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 \((\mu g \ day^{-1})\) 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.8,9 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 extractions.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.12

Saliva is an easily accessible biofluid which is secreted in fluid which is secreted in saliva 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.13 The daily secretion of saliva ranges from 800 to 1500 mL and represents a relatively simple matrix compared to blood and urine.14 Because of the noninvasive nature, ease of collection, and storage, saliva can be helpful for studying a large population and...
Methods have already been established to quantify several heavy metals in saliva and have been used for biomonitoring of lead exposure, mercury release from amalgam fillings, cadmium exposure via smoking addiction, and atrazine exposure from herbicides. 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. However, there have been limited numbers of studies that have detected As in saliva. Fangström et al. 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. 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 the same 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. 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. 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. The detailed descriptions of the study design, recruitment of subjects, and the protocol followed for the interview have been documented in a previous publication.

After the interview, participants were asked to provide saliva and urine samples. Spot urine samples were taken in prewashed (with 5% HNO₃ 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 HNO₃ (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 HNO₃ and H₂O₂ (MERCK). One mL of filtered urine sample was mixed with 3 mL of concentrated HNO₃ (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% H₂O₂ (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) 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 HNO₃ (2% v/v), ethanol (2% v/v), and internal standard (LS) (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. 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. Since the As in water of our study area is primarily composed of inorganic As (As (III) and As (V)), the

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8
the As exposure via drinking water of each participant was estimated following the equation

\[ I_{\text{As},i} (\mu g \text{ day}^{-1}) = (C_{W,i} \times V_i) \]  

(1)

where \( I_{\text{As},i} \) represents the amount of ingested iAs from water, \( C_{W,i} \) represents the concentration of As in drinking water (\( \mu g \text{ L}^{-1} \)), 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

\[ \text{TDI} (\mu g \text{ day}^{-1}) = I_{\text{As},i} + (C_{R,i} \times W_i \times 0.92) \]  

(II)

where TDI represents the total daily ingestion of iAs, \( C_{R,i} \) represents the concentration of As in rice (\( \mu g \text{ kg}^{-1} \)), 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 S1, 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 uncontaminated saliva samples collected from volunteers of different ages and sex. The percentage As recovery ranged from 99% to 101% (Figure S1, 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 shown in Figure 1. Our results are in accordance with the study of Yuan et al.\(^{33}\) which found the mean concentration of saliva As up to 11.9 \( \mu g \text{ L}^{-1} \) for residents of Inner Mongolia, China who were exposed to As concentrations up to 826 \( \mu g \text{ L}^{-1} \) in drinking water. By comparison, the salivary As value of 0.79 ± 2.5 \( \mu g \text{ L}^{-1} \) 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.\(^{24,36,37}\) 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 exposure to As from foods consumed by the participants.\(^{24,36,37}\) 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 \( \mu g \text{ L}^{-1} \)), the major contribution of inorganic As is from rice consumption and for 35% of the cases, it represents the concentration of As in rice (\( C_{R,i} \)), and, therefore, the total amount of As consumed by the participants in our study area accounts to 0.92 of the total daily ingestion of iAs, \( C_{R,i} \), and \( W_i \) represents the volume of daily water intake of each participant, collected during the questionnaire survey (L day\(^{-1}\)).

![Figure 1](https://example.com/figure1.png)

**Table 1. Statistical Table of the Measured As Concentration in Drinking Water, TDI, \( U_{As} \), and \( S_{As} \) of All the Participants**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>x ± SD</th>
<th>median</th>
<th>range</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{W} (\mu g \text{ L}^{-1}) )</td>
<td>16</td>
<td>120 ± 239</td>
<td>18.0</td>
<td>806 ± 2.5</td>
</tr>
<tr>
<td>TDI (( \mu g \text{ day}^{-1} ))</td>
<td>101</td>
<td>235 ± 531</td>
<td>113</td>
<td>3172 ± 19.9</td>
</tr>
<tr>
<td>( U_{As} (\mu g \text{ L}^{-1}) )</td>
<td>101</td>
<td>110 ± 154</td>
<td>67.7</td>
<td>883 ± 0.22</td>
</tr>
<tr>
<td>( S_{As} (\mu g \text{ L}^{-1}) )</td>
<td>101</td>
<td>7.84 ± 12.6</td>
<td>2.99</td>
<td>84.3 ± 0.22</td>
</tr>
</tbody>
</table>

\(^{a}\) \( C_{W} \) = Concentration of As in drinking water; TDI = Total Daily Ingestion of inorganic As; \( U_{As} \) = Urinary As concentration; \( S_{As} \) = Salivary As Concentration.
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 Uₐₐ as well as Sₐₐ was done to evaluate the viability of the excreted As as a measure of As exposure (Figure 2; Table 2). Our study shows that TDI has a positive correlation with both Uₐₐ (r = 0.50; p < 0.05) as well as Sₐₐ (r = 0.68; p < 0.05). This suggests that TDI has a positive correlation with both Uₐₐ and Sₐₐ, signifying As in saliva as a superior reflection of the ingested As compared to urine. A disadvantage that usually persists is interindividual matrix effects past exposure.

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

<table>
<thead>
<tr>
<th>L_TDI</th>
<th>L_Uₐₐ</th>
<th>L_Sₐₐ</th>
</tr>
</thead>
<tbody>
<tr>
<td>L_TDI</td>
<td>r = 0.50; p &lt; 0.05</td>
<td>r = 0.68; p &lt; 0.05</td>
</tr>
<tr>
<td>Rsq = 0.25</td>
<td>Rsq = 0.46</td>
<td></td>
</tr>
<tr>
<td>Adj Rsq = 0.24</td>
<td>Adj Rsq = 0.45</td>
<td></td>
</tr>
<tr>
<td>SEE = 0.57</td>
<td>SEE = 0.38</td>
<td></td>
</tr>
<tr>
<td>L_Uₐₐ = -0.25 + 0.92 L_TDI</td>
<td>L_Sₐₐ = -1.52 + 0.99 L_TDI</td>
<td></td>
</tr>
<tr>
<td>L_Sₐₐ</td>
<td>r = 0.68; p &lt; 0.05</td>
<td>r = 0.60; p &lt; 0.05</td>
</tr>
<tr>
<td>Rsq = 0.46</td>
<td>Rsq = 0.36</td>
<td></td>
</tr>
<tr>
<td>Adj Rsq = 0.45</td>
<td>Adj Rsq = 0.36</td>
<td></td>
</tr>
<tr>
<td>SEE = 0.38</td>
<td>SEE = 0.42</td>
<td></td>
</tr>
<tr>
<td>L_Sₐₐ = -1.52 + 0.99 L_TDI</td>
<td>L_Sₐₐ = -0.24 + 0.48 L_Uₐₐ</td>
<td></td>
</tr>
</tbody>
</table>

*aL_TDI - Log Total Daily Ingestion of As; L_Uₐₐ - Log of As concentration in urine; L_Sₐₐ - Log of As concentration in saliva; r – Pearson correlation coefficient; SEE – Standard Error of the Estimate.

There are several limitations for the use of urine in As epidemiological studies. Uₐₐ gives information about the excretion and the metabolism of As but falls silent about the actual tissue burden. Thus any factors that affect the metabolism of As can have a severe impact on the concentration of Uₐₐ. 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 arsenucholine. Organo As are nontoxic and chemically stable and are excreted rapidly intact, but its consumption can significantly increase the concentration of Uₐₐ. 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. 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. Although spot urine is the preferred collection procedure for urine samples, the major disadvantage that usually persists is interindividual matrix
we observed that ethnic barrier is particularly village women, were very reluctant to provide samples. Our there are several considered for the interpretation of the result. Additionally carbon metabolism as a predictor of creatinine must also be variable in multiple regression analysis, and the role of one-
urinary creatinine should be included as an independent

Moreover, concentration of creatinine is significant related to
the concentration of the As metabolites present in the urine, and changes for creatinine adjustment may give erroneous results.47 Gamble and Liu48 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.

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

<table>
<thead>
<tr>
<th>variables</th>
<th>sample number</th>
<th>urinary As concentration</th>
<th>saliva As concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean ± SD</td>
<td>95% CI for mean*</td>
</tr>
<tr>
<td>sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>48</td>
<td>143 ± 160</td>
<td>1.81–2.08</td>
</tr>
<tr>
<td>female</td>
<td>53</td>
<td>79.9 ± 144</td>
<td>1.24–1.63</td>
</tr>
<tr>
<td>age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35</td>
<td>11</td>
<td>22.8 ± 27.6</td>
<td>0.79–1.46</td>
</tr>
<tr>
<td>36–45</td>
<td>42</td>
<td>95.9 ± 150</td>
<td>1.39–1.81</td>
</tr>
<tr>
<td>46–55</td>
<td>35</td>
<td>156 ± 183</td>
<td>1.70–2.11</td>
</tr>
<tr>
<td>&gt;55</td>
<td>13</td>
<td>102 ± 106</td>
<td>1.44–2.08</td>
</tr>
<tr>
<td>smoker</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>63</td>
<td>90.4 ± 143</td>
<td>1.34–1.70</td>
</tr>
<tr>
<td>yes</td>
<td>38</td>
<td>142 ± 167</td>
<td>1.78–2.09</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18</td>
<td>22</td>
<td>118 ± 132</td>
<td>1.49–2.04</td>
</tr>
<tr>
<td>18–25</td>
<td>70</td>
<td>107 ± 164</td>
<td>1.48–1.80</td>
</tr>
<tr>
<td>&gt;25</td>
<td>9</td>
<td>114 ± 139</td>
<td>1.17–2.22</td>
</tr>
<tr>
<td>score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>37</td>
<td>31.6 ± 48.3</td>
<td>0.94–1.35</td>
</tr>
<tr>
<td>mild</td>
<td>19</td>
<td>137 ± 185</td>
<td>1.71–2.12</td>
</tr>
<tr>
<td>moderate</td>
<td>38</td>
<td>168 ± 180</td>
<td>1.93–2.18</td>
</tr>
<tr>
<td>severe</td>
<td>7</td>
<td>131 ± 132</td>
<td>1.08–2.47</td>
</tr>
</tbody>
</table>

*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

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

Moreover participants needed to feel the urge of urination to provide urine samples and this was often a time taking process.359 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 UAs 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).373 Previous studies on Asian countries have observed that males, smokers, and older participants are likely to be more affected than their respective counterparts.371,42,50,51 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.53–55 Our study show that the controls have lower concentration of UAs than cases with skin lesions (Table 3).
However, among the various cases of skin lesion, participants
categorized as moderate (score ≤ 4) have a higher concentra-
tion of $U_{As}$ than severe (score ≤ 6) and mild (score ≤ 2) cases
(4.1.3).

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
(4.1.3). 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 (4.1.3). 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 $U_{As}$
and 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
in which 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

S 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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