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# Interplay of arsenic exposure and cigarette smoking on oxidative DNA damage in healthy males

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## Abstract

**Background** Long-term exposure to inorganic arsenic (As) and cigarette smoking has been associated with adverse health effects such as cancer, cardiovascular disease, and respiratory disease. Oxidative stress is one of the most well-known damage mechanisms for both. However, studies on the association with induced 8-hydroxy-2-deoxyguanosine (8-OHdG) in Iran are scarce, with no study on the interactive effect of As exposure and smoking in the literature.

**Objective** This stratified cross-sectional study aimed to assess urinary and serum 8-OHdG levels in the relation to As exposure from drinking water, smoking and their interaction effect.

**Methods** This study was based on 132 healthy male subjects living in villages of Hashtroud County, Iran (2021). All participants were categorized into four groups: (i) non-As exposed, never smokers ( $n = 33$ ); (ii) non-As exposed, active smokers ( $n = 33$ ); (iii) As exposed, never smokers ( $n = 33$ ); and (iv) As exposed, active smokers ( $n = 33$ ). Data on demographic and lifestyle factors were collected, and urinary and serum levels of 8-OHdG were determined by enzyme-linked immunosorbent assay (ELISA). The smoker's daily cigarette consumption and the duration of smoking were self-reported data.

**Results** Participants consuming drinking water with an As concentration  $> 10 \mu\text{g/L}$  had significantly higher urinary total arsenic (U-tAs) concentrations (median 26.96, IQR 21.35–37.17)  $\mu\text{g/g Cr}$  compared to the reference group (median 19.33, IQR 17.29–23.26)  $\mu\text{g/g Cr}$ . There was a significant difference in the serum concentration of 8-OHdG between groups (i) and (iii), (iv) and also between groups (ii) and (iv). We did not find a significant interaction effect of As exposure and smoking on the both urinary and serum 8-OHdG levels ( $P > 0.05$ ). Serum 8-OHdG (S-8OHdG) was associated with average daily intake of As (As-ADI) ( $\beta = 0.32$ ; 95% CI 0.01, 0.04,  $P$ -value = 0.001) and cigarettes smoked per day ( $\beta = 0.3$ ; 95% CI 0.00, 0.043,  $P$ -value = 0.046). While As-ADI ( $\beta = 0.16$ ; 95% CI 0.001, 0.004,  $P$ -value = 0.01) and U-tAs ( $\beta = 0.6$ ; 95% CI 0.006, 0.009,  $P$ -value  $< 0.001$ ) were associated with increased urinary 8-OHdG (U-8OHdG), moderate physical activity ( $\beta = -0.15$ ; 95% CI  $-0.07$ ,  $-0.008$ ,  $P$ -value  $< 0.015$ ) inversely decreased biomarker levels.

**Conclusion** Our findings suggest that As exposure and smoking are potential risk factors for oxidative DNA damage, and it is strongly recommended to pay more attention to the role of lifestyle factors in future studies.

**Keywords** Arsenic, Biomonitoring, Drinking water, Interaction, Smoking, Urinary creatinine

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## Introduction

Currently, there is a widespread prevalence of environmental toxicant exposure within populations. Environmental pollutants have the ability to cause an array of both acute and chronic health issues, including endocrine disruption, impaired immune system function, neurological disorders, and potentially carcinogenic effects. These pollutants also have impacts on ecosystems [1]. Among the pollutants considered, a noteworthy category is represented by heavy metals, which have raised mounting concerns due to their toxicity. Certain heavy metals/metalloids, such as chromium (Cr), arsenic (As), cadmium (Cd), and lead (Pb), are classified as priority pollutants due to their pervasive occurrence in the environment, prevalent human exposure, and pronounced toxicity even at low concentrations [2].

Arsenic, a ubiquitous metalloid, is extensively distributed in the environment via natural and anthropogenic sources. Chronic exposure to arsenic, primarily from consumption of contaminated drinking water and certain food sources, has been linked to a spectrum of health conditions such as skin diseases, respiratory disorders, peripheral vascular disorders, diabetes mellitus, and diverse cancers [3, 4]. The prevalence of high arsenic concentrations in groundwater potentially endanger 94 to 220 million people worldwide [5]. The ramifications of arsenic exposure exhibit considerable variation and depend on factors such as concentration, speciation, duration of exposure, and individual attributes [6]. Furthermore, the complex pathogenesis of arsenic-induced toxicity is intertwined with multiple determinants, among which oxidative stress stands out as a well recognized and extensively studied mechanism [7]. The oxidative stress stems from an excessive production of reactive oxygen species (ROS), during which the elevated ROS production overwhelms the counteracting antioxidant mechanism [8]. Arsenic contributes to the generation of ROS while methylation is in progress and promotes diverse forms of DNA damage that culminate in cell apoptosis or DNA mutations [9, 10]. The study of oxidative DNA damage holds paramount importance due to the association between DNA impairments caused by oxidative stress and the development of various health issues, such as cancer, cardiovascular diseases, neurodegenerative disorders and aging [11–13]. Moreover, understanding the fundamental mechanisms can provide insights into cellular reactions to oxidative stress, DNA repair pathways and, in particular, the development of preventive and therapeutic approaches targeted at combating diseases linked to oxidative stress. An outcome of oxidative DNA damage is the formation of 8-OHdG, which serves as a reliable biomarker for quantifying the extent of such damage in the whole body through its urinary levels [14].

Chung et al. [15] have reported heightened U-8OHdG concentrations even at low levels of urinary arsenic exposure. Notably, biomonitoring studies that use spot urine samples should employ normalization techniques to minimize the effect of differences in the hydration status of participants on urinary biomarker concentrations [16]. Though there is no consensus on the most robust method [17], a review of previous studies indicates that the most common correction measure for U-As and U-8OHdG is creatinine adjustment [18–22].

Acknowledging the undeniable influence of genetic factors and modifiable lifestyle habits on the oxidative stress response is essential [23]. Smoking, for instance, stands as significant factor in promoting oxidative stress, increasing the risk of DNA damage and mutations in genes associated with tumorigenesis [24]. In addition to carcinogenesis, there is solid evidence indicating that smoking is linked to various cardiovascular, pulmonary and, neurological diseases [25]. While several studies have highlighted elevated levels of 8-OHdG in smokers [26–29], some have reported inconclusive findings [30–32]. Discrepancies can be attributed to variations in toxic and ROS-inducing compounds within cigarette brands, the daily consumption of cigarettes, the duration of smoking, and the sample size of active smokers in the studies.

Notably, the available literature does not definitively determine whether the effect of arsenic exposure on 8-OHdG levels differs between smokers and non-smokers. Specifically, investigations on the interplay between arsenic exposure and smoking in accentuating oxidative stress remain largely unknown.

In Iran, instances of arsenic contamination of groundwater resources have manifested in various regions, particularly in the western, northwestern, southeastern, and northeastern areas [33]. Hashtroud County, situated near the Sahand volcanic mountains in northwestern Iran, has exhibited anomalies in water resources attributed to geogenic origins [34, 35]. Dastgiri et al. [36] found elevated blood pressure and a higher prevalence of skin lesions in residents of one of the arsenic-affected villages compared to the control village of the county.

In relation to the aforementioned, the main objective of this study was to explore the potential interaction effect resulting from the combined exposure to arsenic through drinking water and smoking on oxidative DNA damage. The deleterious effects of arsenic also target the blood among other organs, however the number of studies investigating the elevation of 8-OHdG in serum or plasma in response to arsenic exposure, especially in the healthy population, is much more limited, so the concentration of this biomarker was investigated in both serum and urine specimens

in relation to As exposure and smoking. Furthermore, the study sought to analyze the relationship between some lifestyle factors and fluctuations in 8-OHdG concentrations.

### Materials and methods

#### Study area

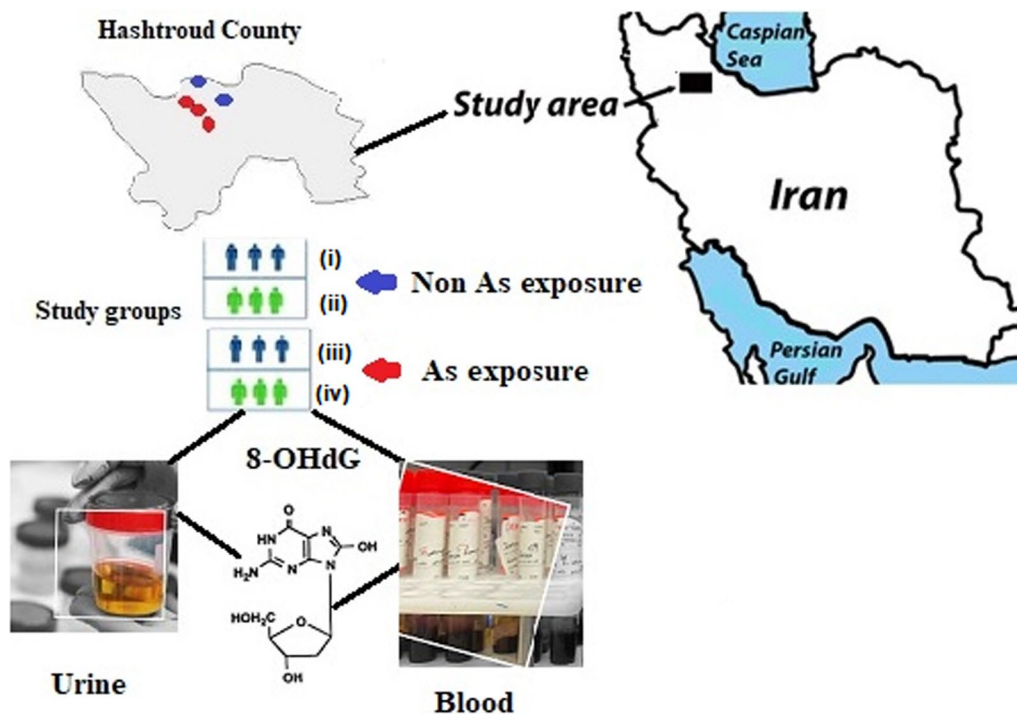
The present study was conducted in some villages of Hashtroud County, East Azerbaijan Province, north-west of Iran, with an area of 1990 km<sup>2</sup> and located at 37° 39' N and 47° 19' E (Fig. 1). According to the last census in Iran (2016), the county's population was over 57,000, with 62% of those living in rural areas and the others in urban areas. Based on the Air Quality Index (AQI), air pollution in the villages of the study area is in most cases lower than the known level that has adverse effects on health (AQI < 100) [37]. The AQI is a well-known index that ranges between 0 and 500, whereby values below 100 are generally considered satisfactory [38].

Groundwater is the main source of drinking water in the study area, and Sheikhi et al. [39] recently reported naturally occurring arsenic contamination in 40% of drinking water samples from Hashtroud's rural communities at concentrations well above the WHO guidelines' maximum permissible value (10 µg/L).

#### Study population

The stratified cross-sectional study was based on 132 healthy male subjects who were invited through the village health worker and recruited if they met the inclusion criteria and signed the consent form to participate. The study was conducted in September 2021, and our inclusion criteria were an age between 18 and 65 years, exclusively drinking water from village groundwater resources for the last 5 years or longer, and did not have any known chronic and infectious diseases. Subjects were excluded from the study if they reported living in the village for less than 5 years, using a water source other than the village drinking water source for drinking, suffering from chronic diseases such as cancer, cardiovascular diseases, hypertension, diabetes, respiratory diseases (chronic obstructive pulmonary disease and asthma), kidney and liver diseases, infectious diseases such as hepatitis, mental-psychological diseases, and subjects currently receiving medical treatment.

The identification of eligible individuals was conducted by considering the study inclusion and exclusion criteria, with the assistance of healthcare personnel of villages and by utilizing electronic health records. Subsequently, the pertinent list was prepared. Individuals were contacted, an explanation was provided regarding the significance of the research and the procedure for involvement, then the volunteers were invited to take part in the study.



**Fig. 1** Study area and sampling strategy

The participants had similar characteristics in terms of ethnicity, culture and socioeconomic status and were divided into four groups (33 subjects in each group) based on their exposure to As via drinking water and their smoking status. Since we know that oxidative damage can be caused by various other factors, and it is very difficult to control for all of them, we attempted to partially control for some of these confounding factors by including subjects in the study who had similar occupations, work conditions, and dietary habits, with the main difference being the exposure conditions mentioned. In fact, we tried the exposure to other stressors was almost similar in all groups. The subjects in groups (i) and (ii) lived in rural communities with a drinking water arsenic concentrations less than 10 µg/L. They were divided into two categories based on their smoking habits: those who had never smoked (group i) and those who were active smokers (group ii). The following two groups were subjected to drinking water containing arsenic levels exceeding 10 µg/L and comprised the never smokers (group iii) and the active smokers (group iv).

The involvement of the Behvarz was sought to confirm that the never smokers were indeed not passive smokers, since none of their family members smoked. Smoking is an uncommon behavior among females in the study area, and no women reported being smokers. Therefore, our study includes only males.

All participants who were recruited for study demonstrated their agreement by providing written informed consent prior to their involvement. The study protocol and informed consent were approved by the Academic Committee for Research Ethics (Human Studies) of Tabriz University of Medical Sciences (Approval Number: IR.TBZMED.REC.1399.708).

#### Demographic and lifestyle data collection

Data related to demographic characteristics and some lifestyle factors, including age, level of education, source of drinking water, daily water consumption rate, number of cigarettes smoked per day and smoking duration were collected through a face-to-face interview. In addition, the Persian version of International Physical Activity Questionnaire (IPAQ) was used to estimate habitual practice of physical activities of participants [40]. To measure the total physical activity, the Metabolic Equivalent Task (MET-min/week) was calculated by summing the adjusted durations (minutes × days) for each type of activity using the following values: walking = 3.3 METs, moderate activity = 4.0 METs, and vigorous activity = 8.0 METs. Then, based on the final scores, the total physical activity was divided into three categories: low, moderate

and, high. Participants who reported a total activity time > 960 min per day were considered as outlier data and excluded from the study [41].

#### Biological samples

Subsequently, biometric data were recorded, and the "clean catch" of urine samples after the removal of the first few ml of the first morning urine were collected in 50 ml disposable polyethylene bottles between 9:00 and 10:00 am. Participants were also asked to fast for at least 8 h overnight before blood sampling. About 2.5 ml peripheral venous blood samples were obtained by venipuncture with a syringe and serum separator tube on the same morning. The samples were centrifuged at 3500 rpm for 10 min, then the serum was aspirated.

Biological samples were immediately transported in a portable cooler with cooling packs to the laboratory for further analysis. In the laboratory, a 5-ml aliquot was separated from each urine sample to measure urinary creatinine concentration within 24 h. The residue was divided into aliquots and then stored with the serum samples at -70 °C until biomarker analysis.

#### Drinking water sampling and As determination in water and urine

A total of 21 drinking water samples (at least two or three samples from each well and spring in each community serving the source population) were collected in acid-washed plastic containers for further analysis.

In the laboratory, all glassware was washed with dilute HNO<sub>3</sub> and rinsed with ultrapure water. Total As content in drinking water and urine samples was analyzed by the hydride generation method with an atomic absorption spectrometer (Perkin-Elmer AAnalyst 600) coupled to the hydride generation system (Perkin-Elmer FIAS 400).

For urine samples, a microwave digestion system (Multiwave GO, Anton-Paar, Graz, Austria) was used to digest 5 ml sample with 5 ml HNO<sub>3</sub> and 2 ml H<sub>2</sub>O<sub>2</sub> for 10 min at 100°C. After digestion, the samples were adjusted to a final volume of 25 ml with ultrapure water.

For quality control, blanks, duplicates, and spiked pooled urine samples were analyzed to confirm the accuracy of the analysis. Spike recovery values ranged from 81 to 118%. The LOD was 0.1 µg/L and the LOQ averaged 1 µg/L.

Urinary creatinine (U-Cr) was determined by a routine clinical method based on the Jaffe reaction for all urine samples according to the method by Butler [42]. The obtained concentrations of As were normalized by U-Cr concentrations.



### Determination of As-ADI

The average daily intake of As through drinking water ingestion was calculated using the following equation [43]:

$$\text{ADI} = \frac{\text{DWAs} \times \text{IR}}{\text{BW}}, \quad (1)$$

where ADI is expressed as average daily intake of As (mg/kg/day), DWAs is drinking water arsenic concentration (mg/L), IR is daily ingestion rate (L/day) (self-reported by participant), and BW is body weight (kg) (measured during study).

### Determination of urinary and serum 8-OHdG levels

The 8-OHdG content in urine and serum samples was determined using an ELISA kit (ZellBio GmbH, Germany; kit cat. no. ZB-11436C-H9648). The kit uses an enzyme-linked immunosorbent assay based on the double antibody sandwich technique [44]. The ELISA kit is influenced by macromolecular components such as protein. Therefore, pretreatment with Amicon Ultra centrifugal filters (Fisher Scientific) was performed prior to serum 8-OHdG measurements.

According to the manufacturer's protocol, the standards, test samples, 8-OHdG antibody, and streptavidin–horseradish peroxidase (HRP) conjugate reagent were added to the wells, mixed, and incubated for 60 min. Then, the unbound conjugates were washed five times with a wash buffer. The chromogen solution was used to produce a blue color, which changed to a yellow color after the acidic stop solution was added. The density of the yellow color and the concentration of 8-OHdG are positively correlated. Finally, the optical density (O.D.) of each well was measured within 10 min using a microplate reader set to 450 nm, and then the concentration was calculated by comparison with a standard curve. Each sample was analyzed in duplicate.

The sensitivity of the method was 0.5 ng/mL, and the procedure had an intra- and inter-assay coefficient of variation of 8.3 and 10.9%, respectively.

Urinary 8-OHdG concentrations were adjusted for U-Cr values. No adjustment for hydration status is required for serum concentrations because blood dilution is relatively stable [45].

### Statistical analysis

Data were expressed as mean  $\pm$  standard deviation (SD) when normally distributed, whereas non-normal data were expressed as the median and interquartile range (IQR): 1st and 3rd quartiles (Q1–Q3). The normality test was performed using Kolmogorov–Smirnov test.

The Levene test was employed to assess the homogeneity of variances.

Comparison between study groups in age, water consumption, As-ADI, U-Cr, and Cr-adjusted concentrations of U-tAs and U-8OHdG was performed using the Kruskal–Wallis test. If there was a statistically significant difference between the medians of four independent groups, we conducted a post hoc test, Dunn's test, to determine exactly which groups are different.

In addition, weight, BMI (body mass index), and S-8OHdG were compared between groups using a one-way ANOVA. The comparison of mean differences between the four groups was then carried out using Fisher's LSD post hoc analysis. Categorical data were analyzed using Pearson's Chi-square test.

Using Spearman's rank correlation, degrees of associations between U-tAs and U-8OHdG, U-tAs and S-8OHdG and U-8OHdG and S-8OHdG were investigated.

General linear model (GLM) was applied to explore the interactive effect of As exposure and cigarette smoking on the urinary and serum 8-OHdG levels.

The multiple linear regression (MLR) model was applied to investigate the association between 8-OHdG concentrations and As-ADI, U-tAs, smoking duration, number of cigarettes smoked per day and physical activity. The model for U-8OHdG was run with log-transformed values, and both models (U-8OHdG, S-8OHdG) were adjusted for age and BMI as covariates. The regression coefficients and 95% confidence intervals (CIs) for each independent variable were determined.

All analyses were performed with SPSS version 21 (IBM software) and GraphPad Prism, version 9 (GraphPad Software, La Jolla, California).

## Results

### Characteristics of the study participants

The information on the participant's characteristics and As exposure status of the study groups are presented in Table 1. The median age of the study participants was 46 years and the mean BMI was  $26.33 \pm 3.64$  kg/m<sup>2</sup>. More than 85% had at least a primary school education level. In addition, all respondents reported never drinking alcohol, and most of them worked as farmers (about 90%). Smoker participants reported an average of 14.06 cigarettes per day and smoked for an average of 20.35 (2–40) years. 48.5% had moderate and vigorous physical activity for 150 min or more per week. No statistically significant differences were between the four groups in terms of age, weight, BMI, water consumption and physical activity ( $P > 0.05$ ).

**Table 1** Descriptive information on general characteristics of study subjects, and As exposure

Parameters	Overall (n = 132)	Group (i) (n = 33)	Group (ii) (n = 33)	Group (iii) (n = 33)	Group (iv) (n = 33)	P-values
Age (years) Median (IQR)	46 (35.25–55)	39 (28.5–55)	52 (44–57)	42 (34.5–59.5)	43 (36.5–52)	0.06 <sup>a</sup>
Weight (kg) Mean ± SD	76.6 ± 12.23	74.5 ± 10.4	76 ± 13.56	78.8 ± 9.86	77 ± 14.58	0.54 <sup>a</sup>
BMI (kg/m <sup>2</sup> ) Mean ± SD	26.3 ± 3.64	25.5 ± 3.6	26.3 ± 3.8	27.2 ± 3.35	26.3 ± 3.8	0.31 <sup>b</sup>
Education, n (%)						
Illiterate	18 (13.6)	4 (12.1)	8 (24.2)	5 (15)	1 (3)	<b>0.03<sup>c,e</sup></b>
Primary school	49 (37.1)	12 (36.3)	14 (42.2)	8 (24.2)	15 (45.4)	
Secondary school	41 (31)	10 (30.3)	8 (24.2)	8 (24.2)	15 (45.4)	
Diploma	18 (13.6)	5 (15)	2 (6)	10 (30.3)	1 (3)	
Bachelor or higher	6 (4.5)	2 (6)	1 (3)	2 (6)	1 (3)	
Physical activity, n (%)						0.88 <sup>c</sup>
Low	64 (48.5)	13 (39.4)	17 (51.5)	17 (51.5)	17 (51.5)	
Moderate	52 (39.4)	16 (48.5)	13 (39.4)	12 (36.3)	11 (33.3)	
High	16 (12.1)	4 (12.1)	3 (9)	4 (12.1)	5 (15.1)	
Smoking duration (year) Mean ± SD	–	–	20.63 ± 9.4	–	20.06 ± 7.46	0.78 <sup>d</sup>
Cigarettes smoked per day Mean ± SD	–	–	13.09 ± 6.6	–	15.03 ± 6.28	0.18 <sup>d</sup>
Water consumption (L/day) Median	1.5	1.3	1.8	1.4	1.6	0.17 <sup>a</sup>
Arsenic intake ADI (mg/kg/day) Median (IQR)	5.7 × 10 <sup>-4</sup> (9 × 10 <sup>-5</sup> –1.5 × 10 <sup>-3</sup> )	8 × 10 <sup>-5</sup> (4.8 × 10 <sup>-5</sup> –1 × 10 <sup>-4</sup> )	1.2 × 10 <sup>-4</sup> (4.7 × 10 <sup>-5</sup> –2.5 × 10 <sup>-4</sup> )	1.1 × 10 <sup>-3</sup> (8.1 × 10 <sup>-4</sup> –1.8 × 10 <sup>-3</sup> )	1.5 × 10 <sup>-3</sup> (1.2 × 10 <sup>-3</sup> –1.9 × 10 <sup>-3</sup> )	<b>0.001<sup>a,e</sup></b>

<sup>a</sup> Comparison between the groups was performed by Kruskal–Wallis tests

<sup>b</sup> Mean values were compared between the groups using ANOVA tests

<sup>c</sup>  $\chi^2$  test

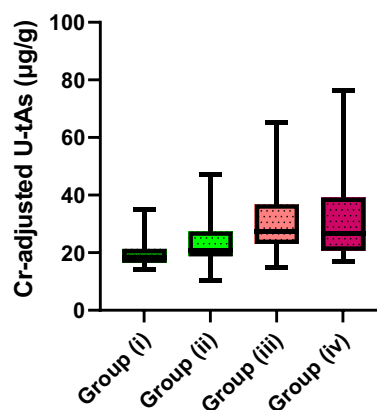
<sup>d</sup> Comparison between two groups was performed by Mann–Whitney test

<sup>e</sup> Statistically significant

**Drinking water and urinary As concentrations**

Arsenic levels in drinking water ranged from 1.1 to 155 µg/L, and the mean concentration in drinking water samples from villages with As contamination (groups (iii) and (iv)) was about 20 times higher than the other villages (groups (i) and (ii)). The participants in groups (i) to (iv) had an average arsenic concentration in their drinking water of 3.9 ± 0.23, 2.5 ± 0.2, 70.2 ± 5.3, and 66.8 ± 6.6 µg/L, respectively. According to Table 1, no statistically significant difference in daily water consumption was found among the study groups, while groups (iii) and (iv) had significantly higher As-ADI compared to groups (i) and (ii) (P < 0.05), which is attributed to the higher As concentrations in their drinking water.

The median concentration of urinary As for overall subjects was 22.63 µg/g Cr and ranged from 10.46 to 76.25 µg/g Cr. The Kruskal–Wallis test followed by Dunn’s post-test revealed a significant difference in U-tAs concentration between group (i) and both groups (iii) and (iv) (P < 0.05), which was also the case for group



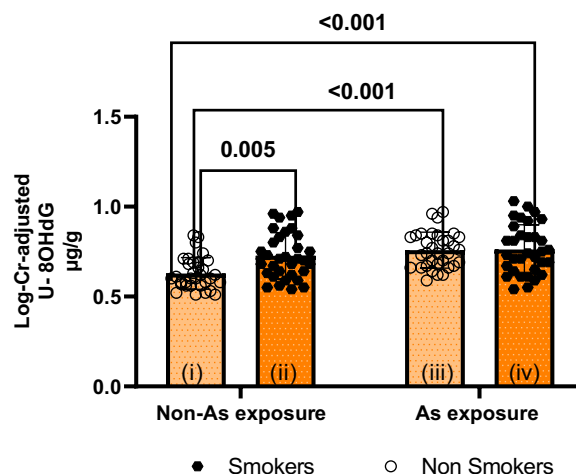
**Fig. 2** U-tAs concentrations among study groups

(ii) (Fig. 2). There was no significant difference between smokers and non-smokers within the arsenic-exposed and non-exposed individuals.

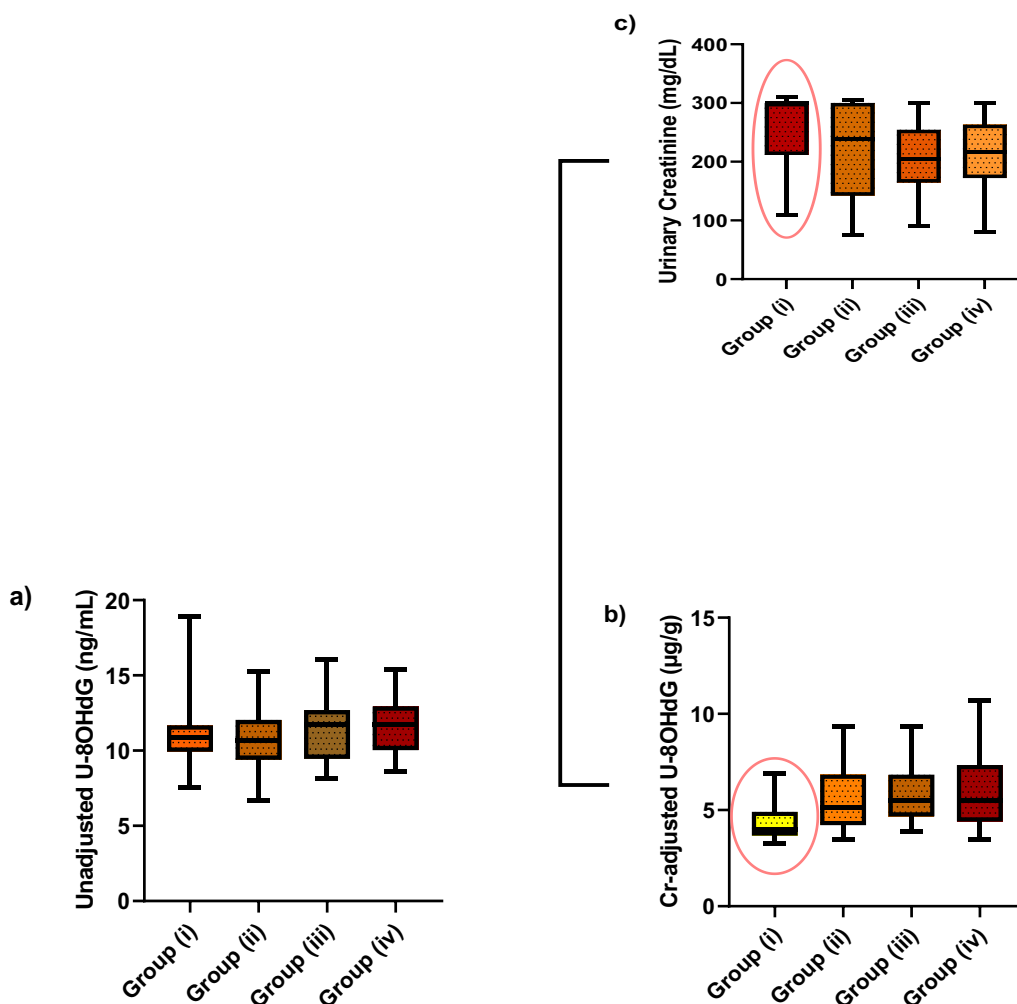
### Urinary 8-OHdG concentrations

The 8-OHdG concentrations in the urine samples of the participants from the groups with As exposure ((iii) and (iv)) (median=4.52, 1thQ-3thQ=3.8-5.53 µg/g Cr) were significantly higher than in the other groups (median=5.5, 1thQ-3thQ=4.65-6.92 µg/g Cr) ( $P < 0.001$ ).

It is noteworthy that the Kruskal–Wallis test showed no significant difference between the four study groups before the creatinine adjustment. However, after adjustment, the significant difference was observed only between group (i) and all other groups. Because group (i) had a significantly higher urinary creatinine concentration, which decreased the adjusted concentration of U-8OHdG compared to others (Fig. 3a–c). Such cases may lead to a misleading interpretation. Figure 4 illustrates a comparison of Cr-adjusted U-8OHdG levels



**Fig. 4** Comparison of Cr-adjusted U-8OHdG concentrations in different scenarios of arsenic exposure and smoking



**Fig. 3** Urinary 8-OHdG levels in subjects from the study groups. **a** Unadjusted concentrations (ng/mL); **b** creatinine-adjusted concentrations (µg/g); and **c** urinary creatinine concentrations by study groups (mg/dL)

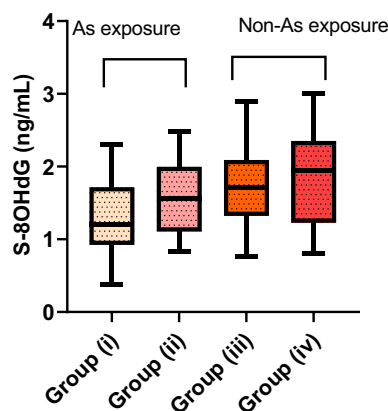
across different circumstances of arsenic exposure and smoking.

The strong, significant correlation was established between urinary arsenic and 8-OHdG concentrations (Spearman's rho=0.7;  $p < 0.001$ ) (Fig. 5a).

**Serum 8-OHdG levels and correlation with U-8-OHdG**

We investigated the sensitivity of serum 8-OHdG to detect an increase in oxidative damage induced by arsenic exposure and/or cigarette smoking. The mean concentrations of the biomarker in serum specimens of subjects in groups (iii) and (iv) were  $1.71 \pm 0.57$  and  $1.91 \pm 0.66$  ng/mL, respectively. For groups (i) and (ii), the mean (SD) of S-8-OHdG levels were 1.34 (0.51) and 1.58 (0.48) ng/mL, respectively (Fig. 6). A comparison between the study groups using one-way ANOVA and Fisher's LSD post hoc analysis revealed significantly higher S-8OHdG concentrations in both groups (iv) and (iii) compared to group (i) ( $P < 0.05$ ), as is evident in Fig. 7. In addition, a significant difference was also found between group (iv) and group (ii).

Regarding the correlation of 8-OHdG concentrations between serum and urine, we found a positive and relatively weak relationship ( $r = 0.43$ ,  $p < 0.001$ ) (Fig. 5b). There was another weak significant correlation between

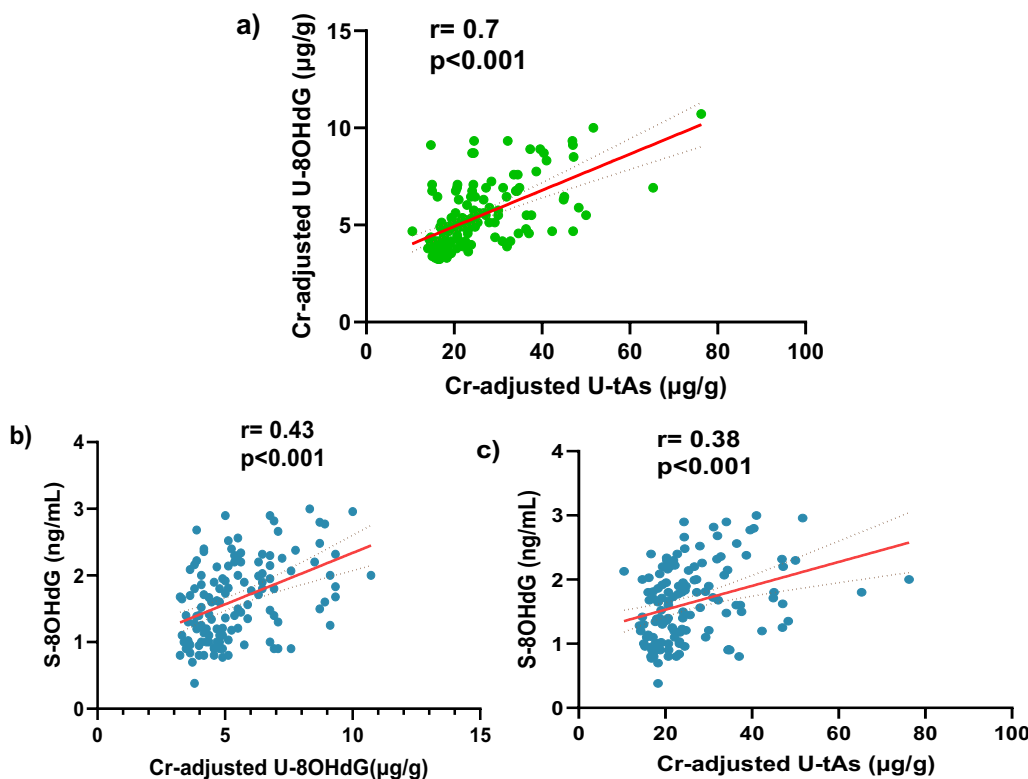


**Fig. 6** Serum 8-OHdG levels in subjects from the study groups

U-tAs and S-8OHdG concentrations as shown in Fig. 5(c) ( $r = 0.38$ ,  $p < 0.001$ ).

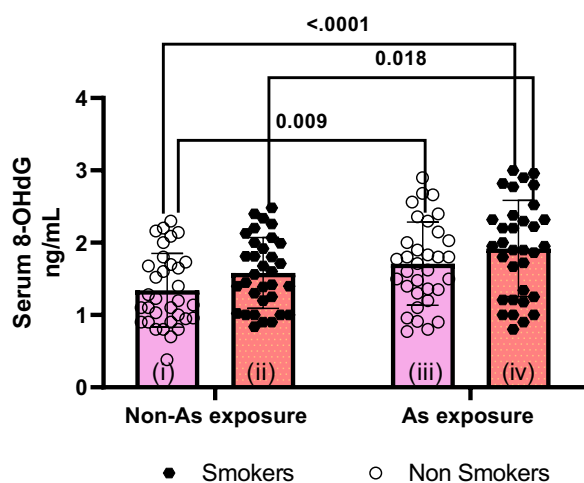
**Interaction or independent effect of arsenic exposure and cigarette smoking on 8-OHdG**

The results of the GML analysis on urinary and serum levels of the effect biomarker of DNA damage are summarized in Table 2. In both cases, arsenic exposure



**Fig. 5** The results of Spearman correlation analysis for statistically significant correlations ( $p < 0.05$ ). **a** U-8OHdG and U-tAs, **b** S-8OHdG and U-8OHdG, **c** S-8OHdG and U-tAs  $r$ —correlation coefficient,  $p$ —the probability level





**Fig. 7** Comparison of S-8OHdG concentrations in different scenarios of arsenic exposure and smoking

( $F_{(U-8OHdG)} = 19.37, P_{(U-8OHdG)} < 0.001$ ), ( $F_{(S-8OHdG)} = 13.22, P_{(S-8OHdG)} < 0.001$ ) and smoking ( $F_{(U-8OHdG)} = 4.13, P_{(U-8OHdG)} = 0.044$ ), ( $F_{(S-8OHdG)} = 3.97, P_{(S-8OHdG)} = 0.048$ ) were independently significant predictors of 8-OHdG, but the interaction of the joint exposure was not significant ( $P > 0.05$ ). The F value for the covariate age was significant, indicating that age is a significant predictor of U-8OHdG.

**Multiple linear regression model**

Table 3 presents the results of the MLR analysis to explore the association between 8-OHdG and independent variables affecting its urinary and serum levels. Both urine and serum concentrations were significantly associated with As-ADI ( $\beta_{(U-8OHdG)} = 0.16, P_{(U-8OHdG)} = 0.01$ ), and ( $\beta_{(S-8OHdG)} = 0.32, P_{(S-8OHdG)} = 0.001$ ). While U-8OHdG level was negatively associated with moderate physical activity ( $\beta_{(U-8OHdG)} = -0.15, P_{(U-8OHdG)} = 0.015$ ), significant positive associations were found with U-tAs ( $\beta_{(U-8OHdG)} = 0.6, P_{(U-8OHdG)} < 0.001$ ). The number of cigarettes smoked per day was significantly associated with higher levels of S-8-OHdG ( $\beta_{(S-8OHdG)} = 0.3, P_{(S-8OHdG)} = 0.046$ ).

**Discussion**

Several studies have been conducted to evaluate the effect of arsenic exposure through drinking water on the status of oxidative stress. However, the number of studies that simultaneously investigate the influence of individual and lifestyle factors is limited. On the other hand, the interaction effect of one of the most significant lifestyle factors, smoking, with arsenic exposure on oxidative stress has not been determined. In this study, these cases were investigated among a rural male population living in the northwest of Iran.

To estimate the internal As doses, we measured U-tAs in the study groups. Note, it is well known that the excretion of organic As in urine is generally associated with

**Table 2** GLM results for the effect of arsenic exposure and cigarette smoking and their interaction on urinary and serum levels of 8-OHdG biomarker

GLM	df	Mean square	F	P-values
<b>Log-Cr-adjusted-U8OHdG-arsenic exposure*cigarette smoking</b>				
Arsenic exposure	1	0.23	19.37	< 0.001 <sup>a</sup>
Cigarette smoking	1	0.05	4.13	0.044 <sup>a</sup>
Arsenic exposure*cigarette smoking	1	0.03	2.9	0.09
Age	1	0.17	14.15	< 0.001 <sup>a</sup>
BMI	1	0.01	0.85	0.36
R-squared = 0.26 (adjusted R-squared = 0.24)				
<b>S-8OHdG-arsenic exposure*cigarette smoking</b>				
Arsenic exposure	1	4.15	13.22	< 0.001 <sup>a</sup>
Cigarette smoking	1	1.25	3.97	0.048 <sup>a</sup>
Arsenic exposure*cigarette smoking	1	0.01	0.04	0.85
Age	1	1.1	3.51	0.06
BMI	1	0.006	0.02	0.89
R-squared = 0.15 (adjusted R-squared = 0.11)				

Covariates: age, and BMI

<sup>a</sup> Statistically significant

**Table 3** Multiple regression analysis of the association between a) U-8OHdG, and b) S-8OHdG concentrations, and some selected independent factors

Variables	Coefficient ( $\beta$ )	95% confidence interval	P-values
<b>a) Log-Cr-adjusted-U8OHdG (<math>\mu\text{g/g Cr}</math>)</b>			
As-ADI (mg/kg/day)	0.16	0.001, 0.004	0.01 <sup>a</sup>
Smoking duration (years)	0.27	-0.002, 0.002	0.8
Cigarettes smoked per day	0.14	-0.001, 0.005	0.15
U-tAs ( $\mu\text{g/g Cr}$ )	0.6	0.006, 0.009	< 0.001 <sup>a</sup>
Physical activity			
Low	Ref		
Moderate	-0.15	-0.07, -0.008	0.015 <sup>a</sup>
High	0.02	-0.034, 0.05	0.7
<b>b) S-8OHdG (ng/mL)</b>			
As-ADI (mg/kg/day)	0.32	0.01, 0.037	0.001 <sup>a</sup>
Smoking duration (years)	0.3	-0.014, 0.017	0.85
Cigarettes smoked per day	0.3	0.000, 0.043	0.046 <sup>a</sup>
U-tAs ( $\mu\text{g/g Cr}$ )	0.08	-0.006, 0.015	0.37
Physical activity			
Low	Ref		
Moderate	-0.02	-0.21, 0.25	0.84
High	0.07	-0.17, 0.44	0.4

The models were adjusted for age and BMI, <sup>a</sup> Statistically significant

the consumption of seafood (e.g., fishes, crustaceans, bivalves, seaweeds) [46], this is while fish and seafood are not staple food items among Iranian population as the annual per capita fish consumption (about 9.2 kg/year) is well below the world average [47], and also subjects recruited in this study, reported no fish and seafood consumption in the week preceding biospecimens collection and before. Therefore, it can be said that the measured U-tAs can be attributed to the sum of arsenic metabolites (iAs + MMA + DMA). Our measured concentrations were lower compared to other studies conducted in regions with high As drinking water exposure, e.g., Bangladesh (1730.39  $\mu\text{g/g Cr}$ ) [48], Peru (Cairani district: 601.61  $\mu\text{g/g Cr}$ , Camilaca district: 30.24  $\mu\text{g/g Cr}$ ) [49], Shanxi China (124.93  $\mu\text{g/g Cr}$ ) [50], and Cambodia (86.77  $\mu\text{g/g Cr}$ ) [51].

We found that despite significantly higher U-tAs concentrations in subjects exposed to arsenic concentrations > 10  $\mu\text{g/L}$  via drinking water, internal As doses were not negligible in non-exposed subjects. Because urinary arsenic is a valid biomarker for integrating exposure from multiple sources [52], it is reasonable to speculate that subjects in villages with low arsenic levels in drinking water may also have been exposed, likely due to dietary intake, direct soil ingestion from cultivated agricultural products, and dust inhalation.

The levels of urinary 8-OHdG in individuals who were subjected to arsenic exposure were found to be

significantly increased when compared to those with low or no exposure, exhibiting a median value of 5.51  $\mu\text{g/g Cr}$  in contrast to 4.42  $\mu\text{g/g Cr}$ .

A comparison between the four groups showed that Cr-adjusted concentrations were significantly different between group (i) and the other groups, which could lead to misinterpretation. It may seem that the non-exposure to arsenic and/or non-smoking in group (i), necessarily caused such a significant difference. However, since creatinine-adjusted values are calculated by dividing the unadjusted concentrations (micrograms of 8-OHdG per liter of urine) by the creatinine concentrations (grams of creatinine per liter of urine), the higher U-Cr value in group (i) has led to such a difference. Creatinine is the breakdown product of creatine, which is formed in muscle tissue, released into the bloodstream, and excreted in the urine via glomerular filtration of the kidneys [53]. Owing to concerns about variability in U-Cr values as a function of age, sex, race/ethnicity, and body size, it is recommended that the creatinine-adjusted concentration of an analyte for an individual be compared with a reference value obtained on a similar demographic group (e.g., adults with adults, children with children) [16]. In our study, participants lived in similar villages of the same county, and there were no significant differences in their demographic characteristics. Several reasons can be envisioned for this, including the possibility that individuals in group (i) may have more muscle mass or lean

body mass, a gradual decline in U-Cr excretion with age [54] that may have occurred in group (ii), and the possible association between arsenic exposure and U-Cr concentrations (low U-Cr levels in groups ((iii), (iv)), as some studies have reported an association between As metabolism and the relative proportion of di-methyl arsenical species (DMAs) in urine with U-Cr [55–58]. Given cross-sectional analysis, our ability to draw a causal inference is limited, and further studies are warranted.

As far as the 8-OHdG concentrations in serum was concerned, the comparison of the four study groups showed a significantly higher mean concentration in groups (iv) and (iii) compared to group (i). A significant difference was also found between group (iv) and group (ii). Therefore, it can be said that serum or plasma concentrations of 8-OHdG, when individuals are randomly assigned to different groups based on exposure conditions, may well reveal differences in DNA damage between exposure groups. In general, similar studies on healthy subjects are rare. For example, a birth cohort study conducted in Vietnam reported increased 8-OHdG levels in cord blood serum associated with maternal arsenic exposure [59]. In another study, the mean plasma 8-OHdG concentration was found to be significantly higher in chronically low As-exposed women (As, 11–50 µg/L) than in the control group (As < 10 µg/L), (8-OHdG: 122.58 ± 73.53 vs 33.13 ± 12.10 ng/mL) in West Bengal [60].

Although the underlying mechanisms by which arsenic causes such oxidative damage are not yet fully understood, possible mechanisms could include inhibition of antioxidant enzymes such as superoxide dismutase (SOD) and catalase, interface with cellular antioxidant defense systems by reducing levels of intracellular antioxidants such as glutathione, inhibition of DNA repair mechanisms, and alterations in epigenetic processes [7]. The results of the study exhibited that arsenic has the ability to not only cause DNA damage, but also impede the function of a key protein involved in DNA repair. This effect which is observed even at low As concentrations, can further intensify the overall oxidative DNA damage caused by arsenic itself and possibly by other oxidants as well [61]. As in our study, S-8OHdG levels were higher in arsenic-exposed smokers than in unexposed smokers.

According to GLM, the main effects of As exposure and smoking on both U-8OHdG and S-8OHdG concentrations were statistically significant. However, no interaction was found between these factors, suggesting that the effect of As exposure on 8-OHdG levels in smokers was not significantly different from that observed in non-smokers. This may potentially be attributed to the limited sample size of As-exposed subjects in our study, which consequently resulted in insufficient statistical power for the interaction analysis. In

addition, it is possible that the biological mechanisms underlying the interaction between arsenic and smoking are complex and multifactorial, making them challenging to address in a single study.

A significant association between As-ADI and 8-OHdG levels in both urine and serum was found by adjusted MLR analysis. The ADI is an As exposure estimation factor that takes into account both DWAs concentration and water ingestion rate and has been largely overlooked in studies on arsenic-induced oxidative stress, although it may have a strong relationship with oxidative damage. It is particularly important in hot climates where daily consumption of drinking water is high, even when arsenic concentration in water is low. It is therefore recommended to take this into account in similar studies. In addition, urinary arsenic was associated with the increased U-8OHdG. This finding is in a good accordance with other studies that have found a similar association [32, 62–64], and more recently Wang et al. [65] reported a 41.5% increase in 8-OHdG levels for every 10 µg/g Cr increase in urinary arsenic concentration in a meta-analysis study. Regarding the association between 8-OHdG concentrations and physical activity in healthy individuals, previous studies have shown discordant results [66–68]. It can be explained by the hormesis theory, so that while regular and moderate exercise produces beneficial adaptive effects against oxidative stress, vigorous exercise may lead to increased production of free radicals [69], and in our study, moderate physical activity showed a significant inverse correlation with U-8OHdG levels.

We found that increase in serum 8-OHdG concentrations depended not only on the As-ADI but also on the number of cigarettes smoked per day. Although in this case as well, the results of some studies did not show a significant correlation between 8-OHdG levels and smoking habits, Kulikowska-Karpińska and Czerw [70] found such a correlation with the duration of smoking and the number of cigarettes smoked daily.

Some limitations of this study should be pointed out. Firstly, the small sample size, which may increase the random bias. Secondly, the assessment of smoking status was not conducted quantitatively through biochemical measurements. Nevertheless, it relied not only on self-reported data from individuals, but also on data derived from Behvarz and electronic health records.

There may be a relationship between urinary creatinine concentration and arsenic metabolites, and U-Cr may be a marker of arsenic methylation efficiency [55]. However, the urinary arsenic species were not determined due to budget constraints. Finally, exposure to pesticides may also increase the risk of oxidative stress [71]. Although the majority of participants were primarily involved in

wheat farming and spraying was predominantly carried out by others with tractors, detailed information on their pesticide exposure was not available.

## Conclusions and future directions

This study investigated the association between arsenic exposure, cigarette smoking and oxidative DNA damage measured as 8-OHdG levels in urine and serum samples of male subjects in the northwest of Iran. The conspicuous augmentation of biomarker concentration in both biological fluids in response to arsenic exposure was apparent. However, interaction effect of joint exposures was not significant, and the increment in question did not exhibit a statistically significant disparity when comparing smokers and non-smokers. Furthermore, our findings suggest that some lifestyle factors may introduce confounding or mediating effects on the relationship between the main exposure factor and related changes, which ought to be appropriately considered in the future similar studies.

It is important to acknowledge that although our study has a regional context, the implications of our findings can extend beyond this context and may be of interest to policy makers and public health officials implementing targeted strategies to reduce As exposure from drinking water and promote healthier lifestyle choices on a worldwide level.

For future studies, it is recommended to also investigate the antioxidant levels, as systemic oxidative stress can be influenced by the content of endogenous antioxidants. Moreover, conducting further studies with a larger sample size and gender-balanced participants is advisable for evaluation of the association between S-8OHdG and arsenic species in blood. Incorporating smoking-related biomarkers such as nicotine/cotinine can enhance the understanding of associations, specially possible interactions.

## Abbreviations

As	Inorganic arsenic
8-OHdG	8-Hydroxy-2-deoxyguanosine
ELISA	Enzyme-linked immunosorbent assay
U-tAs	Urinary total arsenic
U-Cr	Urinary creatinine
DWAs	Arsenic concentration of drinking water
As-ADI	Average daily intake of As
U-8OHdG	Urinary levels of 8-OHdG
S-8OHdG	Serum levels of 8-OHdG
ROS	Reactive oxygen species
IPAQ	International Physical Activity Questionnaire
LOD	Limit of detection
LOQ	Limit of quantification
BMI	Body mass index
GLM	General linear model

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## Author contributions

S-NM: formal analysis, investigation, data curation, writing—original draft. MM: conceptualization, funding acquisition, supervision, writing—review and editing. JB: methodology, investigation. AM: study design, methodology, data curation. RD: methodology. LN: methodology, investigation. MM: visualization, writing—review and editing.

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## Availability of data and materials

All data analyzed or generated during study are included in this published paper.

## Declarations

### Ethics approval and consent to participate

This study was in accordance with ethical consideration of the Academic Committee for Research Ethics (Human Studies) of Tabriz University of Medical Sciences (Approval Number: IR.TBZMED.REC.1399.708). All participants provided a written informed consent.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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