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Is Saliva a Potential Biomarker of Arsenic Exposure? A Case-Control Study in West Bengal, India

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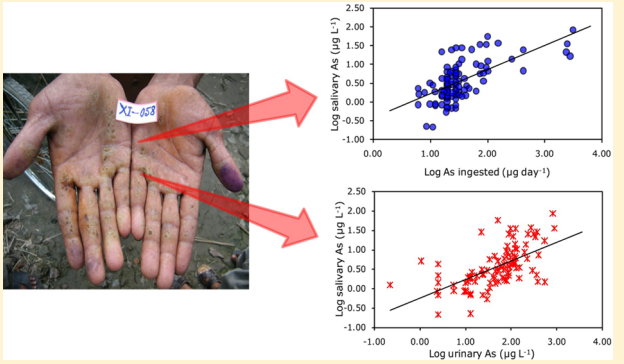
Supporting Information

ABSTRACT: Saliva is a biological fluid that has not been used extensively as a biomonitoring tool in epidemiological studies. This study presents the arsenic (As) concentrations in saliva and urine samples collected from populations of West Bengal, India who had been previously exposed to high As levels in their drinking water. We found a significant ($p < 0.05$) association between the Log transformed Daily Ingestion of As (μg day^{-1}) and the As concentration in saliva ($r = 0.68$). Additionally, As concentration of saliva and urine also had a significant positive correlation ($r = 0.60$, $p < 0.05$). Male participants, smokers, and cases of skin lesion were independently and significantly associated with an increase in salivary As. Thus our findings show that saliva is a useful biomarker of As exposure in the study population. The study also advocates that measurement of the forms of As in saliva may additionally provide insight into the internal dose and any individual differences in susceptibility to As exposure.



INTRODUCTION

Elevated levels of Arsenic (As) in groundwater have now become a threat to the health of communities in many parts of the world.[1,2] There are several manifestations of arsenicosis (As toxicity due to chronic exposure) which include a range of cardiovascular, hepatic, hematological, endocrine, renal, and dermal diseases as well as cancers of the various organs.[3−5] Previous studies on As exposure and its effects on human beings have used blood, urine, scalp hair, and nail as biomarkers, but each of them has serious drawbacks. Blood collection represents an invasive procedure where the participants are made uncomfortable during the venipuncture. The other limitation pertains to sample storage. Additionally, due to increased awareness of spreading disease via blood contamination, participants are becoming increasingly more reluctant to provide blood samples for research. Although, hair and nail can be collected by a noninvasive method, the problem that mainly persists is related to external contamination.[6,7] Erroneous results might be encountered because of the difficulty in distinguishing between endogenous and exogenous sources of As adsorbed in hair and nail.[6,8] Speciation of As from hair and nail involves digestion of the sample at high temperature and measuring the extract.[9] Reports suggest that species transformation might take place during such extraction.[10] Moreover, As in hair and nail have limited applicability for a population exposed to a high amount of As. Schmitt et al.[11] showed that for a 50-fold increase of As concentration in water for As exposed and nonexposed population in Inner Mongolia, an increase of only 20-fold took place for nail As, suggesting that hair and nail may be saturated with arsenic.

Saliva is an easily accessible biofluid which is secreted in salivary glands including parotid, submandibular, and sublingual glands by active transport of water and ion from plasma. Water is the main constituent of saliva (98%) along with electrolytes, enzymes, mucus, and antibacterial constituents.[12] The daily secretion of saliva ranges from 800 to 1500 mL and represents a relatively simple matrix compared to blood and urine.[13] Because of the noninvasive nature, ease of collection, and storage, saliva can be helpful for studying a large population and

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particularly advantageous when children are involved.[14] Methods have already been established to quantify several heavy metals in saliva[15,16] and have been used for biomonitering of lead exposure,[17,18] mercury release from amalgam fillings,[19,20] cadmium exposure via smoking addiction,[21] and atrazine exposure from herbicides.[22] For lead exposed populations, the concentration of Pb in saliva is closely related to blood, plasma, and hair metal concentration, thus rendering saliva as a potential biomarker of lead exposure.[17,18] However, there have been limited numbers of studies that have detected As in saliva.[23,24] Fängström et al.[25] stated that because of low concentration and lesser variation in As concentration, saliva is unsuitable for use as a biomarker in epidemiological studies. In a different study, Lew et al.[26] did not find any significant relationship in As concentration and speciation pattern in saliva samples from children that were exposed to As via hand to mouth transfer by playing in Chromated Copper Arsenate (CCA) treated wood playground compared to those that did not play in CCA-treated wood. Thus there exists a serious knowledge gap in the use of salivary As as a biomarker in epidemiological study.

The aims of our present study are to (i) develop a simple analytical protocol to determine As in saliva and test the method on saliva samples collected from the people residing in three villages in Nadia district, West Bengal, and (ii) we examine the correlation between As in urine and saliva samples from the study population. The overarching objective of the study is to assess whether saliva is a suitable biomarker for biomonitoring As exposure.

METHODS

Study Population and Sample Collection. Saliva (n = 101) and urine (n = 101) samples were collected from participants of three villages (Chhoto-Itna, Debagram, and Tehatta) of Nadia district, West Bengal, India. Participants were recruited from the cross-sectional study (2006−2007) carried out by Guha Mazumder et al.[27] in Nadia district, West Bengal, India. Local volunteers were employed to identify the selected participants from the study areas who have been residing in the same locality for a minimum of 10 years prior to the interview and were between 18 and 65 years of age. Since the control was also taken from the same areas, such criteria enabled us to compare the case-control who was exposed to compatible As for a long period of time. The region has been documented to have high As concentrations in groundwater.[28] Most of the people of these villages used to consume groundwater with high As levels in the past, but for the past few years (3−4 years) are now relying on safe municipal water supply. However there are a few families that are still using As-contaminated groundwater for drinking purposes. The villages are surrounded by agricultural lands, and cultivation of jute and rice are the most common practice.

Trained interviewers conducted a face-to-face interview with the participants in their residence. Before the interview, an experienced physician conducted a clinical examination of the participants, and those suffering from contagious disease and/or renal dysfunction were excluded from the study. Detailed information (age, body height and weight, body mass index (BMI), occupation, residential years, drinking and smoking habits) about the selected participants was obtained using a questionnaire. Participants were characterized as cases of skin lesions and control (with no skin lesions). The severity of the skin lesions was scored following standard protocol.[27,29,30] The detailed descriptions of the study design, recruitment of subjects, and the protocol followed for the interview have been documented in a previous publication.[31]

After the interview, participants were asked to provide saliva and urine samples. Spot urine samples were taken in prewashed (with 5% HNO_3 acid and then several times with Milli-Q water) polyethylene bottles in the day time (10.00 to 16.00 IST). For saliva, the participants were asked not to eat or drink for 1 h prior to the sample collection. The participants rinsed their mouths with Milli-Q water and discarded the saliva which was formed immediately. After 2−3 min, the participants were given 15 mL LDPE bottles, and the saliva was collected. Both urine and saliva were collected simultaneously in the day time. The minimum saliva sample required was 3 mL. Immediately after the sample collection, the bottle was placed in separate zip lock bag with a printed sticker code of the participant and then stored in a salt−ice mixture and kept frozen until returned to the home laboratory. Later the samples were stored in −20 °C freezer until analysis. Drinking water samples (n = 16) were also collected in acid washed, precleaned polyethylene (PE) bottles from the sources mentioned by the participants as the primary supplier of their drinking water. The water samples were acidified with HNO_3 (pH <2) on the spot and were preserved at 4 °C until further analysis.

This study was approved by the individual Ethical committees of University of Michigan (IRB-Health), DNGM Research Foundation, and University of Kalyani on the Ethics of Research on Human Beings.

Sample Preparation and Analysis. Urine samples were brought to room temperature and filtered with a 0.45 μm syringe filter. The specific gravity of each sample was measured. The concentrations of As in the urine samples were corrected to the mean specific gravity of the samples (1.015 g mL^{-1}). The filtered urine samples were digested with HNO_3 and H_2O_2 (MERCK). One mL of filtered urine sample was mixed with 3 mL of concentrated HNO_3 (suprapur, MERCK), and the solution was heated for ∼4 h at 120 °C until the solution turned colorless. To remove the excess organics, 1 mL of 30% H_2O_2 (MERCK) was added, and the heating was continued. The digestion was marked complete when the evolution of gas from the solution stopped. The digested sample was cooled and measured for As using HG-AAS (Varian, AA220) following the manufacturer instructions. The water samples collected during the survey were also measured for total As using HG-AAS.

As contents of the saliva samples were measured with an inductively coupled plasma/mass spectrometer (ICP-MS) (Agilent 7500c) equipped with collision cell. The samples were thawed to room temperature and centrifuged, and 1 mL of the sample was transferred to a plastic vial. To the sample was added an appropriate amount of HNO_3 (2% v/v), ethanol (2% v/v), and internal standard (I.S) (10 μg L^{-1} Rhodium standard). The volume was made up to 3 mL using Milli-Q water and was analyzed for As following the operational parameter as described in Colon et al.[32]

The reproducibility of the data (±0.2−0.4%) was checked through frequently run laboratory standards. Detection limits were calculated as three times the standard deviation for the reagent blanks.

Estimation of Total Inorganic As Exposure. Studies on dose−response relationships have shown that consumption of inorganic As (iAs) in drinking water is one of the routes of As intake in humans.[29,33,34] Since the As in water of our study area is primarily composed of inorganic As [As (III) and As (V)],[28]

the As exposure via drinking water of each participant was estimated following the equation

$$I_{As,i}\ (\mu g\ day^{-1}) = (C_{W,i} \times V_i) \quad (I)$$

where $I_{As,i}$ represents the amount of ingested iAs from water, $C_{W,i}$ represents the concentration of As in drinking water ($\mu g\ L^{-1}$), and V_i represents the volume of daily water intake of each participant, collected during the questionnaire survey ($L\ day^{-1}$).

In our recent study in the same cohort, Halder et al.[35] has estimated the extent of As ingestion through rice by measuring the As concentration of the household rice samples and from the amount of daily rice consumption for each participant. Out of the 157 participants investigated by Halder et al.,[35] the number of participants recruited in this present study was included, and thus the amount of As intake through rice was quantified. Additionally, it was also reported that the rice consumed by the participants in our study area accounts to 0.92 fractions of the inorganic As, and, therefore, the total amount of iAs ingested was calculated as

$$TDI_i\ (\mu g\ day^{-1}) = I_{As,i} + (C_{R,i} \times W_i \times 0.92) \quad (II)$$

where TDI_i represents the total daily ingestion of iAs, $C_{R,i}$ represents the concentration of As in rice ($\mu g\ kg^{-1}$), and W_i represents the amount of rice consumed daily ($kg\ day^{-1}$) for each participant.

Data Analysis. The detailed statistical analyses were performed using SPSS statistical software, version 17.0 by IBM. Histogram and normal probability plot of the tabulated TDI, As concentration in urine and saliva (Figure SI1, see the Supporting Information) revealed that the distributions were right skewed and deviated from normality. Thus all the data was Log transformed prior to use for statistical analysis.

Linear regression analysis was performed to evaluate the strength of the association between TDI with total urinary As and salivary As. Additionally, regression analysis was also estimated between salivary and urinary As so as to assess the relationship between these parameters. Influence of the different demographic variables on the As concentration in urine and saliva was tested by the analysis of variance (ANOVA). The independent variables include age, gender, smoking status, Body Mass Index (BMI), and score of skin lesion. The variables were later tested for multiple linear regression analysis with As concentration in urine and saliva. Statistical significance was indicated by values of $p < 0.05$.

RESULTS AND DISCUSSION

Analytical Protocol and Quality Control. Standard reference material (SRM) for water (SRM 1643e) and urine (SRM 2670a) from the US National Institute of Standards and Technology (NIST) was used for quality assurance. The As concentration in the standard water reference material was found to be in agreement with the certified value. The measurement of total As in urine was confirmed by means of total As recovered from digesting the SRM of urine using the protocol as that described for urine samples. Our result showed mean a percentage recovery of 99 ± 15% ($n = 8$).

Measuring As in saliva is relatively new and currently there is no SRM for salivary As. Thus in the absence of SRM, our protocol involved in-house secondary standards created by spiking different concentrations of As in noncontaminated saliva samples collected from volunteers of different ages and sex. The percentage As recovery ranged from 99% to 101% (Figure SI2, see the Supporting Information). The results showed good agreement when the spiked saliva samples were diluted 3-fold (data not shown), thus suggesting minimum matrix effect. The details of the effect of alcohol and internal standard on As measurement in spiked saliva samples are given in the Supporting Information (SI).

As Exposure and Total As Concentration in Urine and Saliva. The statistical results of the As level in drinking water, tabulated TDI, As concentration in urine (U_{As}), and saliva (S_{As}) are represented in Table 1, and the Log transformed data are

Table 1. Statistical Table of the Measured As Concentration in Drinking Water, TDI, U_{As}, and S_{As} of All the Participants[a]

medium	N	$x \pm SD$	median	range
C_W ($\mu g\ L^{-1}$)	16	120 ± 239	18.0	806 − 2.50
TDI ($\mu g\ day^{-1}$)	101	235 ± 531	113	3172 − 19.9
U_{As} ($\mu g\ L^{-1}$)	101	110 ± 154	67.7	883 − 0.22
S_{As} ($\mu g\ L^{-1}$)	101	7.84 ± 12.6	2.99	84.3 − 0.22

[a] C_W − Concentration of As in drinking water; TDI − Total Daily Ingestion of inorganic As; U_{As} − Urinary As concentration; S_{As} − Salivary As Concentration.

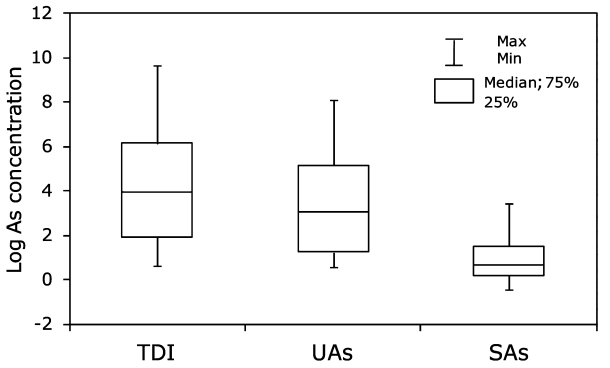

Figure 1. Box plot of Log transformed Total Daily Ingestion of As (TDI), As concentration in urine (U_{As}), and saliva As concentration (S_{As}) of 101 participants. Concentration of TDI is in $\mu g\ day^{-1}$ and concentration of U_{As} and S_{As} is in $\mu g\ L^{-1}$.

shown in Figure 1. Our results are in accordance with the study of Yuan et al.[23] which found the mean concentration of saliva As up to 11.9 $\mu g\ L^{-1}$ for residents of Inner Mongolia, China who were exposed to As concentrations up to 826 $\mu g\ L^{-1}$ in drinking water. By comparison, the salivary As value of 0.79 $\mu g\ L^{-1}$ has been reported for populations of Edmonton, Alberta, Canada who were consuming As concentration <5 $\mu g\ L^{-1}$ in drinking water. Although the groundwater in our study area has a high concentration of As, due to increased social awareness, the participants are now sharing the low As common water sources for drinking purposes.[27] However, the local farmer still uses high As concentration groundwater for irrigation and crop cultivation. Studies have revealed that because of the use of such groundwater for agricultural purposes, there are additional exposures of bioavailable As from foods consumed by the participants.[36,37] In our recent publication on the same participants, Halder et al.[35] have explicitly measured the As exposure from dietary sources, and it was shown that for people consuming safe water (<10 $\mu g\ L^{-1}$), the major contribution of inorganic As is from rice consumption and for 35% of the cases,

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the total As intake from water and rice exceeds the previous provisional tolerable daily intake of 2.1 μg day^{-1} kg^{-1} BW as recommended by WHO. Additionally, for the participants consuming water with As concentration >10−50 μg L^{-1}, the intake of inorganic As from water and rice are almost equal, and, therefore, the cumulative contribution of the As ingested through rice and water may be sufficient to cause a potential threat to the inhabitants of these areas.

Simple regression analysis between TDI and U_{As} as well as S_{As} was done to evaluate the viability of the excreted As as a measure of As exposure (Figure 2; Table 2). Our study shows

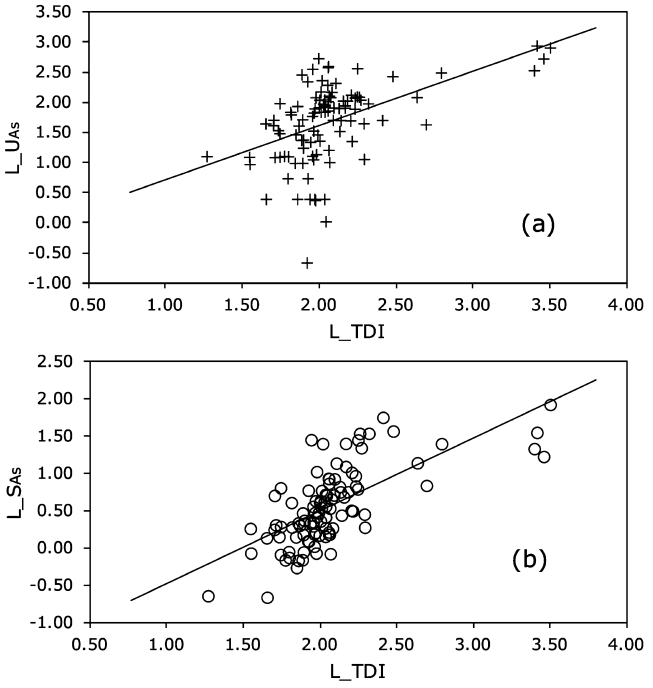

Figure 2. Plot of (a) Log-transformed urine As concentration (U_{As}) versus Total Daily Ingestion of As (TDI) and (b) saliva As concentration (S_{As}) vs Total Daily Ingestion of As (TDI). Concentration of TDI is in μg day^{-1} and concentration of U_{As} and S_{As} is in μg L^{-1}.

that TDI has a positive correlation with both U_{As} (r = 0.50, p < 0.05) as well as S_{As} (r = 0.68, p < 0.05). This suggests that similar to urinary As, salivary As can also act as a predictor of As exposure. However the results of our study shows that S_{As} has a better correlation with TDI than U_{As}, signifying As in saliva as a superior reflection of the ingested As compared to urine. A number of previous epidemiological studies on As have used urinary As as a biomarker of As exposure.[38−42] The ingested As is quickly eliminated within 2−3 h from blood through the kidneys and from urine in 2−3 days.[40] Therefore, urinary As indicates recent exposure. Nevertheless, As concentration in urine reaches a steady state and may thus reflect past exposure for populations exposed to continuous chronic levels.[43] Simple regression analysis was done between U_{As} and S_{As}, and there exists a positive, significant correlation between the two parameters (r = 0.60, p < 0.05; Figure 3). This suggests that ingestion of inorganic As is important in determining the As concentration in saliva. Thus S_{As} can be regarded as a biomarker of As exposure and can be used as a surrogate of urine in As epidemiological studies.

Table 2. Correlation Matrix of the Bivariate Relation between Log Total Daily Ingestion of As, Log As in Urine and Saliva[a]

	L_ TDI	L_U_{As}	L_S_{As}
L_ TDI	−	r = 0.50; p < 0.05 Rsq = 0.25 Adj Rsq = 0.24 SEE = 0.57 L_U_{As} = −0.25 + 0.92 L_TDI	r = 0.68; p < 0.05 Rsq = 0.46 Adj Rsq = 0.45 SEE = 0.38 L_S_{As} = −1.52 + 0.99 L_TDI
L_U_{As}	r = 0.50; p < 0.05 Rsq = 0.25 Adj Rsq = 0.24 SEE = 0.57 L_U_{As} = −0.25 + 0.92 L_TDI	−	r = 0.60; p < 0.05 Rsq = 0.36 Adj Rsq =0.36 SEE = 0.42 L_S_{As} = −0.24 + 0.48 L_U_{As}
L_S_{As}	r = 0.68; p < 0.05 Rsq = 0.46 Adj Rsq = 0.45 SEE = 0.38 L_S_{As} = −1.52 + 0.99 L_TDI	r = 0.60; p < 0.05 Rsq = 0.36 Adj Rsq = 0.36 SEE = 0.42 L_S_{As} = −0.24 + 0.48 L_U_{As}	−

[a]L_TDI - Log Total Daily Ingestion of As; L_U_{As} - Log of As concentration in urine; L_S_{As} − Log of As concentration in saliva; r − Pearson correlation coefficient; SEE − Standard Error of the Estimate.

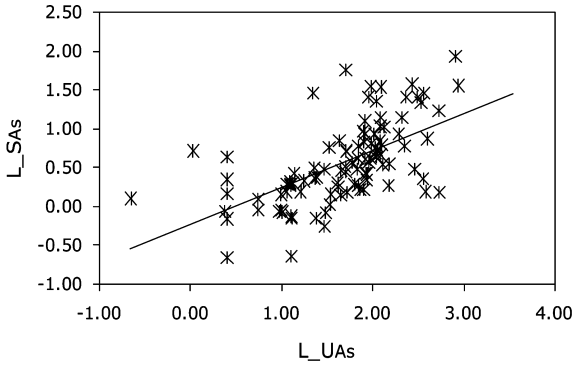

Figure 3. Log-transformed saliva As concentration (S_{As}) versus urine As concentration (U_{As}). Concentration of U_{As} and S_{As} is in μg L^{-1}.

There are several limitations for the use of urine in As epidemiological studies. U_{As} gives information about the excretion and the metabolism of As but falls silent about the actual tissue burden.[44] Thus any factors that affect the metabolism of As can have a severe impact on the concentration of U_{As}. Studies have shown that consumption of seafood and marine fish containing organic As can interfere with the total urinary As and the distribution of As derivatives such as arsenobetaine and arsenocholine.[45] Organo As are nontoxic and chemically stable and are excreted rapidly intact, but its consumption can significantly increase the concentration of U_{As}.[6] Therefore before performing the study, restriction in food consumption needs to be taken, and participants are refrained from consuming seafood for 2−3 days before collection of the urine samples.[45] Sometimes rapid analysis of the urine samples are required as the reduced As species present in urine [MMA(III) and DMA(III)] are rapidly oxidized even when kept frozen, and thus, underestimation of the As species may take place.[38] Although spot urine is the preferred collection procedure for urine samples, the major disadvantage that usually persists is interindividual matrix

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Table 3. Association of Urinary and Salivary As Concentration with Study Variables

		urinary As concentration			saliva As concentration		
variables	sample number	mean ± SD	95% CI for mean*	*p*-value*	mean ± SD	95% CI for mean*	*p*-value*
sex							
male	48	143 ± 160	1.81−2.08	0.00	10.4 ± 12.8	0.58−0.88	0.00
female	53	79.9 ± 144	1.24−1.63		5.56 ± 12.1	0.28−0.54	
age							
<35	11	22.8 ± 27.6	0.79−1.46	0.00	4.83 ± 9.97	−0.11−0.64	0.15
36−45	42	95.9 ± 150	1.39−1.81		7.89 ± 15.7	0.37−0.69	
46−55	35	156 ± 183	1.70−2.11		8.27 ± 9.85	0.47−0.82	
>55	13	102 ± 106	1.44−2.08		9.10 ± 10.4	0.38−0.99	
smoker							
no	63	90.4 ± 143	1.34−1.70	0.00	5.12 ± 11.2	0.29−0.52	0.00
yes	38	142 ± 167	1.78−2.09		12.4 ± 13.7	0.65−0.99	
BMI							
<18	22	118 ± 132	1.49−2.04		8.99 ± 11.4	0.34−0.88	0.87
18−25	70	107 ± 164	1.48−1.80	0.73	7.77 ± 13.6	0.44−0.67	
>25	9	114 ± 139	1.17−2.22		5.60 ± 6.70	0.14−0.89	
score							
control	37	31.6 ± 48.3	0.94−1.35	0.00	2.92 ± 5.95	0.09−0.36	0.00
mild	19	137 ± 185	1.71−2.12		10.1 ± 19.5	0.38−0.87	
moderate	38	168 ± 180	1.93−2.18		9.03 ± 9.47	0.61−0.90	
severe	7	131 ± 132	1.08−2.47		21.2 ± 18.8	0.62−1.61	

*ANOVA of Log transformed dependent variables.

Table 4. Results of Multiple Regression Analysis of Log Transformed Urinary As and Saliva As Concentration with Selected Study Variables

	Log urinary As			Log saliva As		
	beta coefficient ± SE	95% CI	*p*-value	beta coefficient ± SE	95% CI	*p*-value
Log TDI	0.64 ± 0.15	0.38−0.95	0.00	0.77 ± 0.10	0.57−0.97	0.00
sex	0.19 ± 0.19	−0.19−0.58	0.32	−0.30 ± 0.13	−0.55− −0.04	0.02
age	0.01 ± 0.01	0.00−0.02	0.08	0.01 ± 0.00	0.00−0.01	0.16
smoker	−0.02 ± 0.19	−0.39−0.35	0.90	0.40 ± 0.12	0.16−0.65	0.00
BMI	−0.01 ± 0.02	−0.04−0.02	0.50	−0.01 ± 0.01	−0.03−0.01	0.48
score	0.11 ± 0.03	0.04−0.17	0.00	0.09 ± 0.02	0.05−0.13	0.00

variation due to difference in fluid intake, physical activity, and temperature between the individuals.[46] As a result, hydration correction is necessary to account for the differences, while omitting such corrections may lead to highly significant correlations as dilution of urine samples are not accounted. For hydration correction, there are studies where urine As has been normalized with urine creatinine.[47,48] However such a method of adjusting the As-level with the concentration of creatinine in urine has limitations as the excretion of creatinine is dependent on factors such as age, sex, BMI, and race.[49,50] Moreover, concentration of creatinine is significantly related to the concentration of the As metabolites present in the urine, and changes for creatinine adjustment may give erroneous results.[47] Gamble and Liu[48] in their report concluded that urinary creatinine should be included as an independent variable in multiple regression analysis, and the role of one-carbon metabolism as a predictor of creatinine must also be considered for the interpretation of the result. Additionally there are several field problems for the collection of urine samples. Our field experience shows that participants, particularly village women, were very reluctant to provide samples for research. We also observed that ethnic barrier is another important factor for the collection of urine samples, and participants of certain race are conservative and are unwilling to give urine sample even after long persuasion. Moreover participants needed to feel the urge of urination to provide urine samples and this was often a time taking process. Difficulties in the collection of urine samples may be compounded when the studies involve children and especially young children who are still in diapers.[14] Such drawbacks can be eliminated by using saliva as a biomarker since no prior adjustment of the samples is necessary for interpretation of the results, and the samples can be collected easily on the spot in a few minutes.

Factors Regulating As Concentration in Urine and Saliva. Influence of age, sex, smoker, BMI, and score of skin lesion on urinary and saliva As concentration is shown in Table 3. Our results indicate significant association of U_{As} with sex ($p < 0.0001$), age ($p = 0.003$), smoker ($p = 0.002$), and prevalence of skin lesion ($p = 0.000$). However, there were no significant differences of urinary As concentration with BMI ($p = 0.44$). Previous studies on Asian countries have observed that males, smokers, and older participants are likely to be more affected than their respective counterparts.[31,42,50,51] The possible explanation for such an observation has been suggested to decreased methylation capacity of the participants,[42,50] and this has also been reflected in our study (Table 3). Manifestation of skin lesion has been positively associated with As exposure.[51−53] Our study show that the controls have lower concentration of U_{As} than cases with skin lesions (Table 3).

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However, among the various cases of skin lesion, participants categorized as moderate (score ≤4) have a higher concentration of U_{As} than severe (score ≤6) and mild (score ≤2) cases (Table 3).

Similar to urinary As, male participants and smokers had a higher concentration of S_{As} compared to females and nonsmokers, respectively, while association of S_{As} with BMI ($p = 0.871$) and age ($p = 0.440$) was not statistically significant (Table 3). Control had a lower concentration of S_{As}, and the concentration for severe cases was 2-fold higher than the mild and moderate cases of skin lesion (Table 3). Results of multiple regression analysis for U_{As} and S_{As} are shown in Table 4. It is interesting to note that while considering the concurrent effect, TDI and scores of skin lesion had a significant effect on U_{As}, while for saliva, sex, smokers, score, and TDI was positively related with S_{As}. This suggests that compared to U_{As}, S_{As} provides better information about the confounding factors which in turn are directly related to the individual As exposure.

In conclusion, the use of saliva for exposure assessment has several advantages compared to other already established biomarkers. Saliva, secreted in the salivary gland, consists of ingredients of extracellular fluids. Thus the chemical composition and the chemistry are widely different from that of plasma and serum. The metal ions are actively transported from the plasma and thus represent a measure of internal dose. So monitoring saliva data may provide insight to the As metabolic process. This study demonstrated S_{As} as a potent biomarker of As exposure in our study population that has been exposed to high As concentration groundwater in the past. The strong positive correlation between the TDI and S_{As} suggests that As concentration in saliva provides a good reflection of As exposure. Since urine is considered as a surrogate of As intake, the positive correlation between U_{As} and S_{As} strengthens the case for the use of saliva as a biomarker for As exposure.

■ ASSOCIATED CONTENT

Supporting Information

Distribution of As concentration in Total Daily As Intake (TDI), urine and saliva, and the details of the experimental results of the spiked saliva samples. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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