previous studies also reported increased levels of 8 – OHdG in response to arsenic exposure [35–39]. For example, higher urinary 8 – OHdG concentrations were found among Chinese residents in arsenic-affected areas with higher urinary arsenic concentrations [38,39]. Another study also showed that chronic exposure to arsenic significantly increased urinary 8 – OHdG concentrations in a Bangladesh population [37]. This association could be predicted due to the higher urinary arsenic concentrations in the exposed populations (median = 158.3 μg/g creatinine in China, mean = 410 μg/g creatinine in Mongolia, median = 100 μg/g creatinine in Bangladesh) in those previous studies [37,38,40]. Although arsenic exposure levels appeared to be quite low (median = 66.3 μg/g creatinine) in our study population, the effect of arsenic on the 8 – OHdG concentration cannot be neglected. Our finding is supported by previous studies which reported the effects of similar and lower level of arsenic exposure (mean = 45.6 ng/mg creatinine in a Japanese population and mean = 58.3 μg/g creatinine among a Chinese population) in terms of oxidative DNA damage [35,39].

Arsenic is known to be associated with increased production of ROS, such as superoxide, peroxyl and hydroxyl groups, when metabolized in the body [14]. A previous study of Bangladeshi adults also confirmed that arsenic triggers the depletion of glutathione, a critical tripeptide that detoxifies ROS [41]. ROS in living cells are correlated with various pathological conditions including, aging, cancers, chronic inflammation, and neurodegenerative diseases [13]. In accordance, arsenic exposure has been linked with biomarkers of oxidative stress, chronic inflammation, and endothelial dysfunction [42]. Thus, arsenic-induced

(a)



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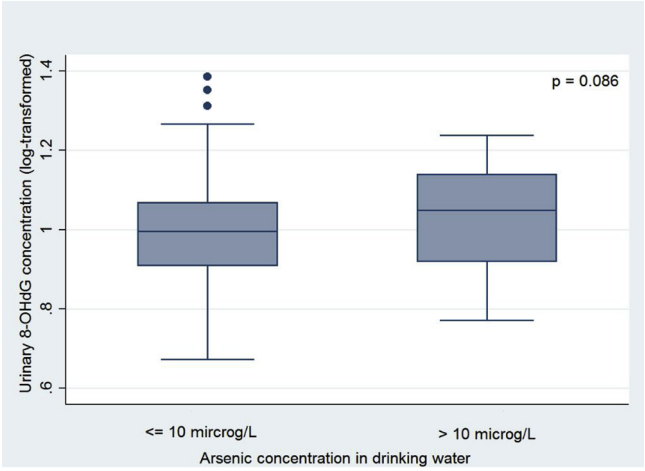

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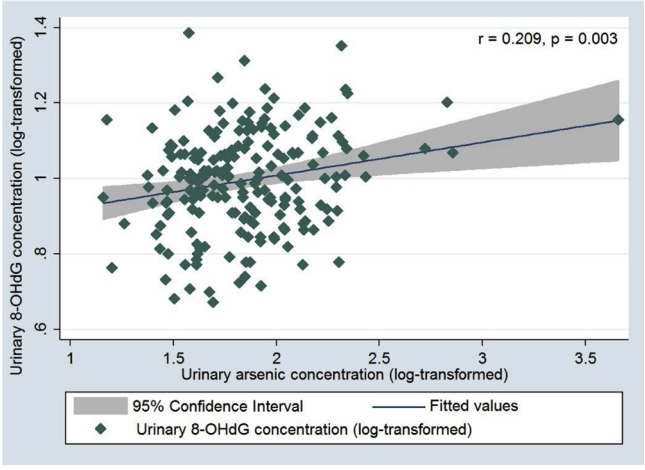

Fig. 3. Correlations between arsenic concentrations in drinking water and urine and urinary 8-hydroxydeoxyguanosine (8−OHdG) concentrations (n = 198): (a) Scatter-plot depicting the association between the arsenic concentrations in drinking water (log-transformed) and urinary 8−OHdG concentration (creatinine-adjusted, log-transformed), (b) a box-plot comparing the urinary 8−OHdG concentration (creatinine-adjusted, log-transformed) and arsenic concentrations in drinking water as categorical variables based on the cut-off point of 10 μg/L, and (c) Scatter-plot depicting the association between the urinary arsenic concentration (creatinine-adjusted, log-transformed) and the urinary 8−OHdG concentration (creatinine-adjusted, log-transformed).

Table 3
Association between urinary arsenic and 8-hydroxydeoxyguanosine (8−OHdG) concentrations by multivariate linear regression (n = 198).

Variables	Coefficient(95% CI)	*p*-value
Urinary As concentration[a]	0.09 (0.03, 0.15)	0.002
Age (years)		
20 and below	ref	
> 20 and ≤ 25	0.01 (−0.04, 0.07)	0.686
> 25 and ≤ 30	0.06 (0.01, 0.12)	0.031
> 30	0.06 (0.01, 0.12)	0.032
Education		
Illiterate	ref	
Able to read and write	0.05 (−0.09, 0.19)	0.485
Primary school completed	0.08 (−0.06, 0.21)	0.267
Middle school completed	0.08 (−0.06, 0.22)	0.262
High school completed	0.04 (−0.10, 0.18)	0.566
Graduate or above	0.13 (−0.06, 0.32)	0.170
Occupation		
Unemployed or housewives	ref	
Farmers	−0.04 (−0.09, 0.00)	0.065
Private sectors	0.07 (−0.11, 0.26)	0.436
Government officers	−1.13 (−0.06, 0.22)	0.092
Own business	−0.01 (−0.10, 0.18)	0.741
Others	0.01 (−0.07, 0.08)	0.885
Primary source of drinking water		
Ground water (well/ hand pump/ motor pump)	ref	
River/lake/ household water supply	0.09 (−0.12, 0.29)	0.404
Others	0.02 (−0.06, 0.10)	0.608
Any treatment before drinking		
No	ref	
Yes	0.03 (−0.03, 0.09)	0.286
Rice cooking method		
Traditional boiling	ref	
Rice cocker	0.01 (−0.05, 0.07)	0.774
Smoking status		
No exposure at all	ref	
Have ever smoked or was exposed to passive smoking	0.02 (−0.02, 0.06)	0.372

[a] log-transformed value; CI: Confidence interval.

ROS production is a possible mechanisms of arsenic-related health outcomes.

Arsenic concentrations in urine and drinking water were significantly and positively correlated; however, no significant linear association was found between arsenic concentrations in drinking water and urinary 8−OHdG concentrations. Although not statistically significant, a trend toward higher urinary 8−OHdG concentrations was observed among the subjects with drinking water arsenic concentration > 10 μg/L. The probable reason is that variations in the amount ingested determine the differences in the body despite having the same drinking water source [40]. A study conducted in Bangladesh also supported differences in the amount ingested by showing that arsenic intake from water differed according to individuals' physical activity levels [43]. Individuals' differences in arsenic metabolism may result in variations in arsenic toxicity and retention in the body [40,44].

In this study, urinary 8−OHdG levels differed significantly among the age groups. Older age groups had higher urinary 8−OHdG concentrations than did the younger age groups. This finding is conceivable, considering the increase in oxidative stress by ROS formation as a major contributor of cellular aging [45]. An increase in intracellular ROS may have an impact at the cellular level, thereafter influencing the regulation of physiological functions with increasing age [45]. Another possible reason could be that older people might have longer duration of exposure to arsenic, resulting in a higher arsenic-induced oxidative stress. Although the association between

tobacco smoking and urinary 8−OHdG has been reported previously [46], we did not find any significant association between tobacco smoking and the urinary 8−OHdG level, likely because our study population contained only few active smokers (2.1%) and passive exposure to tobacco smoke may have a lesser effect on the 8−OHdG concentration.

Some limitations of this study should be considered. First, urinary excretion of 8−OHdG may reflect other active inflammatory and renal functions [21]. To minimize variation in urinary 8−OHdG concentrations, this study included subjects with no diagnosed medical disease, and all samples were adjusted for the urinary creatinine level. Second, an arsenic speciation analysis should be performed, as the toxicity of arsenic species varies, and methylation may influence oxidative damage [39]. However, survey responses revealed no excessive fish or seafood consumption in our study population. Although pregnancy may induce oxidative stress due to increased metabolic activity [47], the effect of pregnancy was not relevant in this study since all the participants were pregnant. In addition, the effects of other environmental factors such as polycyclic aromatic hydrocarbons, particulate matter and other heavy metals should be considered [35,37,39,48].

5. Conclusions

This study showed that higher urinary arsenic concentrations were associated with higher levels of urinary 8−OHdG, reflecting an increased oxidative DNA damage status. Despite having a significant positive association between drinking water and urinary arsenic concentrations, no correlation was found between drinking water arsenic concentrations and urinary 8−OHdG concentrations in this study. Further studies should consider the individual variations in the intake and metabolism of arsenic while tracing the sources of arsenic-induced oxidative stress. Nevertheless, the findings of this study provide evidence that may address the role of arsenic-induced oxidative stress in the pathophysiology of arsenic related diseases, including cancers.

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Conflicts of interest

We have no conflict of interest to declare.

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