**A Pathway-based Analysis of Urinary Arsenic Metabolites and Skin Lesions**

Molly L. Kile*, Elaine Hoffman, Ema G. Rodrigues, Carrie V. Breton, Quazi Quamruzzaman, Mahmuder Rahman, Golam Mahiuddin, Yu-Mei Hsueh, and David C. Christiani

* Correspondence to Dr. Molly L. Kile, Department of Environmental Health, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115 (e-mail: mkile@hsph.harvard.edu).

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Inorganic arsenic is metabolized to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). Limited evidence suggests that the ability to fully metabolize arsenic into DMA influences susceptibility to disease. To determine whether percentage of MMA was predictive of disease, the authors used data from a case-control study conducted in Bangladesh (2001–2003). Persons who were diagnosed with keratosis, melanosis, Bowen’s disease, or squamous cell carcinoma were matched on age, sex, and village to persons without these conditions. This analysis was restricted to persons who had no missing data on covariates (859 cases, 868 controls). A path analysis was used to evaluate simultaneously the association between the percentage of all urinary arsenic metabolites and the odds of skin lesions using PROC CALIS in SAS, version 9.1 (SAS Institute, Inc., Cary, North Carolina) and Mplus, version 6.1 (Muthe´n & Muthe´n, Los Angeles, California). The odds of skin lesions were significantly associated with log10 percentage of MMA (adjusted odds ratio (ORadj) = 1.56, 95% confidence interval (CI): 1.15, 2.12) but not log10 percentage of inorganic arsenic (ORadj = 1.06, 95% CI: 0.75, 1.50) or log10 percentage of DMA (ORadj = 1.07, 95% CI: 0.33, 3.46). This novel analysis confirmed that persons who excrete a higher proportion of MMA have a greater risk of skin lesions after data are adequately controlled for urinary arsenic metabolites, current arsenic exposure, and other risk factors.

Abbreviations: CI, confidence interval; DMA, dimethylarsinic acid; %iAs, percentage of inorganic arsenic; MMA, monomethylarsonic acid; ORadj, adjusted odds ratio; UAMs, urinary arsenic metabolites.
currently being exposed to arsenic also found that %MMA or MMA:DMA ratio confers a greater risk of skin lesions (20–22). However, all of these studies examined the association between disease and each UAM in separate models. Given the complex, collinear relation between arsenic exposure and UAMs expressed as a ratio or a percentage, evaluation of each metabolite in separate models makes it difficult to interpret whether the observed relations are associated with a single metabolite.

Therefore, we conducted a path analysis to evaluate the association between all UAMs and the odds of skin lesions in a population-based case-control study in Bangladesh. Path analysis is an intuitive statistical method that can be used when the relation between exposure and outcome is mediated by other variables. It is used to establish how well a statistical model accounts for existing correlations among variables in observed data and is particularly well suited for analysis of variables with complex relations. This approach allowed us to test the hypothesis that %MMA was an independent risk factor for skin lesions while controlling for all UAMs, current arsenic exposure from drinking water, and other covariates in a statistically efficient manner.

MATERIALS AND METHODS

Participant selection

Nine hundred cases and 900 controls were recruited from the Pabna district of Bangladesh through the Pabna Community Clinic, a rural health-care clinic affiliated with Dhaka Community Hospital (Dhaka, Bangladesh). All volunteers provided informed consent before participating in the study. Two physicians who were trained to characterize arsenic-related skin lesions visually screened volunteers in their homes to identify eligible cases and controls. Cases were defined as persons who had 1 or more types of skin lesions: diffuse/spotted keratosis, diffuse/spotted melanosis, hyperkeratosis, leukomelanosis, Bowen’s disease, or squamous cell carcinoma. In a subset of cases, including all suspected carcinomas, lesions were histologically confirmed. Controls were selected from the same communities as the cases and did not have any visible skin lesions. One control was selected per case and was matched to the case on sex and age (within 3 years). A more detailed description of the study population and activities has been published previously (21, 23). The institutional review boards of the Harvard School of Public Health and Dhaka Community Hospital approved the protocol for this study.

For the statistical data set, we excluded 48 participants whose drinking water was not measured for arsenic (24 controls, 24 cases), 6 participants who did not provide information on betel nut usage (2 controls, 4 cases), 2 participants who did not provide information on their educational status (1 control, 1 case), and 16 participants who did not report the duration of time for which they had used their current tube well (5 controls, 11 cases). We also omitted 1 case who had a body mass index (weight (kg)/height (m)²) of 61.4, which was implausible. This left a total of 1,729 persons (868 controls, 859 cases) whose information was included in this analysis.

Urine sample collection and analysis

Briefly, spot urine samples were collected in sterile urine collection cups (VWR International, West Chester, Pennsylvania). An aliquot of urine was transferred into 15-mL polyethylene tubes (BD Falcon, BD Bioscience, Bedford, Massachusetts), frozen at −20°C, and shipped on dry ice to Taipei Medical University (Taipei, Taiwan). Briefly, urine samples were thawed at room temperature, dispersed by ultrasonic wave, and filtered through a Sep-Pak C18 column to remove protein (Mallinckrodt Baker, Inc., Phillipsburg, New Jersey). Urinary arsenic species, arsenite, arsenate, MMA, and DMA were separated by high performance liquid chromatography (Waters 501; Waters Associates, Milford, Massachusetts) using a Nucleosil 10-µm SB 100A column (Phenomenex, Torrance, California). Individual species were then detected using hydride generation atomic absorption spectrometry (Perkin-Elmer Flow Injection Analysis System 400-AA 100; Perkin-Elmer, Waltham, Massachusetts) as described by Hsueh et al. (24).

This method detects arsenite, arsenate, MMA, and DMA and eliminates interference from arsenobetaine and arsenocholine, nontoxic organic arsenic species found in seafood. We summed the reported values for arsenite and arsenate to quantify the amount of inorganic arsenic in urine. We calculated the relative proportion of each arsenic species (percentage of inorganic arsenic (%iAs), %MMA, and percentage of DMA (%DMA)) by dividing the concentration of each species by the total urinary arsenic concentration (inorganic arsenic + MMA + DMA) and multiplying by 100. Urinary creatinine concentration was measured by means of the kinetic Jaffe method using a Hitachi 7170S autoanalyzer (Hitachi Ltd., Tokyo, Japan).

Quality control procedures included analyzing samples in duplicate and spiking samples with arsenite, arsenate, MMA, and DMA. All replicate values were within 5% of the initial values, and all spiked samples were within 10%. The average limits of detection for arsenite, arsenate, MMA, and DMA were 0.05 µg/L, 0.12 µg/L, 0.05 µg/L, and 0.06 µg/L, respectively (25). Of the 1,729 urine samples included in this analysis, 300, 241, 49, and 2 were below the limit of detection for arsenite, arsenate, MMA, and DMA, respectively. For these samples, we used the limit of detection for the UAM concentration.

Drinking water collection and analysis

Approximately 50 mL of water was collected from the tube well identified by the participant as his or her primary source of drinking water. The water was preserved with reagent-grade nitric acid (Merck, Darmstadt, Germany) to a pH less than 2 and kept at room temperature until analysis. Arsenic was quantified by means of inductively coupled plasma mass spectrometry using US Environmental Protection Agency method 200.8 (Environmental Laboratory Services, North Syracuse, New York). Analysis was validated using PlasmaCAL multielement QC standard #1 solution (SCP Science, Inc., Baie d’Urfé, Quebec, Canada). In total, 234 water samples were below the limit of detection of 1 µg of arsenic per liter and were assigned a value of 0.5 µg/L.
Statistical analysis

Descriptive characteristics of the cases and controls were compared using the $\chi^2$ test for categorical data, the $t$ test for comparison of mean values, and Wilcoxon’s rank-sum test for comparison of median values. Data on drinking water arsenic, UAMs (%iAs, %MMA, and %DMA), and creatinine were log$_{10}$-transformed to make these variables approximate a normal distribution. Data on age, body mass index, duration of current tube-well use, and education were centered at their respective means. Among controls, general linear models were used to identify factors that were significantly associated with drinking water arsenic, %iAs, %MMA, and %DMA.

In this path analysis, the relation between UAMs and case status was estimated using raw data, not covariance matrices, with PROC CALIS in SAS, version 9.1.3 (SAS Institute, Inc., Cary, North Carolina), and Mplus, version 5 (Muthén & Muthén, Los Angeles, California). The paths incorporated factors that significantly predicted log$_{10}$ %iAs, %MMA, and %DMA. The process of selecting the model involved simultaneously fitting a series of linear regressions and a logistic model for case status and then selecting the model which best fitted the observed covariances among the UAMs and covariates. Ultimately, we chose the most biologically plausible model that conformed to standard path analysis goodness-of-fit indices, which included a $\chi^2$ test ($\chi^2 P > 0.05$), the root mean square error of approximation, root mean square residuals, Bentler’s comparative fit index, Bentler and Bonnet’s normed fit index, and Bentler and Bonnet’s non-normed fit index. The final path analysis was performed in Mplus, accounted for binary outcomes using maximum likelihood and the expectation-maximization algorithm, and included 3 linear regression models (%iAs, %MMA, and %DMA) and a logistic regression for case-control status controlling for arsenic exposure and other covariates. Mplus does not support conditional logistic regression in path analysis, so matching variables (age and sex) were included in all regressions. We also evaluated the association between UAMs and skin lesions using separate conditional logistic regression models that included the same covariates as described for the path analysis.

RESULTS

The median drinking water arsenic concentration was 23.0 $\mu$g/L (range, 1–1,480 $\mu$g/L). The most common skin lesions diagnosed were leukomelanosis (46.4%) and melanosi (44.0%). In addition, 99 persons (11.5%) were diagnosed with more than 1 type of skin lesion. Persons with skin lesions were more likely to drink from tube wells containing higher concentrations of arsenic (39.0 $\mu$g/L vs. 11.4 $\mu$g/L), to have higher concentrations of arsenic in their urine (72.5 $\mu$g/L vs. 54.0 $\mu$g/L), to have a higher proportion of MMA in their urine (13.3% vs. 12.0%), and to have a lower proportion of DMA in their urine (74.6% vs. 75.9%) compared with persons without skin lesions. Persons with skin lesions reported having used their current tube well for fewer years compared with persons without skin lesions (7.7 years vs. 9.4 years)—an average of 25% of their lifetime as compared with 31% for persons without skin lesions. Persons who had skin lesions were more likely to chew betel nuts (28.8% vs. 23.9%) and to have a lower educational status than persons without skin lesions (Table 1). No difference was observed between cases and controls for age and sex, which were matching criteria. The distributions of body mass index, creatinine, and %iAs in urine did not differ between cases and controls.

Predictors of UAMs

In general linear regression models, we evaluated the association between several factors and UAMs to identify covariates that would be considered in the path analysis. We limited this analysis to controls in order to avoid potential reverse causality. Among controls, the proportion of UAMs was significantly associated with educational attainment, body mass index, age, sex, urinary creatinine, duration of current tube-well use, and drinking water arsenic levels (Table 2). Men had higher %MMA and lower %DMA compared with women. Body mass index had a linear association with methylated urinary metabolites, with increasing body mass index being associated with lower %MMA and higher %DMA. Drinking water arsenic levels were also associated with higher %iAs and %MMA but lower %DMA, suggesting that arsenic metabolism may have been saturated.

Path analysis

The a priori path analysis was based on the premise that arsenic exposure had direct and indirect effects on all UAMs (Figure 1, part A)—specifically, that %iAs had a direct effect on %MMA and an indirect effect on %DMA and that %MMA had a direct effect on %DMA. Additionally, all UAMs were assumed to have a direct effect on skin lesions. Additional covariates that were associated with %iAs, %MMA, and %DMA or were considered to be risk factors were included in the path analysis if they improved the fit of the model. The final path analysis conformed to all model fit statistics (Table 3 and Figure 1, part B) and explained 7%, 13%, and 35% of the observed variability in %iAs, %MMA, and %DMA, respectively.

All standardized path coefficients in the models were statistically significant except for the effect of water arsenic to %DMA (Table 4). The standardized path coefficients for the effect of %iAs and %MMA to %DMA were very large (−0.47 and −0.27), indicating a strong relation between these variables in the path (Table 4 and Table 5). The direct effect of drinking water arsenic to %DMA was much smaller than the indirect effect (0.01 vs. 0.15), which suggested that arsenic exposure was mediated by %iAs and %MMA in the path to %DMA. In contrast, the direct effect of drinking water arsenic to %MMA was much larger than the indirect effect (0.22 vs. 0.02), and the direct effect of %iAs was stronger than the indirect effect to %DMA (−0.47 vs. −0.03). These results conform to the understanding that arsenic exposure is correlated with each UAM and that inorganic arsenic is methylated to MMA, and then MMA is methylated to DMA.

To explore sex differences, we stratified the path analysis. The sex-stratified analysis explained 8%, 12%, and 30% of the observed variability in %iAs, %MMA, and %DMA for males and 8%, 7%, and 47% of the observed variability in
%iAs, %MMA, and %DMA for females, respectively. This suggested different methylation capacities for males and females, with a stronger relation between %iAs and %DMA for females than for males (−0.59 vs. −0.44) but similar relations between %MMA and %DMA for females and males (−0.26 vs. −0.28). This may imply that men and women methylate arsenic differently from %iAs to %DMA.

The association between \( \log_{10} \%iAs \), %MMA, and %DMA and skin lesions was evaluated via the logistic regression portion of the path analysis. Only %MMA was significantly associated with an increased risk of skin lesions (adjusted odds ratio (OR\(_{adj}\)) = 1.56, 95% confidence interval (CI): 1.15, 2.12). There was no significant association of %iAs or %DMA with the odds of skin lesions (\( \log_{10} \%iAs: OR_{adj} = 1.06, 95\% CI: 0.75, 1.50; \log_{10} \%DMA: OR_{adj} = 1.07, 95\% CI: 0.33, 3.46 \)). In the sex-stratified models, the odds ratios for the relation between %MMA and skin lesions remained approximately the same for males and females (males: OR\(_{adj}\) = 1.53, 95% CI: 1.03, 2.28; females: OR\(_{adj}\) = 1.61, 95% CI: 0.99, 2.61). However, the confidence intervals were larger in the sex-stratified model, which was probably due to the smaller sample size of the stratified models.

### Conditional logistic regression

We compared the results obtained in the path analysis using conditional logistic regression models that adjusted for the same covariates (water arsenic, sex, age, body mass index, education, betel nut chewing, and \( \log_{10} \) creatinine).
The results from these separate conditional logistic regression models were similar to those observed in the path analysis where the odds of skin lesions increased with log_{10} %MMA (OR_{adj} = 1.56, 95% CI: 0.91, 2.47). No association was observed between the odds of skin lesions and log_{10} %iAs (OR_{adj} = 1.08, 95% CI: 0.69, 1.68) or log_{10} %DMA (OR_{adj} = 0.62, 95% CI: 0.19, 2.04). In comparison, it is interesting to note the greater precision of the path analysis, as indicated by narrower confidence intervals.

**DISCUSSION**

Integrating an a priori understanding of arsenic metabolism and significant covariates allowed us to construct a path analysis that tested the independent effects of each urinary arsenic species on the odds of disease while simultaneously controlling for collinear variables in a statistically efficient manner. This analysis confirmed that persons with a higher proportion of MMA in their urine and subsequently a limited capacity to methylate inorganic arsenic to DMA were more susceptible to arsenic toxicity, as reflected by higher odds of skin lesions. No association between %iAs or %DMA and skin lesions was observed.

**Methylation of arsenic** was originally thought to be a detoxification pathway. However, there is growing evidence that arsenic metabolism produces toxic intermediates. Experimental studies in cell cultures suggest that intermediate trivalent arsenic species (arsenite, MMA_{3}, and DMA_{3})
are more toxic than pentavalent arsenic species (arsenate, MMA$_5$, and DMA$_5$) (25). Of the trivalent species, MMA$_3$ appears to be the most potent; it is capable of inducing reactive oxygen species, cellular stress, and oxidative DNA damage at levels 50 times lower than arsenite (26). MMA$_3$ also has the highest affinity for thiol ligands and forms stable complexes with proteins (27). In addition, in vitro studies in human hepatocytes show that the capacities for inorganic arsenic methylation are saturable and that moderate concentrations of inorganic arsenic inhibit DMA synthesis, resulting in accumulation of inorganic arsenic and MMA in cells (28).

Among controls who had relatively low exposure to arsenic from drinking water (average = 65.5 µg/L; median, 11.4 µg/L), we observed that arsenic exposure was significantly associated with a dose-dependent increase in %iAs and %MMA in the control group. This suggested that arsenic methylation may be a saturable process, specifically the conversion of MMA to DMA. A similar dose-dependent increase in %iAs and %MMA was reported by Ahsan et al. (20). Both our study and that by Ahsan et al. had similar case-control designs and were conducted in Bangladesh, where arsenic exposure is ongoing. While average drinking water arsenic exposures were similar between the 2 studies, the average age of participants was lower in our study (33.3 years for cases and 33.5 years for controls) than in the study by Ahsan et al. (44.4 years for cases and 36.6 years for controls). In addition, the duration of exposure in our study was slightly longer (the average duration of current tube-well use for controls was 9.5 years) than the duration of current tube-well use reported by Ahsan et al. (20) (8.9 years for cases and 7.1 years for controls). Males also had a higher concentration of MMA and a higher risk of skin lesions, which has been reported in many other studies (20, 22, 29, 30).

This study did have limitations. It was a cross-sectional study, and arsenic metabolites were measured in prevalent cases. As such, it is possible that the disease process influenced arsenic metabolism, and we were not able to determine whether there was a temporal relation between %MMA and skin lesions. In addition, we used a single urine sample to evaluate a participant’s arsenic methylation capacity. While previous studies have suggested that UAMs

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Figure 1. A) The a priori path analysis illustrating the set of simple linear regression models describing the relation between arsenic (As) exposure and urinary arsenic metabolites, Bangladesh, 2001–2003. B) The final path analysis including the standardized path coefficients ($r$) for each path and the odds ratio (OR) and 95% confidence interval (CI) for the association between each urinary arsenic metabolite and skin lesions. BMI, body mass index; %DMA, percentage of dimethylarsinic acid; %iAs, percentage of inorganic arsenic; %MMA, percentage of monomethylarsonic acid.
are relatively stable over time (13, 31), a 2-year biomonitoring study conducted by our research group observed that the percentage of UAMs was poorly correlated within the individual (14). This variability in our UAM measurement was probably random, so it is possible that %MMA has an even stronger effect than what we observed. In addition, we did not specifically ask about seafood consumption, which can contribute to urinary MMA and DMA, prior to the collection of the urine sample (32, 33). However, a duplicate diet study conducted by our group in a similar population in Pabna District suggests that seafood intake is not common, with 80% of participants reporting that they never ate sea-

Table 3.  Fit Indices for the Final Path Analysis Model (Figure 1, Part B) Describing the Association Between Urinary Arsenic Metabolites and the Odds of Skin Lesions, Bangladesh, 2001–2003

<table>
<thead>
<tr>
<th>Index</th>
<th>Criterion for “Good Fit”</th>
<th>Fitted Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\chi^2$ P value</td>
<td>$&gt; 0.05$</td>
<td>0.89</td>
</tr>
<tr>
<td>Root mean square error of approximation</td>
<td>$&lt; 0.05$</td>
<td>$&lt; 0.0001$</td>
</tr>
<tr>
<td>Root mean square residuals</td>
<td>$&lt; 0.05$</td>
<td>0.001</td>
</tr>
<tr>
<td>Bentler’s comparative fit index</td>
<td>$&gt; 0.9$</td>
<td>1.00</td>
</tr>
<tr>
<td>Bentler and Bonnet’s normed fit index</td>
<td>$&gt; 0.9$</td>
<td>1.00</td>
</tr>
<tr>
<td>Bentler and Bonnet’s non-normed fit index</td>
<td>$&gt; 0.9$</td>
<td>1.02</td>
</tr>
<tr>
<td>Akaike’s Information Criterion</td>
<td>Smaller is better</td>
<td>-5.11</td>
</tr>
<tr>
<td>Bayesian Information Criterion</td>
<td>Smaller is better</td>
<td>-21.47</td>
</tr>
</tbody>
</table>

Table 4. Standardized Coefficients for Each Path Included in an Analysis of Urinary Arsenic Metabolites and Odds of Skin Lesions, Bangladesh, 2001–2003

<table>
<thead>
<tr>
<th>Effect</th>
<th>Standardized Coefficient</th>
<th>t Statistic</th>
<th>$\rho$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Path from water As to %DMA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\log_{10}$ %MMA</td>
<td>-0.27</td>
<td>-12.81*</td>
<td>0.35</td>
</tr>
<tr>
<td>$\log_{10}$ %iAs</td>
<td>-0.47</td>
<td>-23.20*</td>
<td>0.51</td>
</tr>
<tr>
<td>$\log_{10}$ water As to $\log_{10}$ %DMA</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Path from water As to %MMA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\log_{10}$ %iAs</td>
<td>0.12</td>
<td>5.11*</td>
<td>0.13</td>
</tr>
<tr>
<td>$\log_{10}$ water As to $\log_{10}$ %MMA</td>
<td>0.22</td>
<td>9.54*</td>
<td></td>
</tr>
<tr>
<td>Path from water As to %iAs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\log_{10}$ water As to $\log_{10}$ %iAs</td>
<td>0.18</td>
<td>7.64*</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Table 5. Direct and Indirect Effect Estimates for Each Path Included in an Analysis of Urinary Arsenic Metabolites and Odds of Skin Lesions, Bangladesh, 2001–2003

<table>
<thead>
<tr>
<th>Effect</th>
<th>$\log_{10}$ Water As</th>
<th>$\log_{10}$ %iAs</th>
<th>$\log_{10}$ %MMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standardized direct effects</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>$\log_{10}$ %iAs</td>
<td>0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\log_{10}$ %MMA</td>
<td>0.22</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>$\log_{10}$ %DMA</td>
<td>0.01</td>
<td>-0.47</td>
<td>-0.27</td>
</tr>
<tr>
<td>Standardized indirect effects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\log_{10}$ %MMA: (water As to iAs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\log_{10}$ %DMA: ( [water As to iAs] + [water As to MMA] - [water As to MMA] )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\log_{10}$ %DMA: ( direct - indirect)</td>
<td>-0.15</td>
<td>-0.03</td>
<td></td>
</tr>
<tr>
<td>Standardized total effects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\log_{10}$ %MMA: ( direct + indirect)</td>
<td>0.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\log_{10}$ %DMA: ( direct + indirect)</td>
<td>-0.14</td>
<td>-0.50</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: As, arsenic; DMA, dimethylarsinic acid; iAs, inorganic arsenic; MMA, monomethylarsonic acid.

This study also had several strengths. It used data from a large population-based case-control study that was designed to examine the effects of chronic exposure to lower levels of arsenic. This epidemiologic study took place in Pabna District shortly after the completion of a water quality survey and where chronic arsenic exposure from drinking water was ongoing. While some persons had very high arsenic exposure, overall, drinking water arsenic levels were relatively low, making our results generalizable to a wide range of exposures. In addition, arsenic exposure, UAMs, and diagnosis of skin lesions all occurred at the same time, and cases and controls were examined by the same physician, minimizing the possibility of bias. Finally, the path analysis efficiently isolated the independent effects of %iAs, %MMA, and %DMA on the odds of skin lesions by simultaneously fitting several regression models (linear

and logistic), estimated direct and indirect effects of arsenic exposure by parsing the correlations among the UAMs, and appropriately controlled for collinear variables (35–37).

While reducing arsenic exposure through the provision of safe drinking water must remain the priority for this region, improving arsenic metabolism through dietary interventions may also be helpful for reducing arsenic toxicity. Specifically, folate supplementation has been shown to influence arsenic metabolism by decreasing %MMA and increasing %DMA in urine (38, 39). A nested case-control study of persons who developed skin lesions during a 2-year follow-up period also found an increased risk of skin lesions for persons who were folate-deficient (ORadj = 1.8, 95% CI: 1.1, 2.9) (40). In addition, dietary intakes of niacin have been shown to modestly decrease %MMA in urine (41). Folate deficiency is common in Bangladesh, with the authors of a recent survey reporting that 38.6% of women and 57.0% of men residing in arsenic-endemic regions are folate-deficient (<9 nmol/L) (42).

In conclusion, skin lesions are a well-known early sign of chronic arsenic toxicity and are associated with the majority of arsenic-induced basal and squamous cell skin cancers (2, 9, 43). A person’s ability to methylate arsenic, as indicated by a higher proportion of MMA in urine, was associated with a greater risk of skin lesions in this study. This could help explain why some of the participants appeared to be more susceptible to chronic arsenic toxicity.

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Author affiliations: Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts (Molly L. Kile, Ema G. Rodrigues, David C. Christiani); Department of Biostatistics, Harvard School of Public Health, Boston, Massachusetts (Elaine Hoffman); Department of Occupational and Environmental Health, Keck School of Medicine, University of Southern California, Los Angeles, California (Carrie V. Breton); Dhaka Community Hospital, Dhaka, Bangladesh (Quazi Quamruzzaman, Mahmuder Rahman, Golam Mahiuddin); and Department of Public Health, School of Medicine, Taipei Medical University, Taipei, Taiwan (Yu-Mei Hsueh).

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