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# Urinary arsenic species in an arsenic-affected area of West Bengal, India†

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**Arsenic contamination of groundwater and associated medical problems have long been reported in the Mushidabad district, one of nine arsenic-affected districts in West Bengal, India. In order to estimate people's total exposure to arsenic, we visited 12 arsenic-affected families in that area during 4–7 December 2000 and collected seven tubewell waters used for drinking, cooking and other household purposes and 51 urine samples from those families. The arsenic concentrations in drinking water ranged from 2.7 to 170 ppb. Those families designated A–E, G–I and J took in arsenic concentrations of 72.6 ppb, 154 ppb and 170 ppb respectively. The concentrations of arsenite, arsenate, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) in urine (corrected for creatinine level in the urine), obtained from 51 persons, ranged from 0 to 796.9 ppb (mg creatinine/ml urine)$^{-1}$, from 0 to 1635.2 ppb (mg creatinine/ml urine)$^{-1}$, from 2.1 to 411.0 ppb (mg creatinine/ml urine)$^{-1}$ and from 8.3 to 2017.5 ppb (mg creatinine/ml urine)$^{-1}$ respectively. The average concentration of total arsenic was 59.2 ppb (mg creatinine/ml urine)$^{-1}$. On comparison of the ratios of (MMA + DMA) to total arsenic, the average proportion of (MMA + DMA) was 83.2%, but the proportions were 27.3% and 16.5% for two of the children (2 years old and 13 years old respectively). This result suggested that they might be damaged due to the methylating capacity. When estimating arsenic species in urine obtained from families A–E, G–I and J, these family members normally metabolized the inorganic arsenic to MMA and DMA and eliminated these as such in comparison with an intake of inorganic arsenic from the tubewell water. The arsenic species in urine from people having the same food and life habits showed the same profile in both men and women. There was a good correlation ($p < 0.05$) between the ages of 19 persons in families A–E and the values of (MMA + DMA) or total arsenic in urine.** 

**KEYWORDS:** arsenic species; urine; tubewell water; HPLC–ICP-MS; West Bengal; India; As(III); As(V); MMA; DMA

## INTRODUCTION

The world's two biggest cases of arsenic contamination of groundwater are in Bangladesh and West Bengal, India. More than 100 million people are living in arsenic-affected districts in these two countries. Arsenic contamination of groundwater and the associated medical problems have been reported all over the world. The arsenic contamination incident in well waters in Taiwan (1961–85) is well known.[1] Black-foot disease was noted, as were other arsenical manifestations such as hyperkeratosis, spotted melanosis and diffuse keratosis. The arsenic concentration in well waters ranged from 10 to 1820 ppb. At Monte Quemado, Cordoba Province, Argentina, similar groundwater arsenic problem was reported.[2] Recently, arsenic-contaminated groundwater in Vietnam has been reported.[3,4]

In 1987, Chakraborti and Saha[5] reported arsenical skin manifestations in five districts of West Bengal. Since 1989, this group has continued to report on the arsenic incident in West Bengal, India.[6–10] The district of Mushidabad is located near to the border with Bangladesh and the groundwater is

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highly contaminated with arsenic. In order to estimate the total exposure of humans to arsenic, we visited that area during 4–7 December 2000 with Dr Chakraborti's group and collected seven tubewell water and 51 urine samples from 12 arsenic-affected families.

We determined the arsenic species in urine by using high-performance liquid chromatography–conductively coupled plasma mass spectrometry (HPLC–ICP-MS) and report the correlation between arsenic species in urine and arsenic in tubewell waters.

## MATERIAL AND METHODS

### Reagents and samples

Sodium arsenite (As(III)) and sodium arsenate (As(V)) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and arsenobetaine were obtained from Tri Chemical Lab. (Yamanashi, Japan). Other chemicals (analytical grade) were also from Wako Pure Chemical Industries (Osaka, Japan).

Stock standard solutions, each of 1000 ppm, were obtained by weighing accurate amounts of As(III), As(V), MMA and DMA and dissolving them in MilliQ water. These stock standard solutions were kept in the refrigerator at 4 °C until required.

Mixed standard solutions, one containing 25 ppb of each arsenic species and one containing 100 ppb of each arsenic species were prepared daily from the stock standard solutions, by the appropriate dilution.

Spot urine samples were collected from 51 persons from 12 arsenic-affected families and were kept in polyethylene bottles. The samples were not subjected to any chemical treatment. After collection, the samples were stored in a cooler icebox.

The tubewell water samples were collected from the hand tubewells, used by family members for drinking and cooking and for other household purposes. The water samples were stored in polyethylene bottles; one drop of nitric acid was added as a preservative, and they were then stored in a cooler icebox.

Both the urine and tubewell water samples were transported from India to Japan and kept in a refrigerator at −30 °C in the laboratory before use. Each family member, their arsenical symptoms, gender and age from the 51 persons studied and the amount of arsenic in their tubewell water are listed in Table 1.

### Instrumentation

A Model Agilent 7500 ICP mass spectrometer (Agilent, DE, USA) was used for detecting the arsenic species. The operating conditions for ICP-MS are shown in Table 2.

The chromatograph had an STM-10A system controller with a Shimadzu 10AC HPLC pump, Shimadzu SIL-10A auto sampler and Shimadzu CTO-10AC column oven. The

**Table 1.** Symptoms, gender and age of inhabitants and arsenic concentrations in tubewell water

| | Symptom | Sex[a] | Age (years) | As (ppb) in water |
|---|---|---|---|---|
| A-1 | + | M | 40 | 72.6 |
| A-2 | + | F | 30 | 72.6 |
| A-3 | | M | 4 | 72.6 |
| A-4 | | M | 8 | 72.6 |
| A-5 | | F | 10 | 72.6 |
| B-1 | | M | 35 | 72.6 |
| B-2 | + | F | 25 | 72.6 |
| B-3 | | M | 2 | 72.6 |
| C-1 | + | M | 30 | 72.6 |
| C-2 | | M | 4 | 72.6 |
| C-3 | | F | 23 | 72.6 |
| D-1 | + | M | 28 | 72.6 |
| D-2 | | M | 3 | 72.6 |
| D-3 | | M | 2 | 72.6 |
| D-4 | | F | 22 | 72.6 |
| E-1 | + | M | 56 | 72.6 |
| E-2 | + | F | 45 | 72.6 |
| E-3 | + | M | 24 | 72.6 |
| E-4 | + | M | 28 | 72.6 |
| E-5 | | F | 20 | 72.6 |
| F-1 | + | M | 45 | 23.9 |
| F-2 | + | F | 35 | 23.9 |
| F-3 | | M | 13 | 23.9 |
| F-4 | | M | 10 | 23.9 |
| F-5 | | M | 7 | 23.9 |
| G-1 | + | M | 40 | 154.0 |
| G-2 | + | F | 15 | 154.0 |
| G-3 | + | F | 33 | 154.0 |
| G-4 | + | F | 12 | 154.0 |
| H-1 | + | M | 55 | 154.0 |
| H-2 | | F | 40 | 154.0 |
| H-3 | | F | 20 | 154.0 |
| H-4 | | F | 20 | 154.0 |
| H-5 | | F | 3 | 154.0 |
| H-6 | + | M | 26 | 154.0 |
| I-1 | + | M | 48 | 154.0 |
| I-2 | + | F | 40 | 154.0 |
| I-3 | | F | 16 | 154.0 |
| J-1 | | M | 32 | 170.0 |
| J-2 | | F | 25 | 170.0 |
| J-3 | | F | 8 | 170.0 |
| J-4 | | F | 6 | 170.0 |
| K-1 | + | M | 45 | 2.7 |
| K-2 | + | F | 35 | 2.7 |
| K-3 | + | F | 13 | 2.7 |
| K-4 | | M | 10 | 2.7 |
| L-1 | + | M | 44 | 7.3 |
| L-2 | + | F | 32 | 7.3 |
| L-3 | | F | 15 | 7.3 |
| L-4 | | F | 9 | 7.3 |
| L-5 | | M | 5 | 7.3 |

[a] M: male; F: female.

**Table 2.** ICP-MS conditions

| | |
|---|---|
| RF power (W) | 1400 |
| RF refraction (W) | 5 |
| Plasma gas flow (l $min^{-1}$) | 15 |
| Carier gas flow (l $min^{-1}$) | 0.8 |
| Monitoring mas | 35 (Cl), 75 (As) |
| Integrating interval (s) | 0.3 |
| Scan number | 1 |

analytical column was a Gel PAK GL-IC-A15 (4.6 mm i.d. × 150 mm) packed with cation-exchange resin (Hitachi Kasei Co. Ltd., Tokyo, Japan). HPLC was performed under the following conditions: mobile phase 10 mM phosphate buffer (pH 6.0), flow rate 1 ml $min^{-1}$, column temperature 35 °C and injection volume 20 µl. The outlet from the separation column was connected directly to the nebulizer of the ICP mass spectrometer using a polyethylene tube of 0.3 mm i.d. when determining the amount of arsenic in tubewell waters, the analytical column was removed from the HPLC system and the HPLC pump was connected with the rheodyne injector and the nebulizer of ICP mass spectrometer.

## HPLC–ICP-MS analysis

After defrosting, 20 µl of urine was injected into the HPLC column and the peak areas of the arsenic species were measured by ICP-MS for 8 min. The amounts of arsenic species were calculated using working curves prepared by using 0, 25 and 100 ppb solutions of arsenic species.

After defrosting, 100 µl of tubewell water was injected into the rheodyne injector and the peak area of arsenic was measured by ICP-MS for 2 min. The amounts of inorganic arsenic were calculated using working curves prepared by using 0.1 to 1000 ppb inorganic arsenic solutions.

After a day of work, the skimmer cone and the sampling cone of the ICP mass spectrometer were always cleaned with water.

## Assay of urinary creatinine

1 ml of urine was diluted with MilliQ water to 10 ml, 0.5 ml of the diluted urine was put into a centrifuge tube and 3 ml of a solution for eliminating the urinary protein was added.

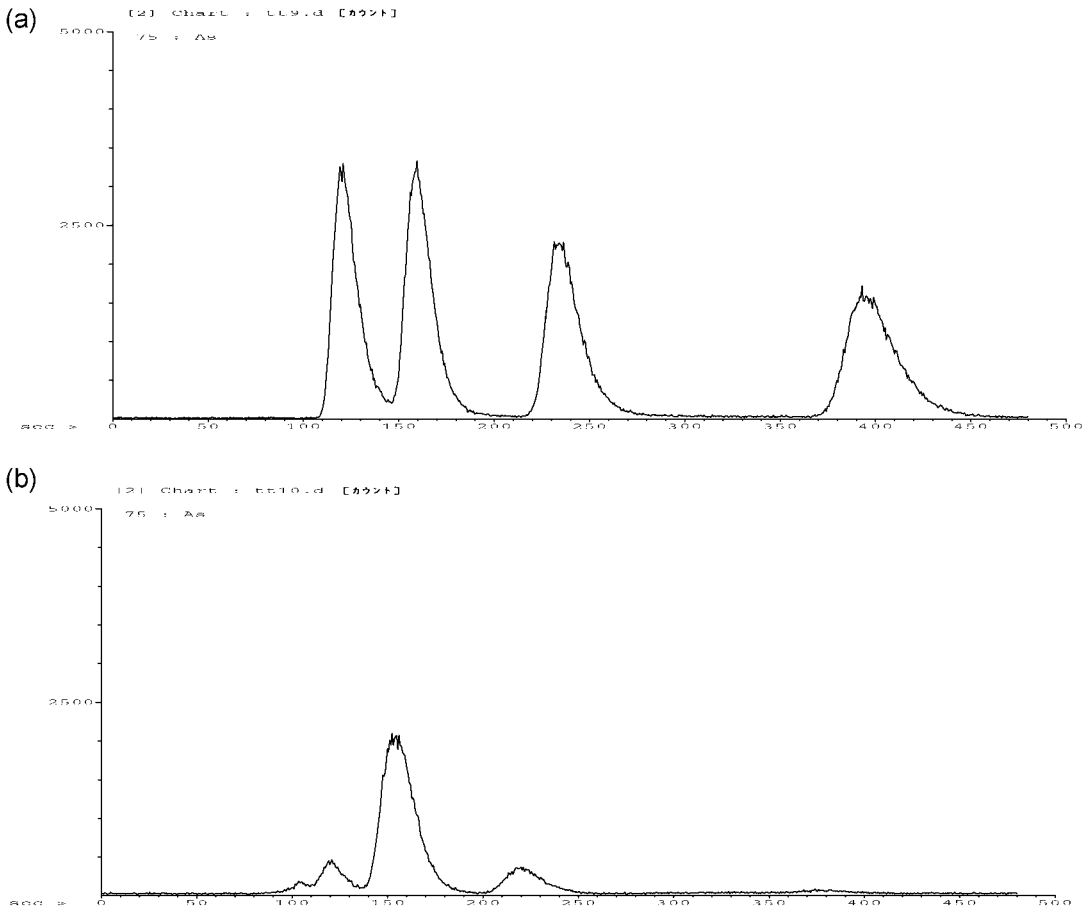


**Figure 1.** HPLC chromatogram for: (a) As(III), DMA, MMA and As(V); (b) arsenic species from D-1 urine.

 

After standing for 10 min, the urinary solution was centrifuged for 10 min at 2500 rpm, 2 ml of supernatant was put into the test tube and 1 ml of picric acid solution and 1 ml of 0.75 M sodium hydroxide solution were added and mixed well. After standing for 20 min in a water bath at 25 to 30 °C, the absorbance at 520 nm was determined. The creatinine standard solutions, which ranged from 0.025 to 0.10 mg $ml^{-1}$, were prepared and the working curve was made. The urinary creatinine was calculated by using the working (calibration) curve.

## RESULTS AND DISCUSSION

In general, the Indian family system is one of an extended family in which the parent and sons' families reside together. One such extended family in this study was comprised of five brothers' families (labeled A to E) and a second was comprised of three (G to I). They used the same tubewell water for drinking, cooking and other household purposes. Dr Chakraborti's group continuously surveyed Mushidabad and checked so arsenic symptoms and the arsenic concentrations of tubewell waters. Their habitants who had skin damage (such as melanosis or keratosis) caused by arsenic are shown in Table 1. When determining total arsenic in tubewell water (Table 1), the arsenic amounts were between 2.7 and 170.0 ppb. The Indian guideline for arsenic in drinking water is below 50 ppb. The question arises as to why seven peoples manifested arsenic symptoms on drinking water containing below 50 ppb arsenic. Several years ago Dr Chakraborti's group examined their tubewell waters and found high concentrations of arsenic (>50 ppb). So they were recommended to stop using their tubewell waters for drinking, cooking and other household purposes. Finally, the families of F, K and I stopped drinking water from the contaminated tubewells and installed a new well. However, but the symptoms of family member; of F, K and I remained during our visit to Mushidabad. There were 26 males and 25 females, and their ages ranged from 2 to 56 years old.

On injecting 20 µl of As(III), As(V), MMA and DMA at 100 ppb, the HPLC chromatogram shown in Fig. 1a was obtained. The retention times $t_R$ of As(III), DMA, MMA and As(V) were 110 s, 150 s, 220 s and 430 s respectively. The HPLC chromatogram obtained from D-1 urine is shown in Fig. 1b. The peaks of As(III), DMA, MMA and As(V) appeared on the chromatogram. The $t_R$ of the small peak at 90 s corresponded to the $t_R$ of standard arsenobetaine (data not shown).

The concentrations of As(III), As(V), MMA and DMA obtained from 51 urine samples are shown in Table 3. Each concentration was corrected for milligrams of creatinine per milliliter of urine. Total arsenic in urine ranged from 13.2 to 2912.5 ppb (mg creatinine/ml urine)$^{-1}$ the average was 590.8 ppb (mg creatinine/ml urine)$^{-1}$. Total arsenic in urine from families K and L, whose tubewell water arsenic concentrations were 2.7 ppb and 7.3 ppb respectively,

**Table 3.** Arsenic species[a] in urine from families A to L

| | As in water | As(III) | As(V) | MMA | DMA | Total |
|---|---|---|---|---|---|---|
| A-1 | 72.6 | 18.8 | 4.7 | 35.1 | 239.2 | 297.8 |
| A-2 | 72.6 | 18.7 | 4.0 | 16.2 | 180.5 | 219.4 |
| A-3 | 72.6 | 22.9 | 10.1 | 48.9 | 295.7 | 377.6 |
| A-4 | 72.6 | 41.9 | 12.1 | 58.4 | 403.0 | 515.4 |
| A-5 | 72.6 | 18.6 | 4.5 | 18.2 | 216.5 | 257.8 |
| B-1 | 72.6 | 23.7 | 6.3 | 17.5 | 141.0 | 188.5 |
| B-2 | 72.6 | 9.4 | 6.6 | 8.6 | 107.6 | 132.2 |
| B-3 | 72.6 | 0.8 | 11.1 | 20.8 | 118.4 | 151.1 |
| C-1 | 72.6 | 21.3 | 4.8 | 26.1 | 162.1 | 214.3 |
| C-2 | 72.6 | 157.2 | 43.7 | 77.9 | 280.3 | 559.1 |
| C-3 | 72.6 | 29.5 | 9.5 | 26.5 | 172.3 | 237.8 |
| D-1 | 72.6 | 30.2 | 11.7 | 41.0 | 189.0 | 271.9 |
| D-2 | 72.6 | 51.3 | 19.1 | 85.0 | 633.9 | 789.3 |
| D-3 | 72.6 | 737.6 | 21.1 | 43.4 | 242.0 | 1044.1 |
| D-4 | 72.6 | 47.5 | 6.4 | 18.0 | 150.6 | 222.5 |
| E-1 | 72.6 | 12.1 | 5.8 | 21.8 | 165.1 | 204.8 |
| E-2 | 72.6 | 17.9 | 4.6 | 17.8 | 212.2 | 252.5 |
| E-3 | 72.6 | 22.8 | 7.7 | 25.1 | 138.1 | 193.7 |
| E-4 | 72.6 | 15.8 | 7.4 | 26.6 | 152.5 | 202.3 |
| E-5 | 72.6 | 66.3 | 10.9 | 44.2 | 155.6 | 277.0 |
| F-1 | 23.9 | 43.7 | 23.1 | 29.1 | 308.0 | 403.9 |
| F-2 | 23.9 | 54.6 | 29.5 | 37.5 | 403.0 | 524.6 |
| F-3 | 23.9 | 796.9 | 1635.2 | 56.3 | 424.1 | 2912.5 |
| F-4 | 23.9 | 45.2 | 10.5 | 32.5 | 517.0 | 605.2 |
| F-5 | 23.9 | 120.8 | 39.8 | 72.1 | 619.0 | 851.7 |
| G-1 | 154.0 | 94.2 | 12.0 | 96.6 | 602.3 | 805.1 |
| G-2 | 154.0 | 166.1 | 13.6 | 132.7 | 1005.3 | 1317.7 |
| G-3 | 154.0 | 70.9 | 13.9 | 131.0 | 884.7 | 1100.5 |
| G-4 | 154.0 | 204.8 | 22.4 | 221.4 | 894.6 | 1343.2 |
| H-1 | 154.0 | 173.5 | 24.9 | 159.8 | 885.2 | 1243.4 |
| H-2 | 154.0 | 139.3 | 22.9 | 124.3 | 752.8 | 1039.3 |
| H-3 | 154.0 | 52.9 | 7.7 | 37.0 | 544.3 | 641.9 |
| H-4 | 154.0 | 334.6 | 8.4 | 411.0 | 1126.0 | 1880.0 |
| H-5 | 154.0 | 258.0 | 42.3 | 298.4 | 2017.5 | 2616.2 |
| H-6 | 154.0 | 101.4 | 12.2 | 60.5 | 233.6 | 407.7 |
| I-1 | 154.0 | 66.1 | 35.5 | 46.5 | 363.6 | 511.7 |
| I-2 | 154.0 | 56.4 | 66.0 | 58.8 | 433.8 | 615.0 |
| I-3 | 154.0 | 91.1 | 39.7 | 70.9 | 466.7 | 668.4 |
| J-1 | 170.0 | 66.4 | 0.0 | 108.5 | 411.5 | 586.4 |
| J-2 | 170.0 | 89.1 | 7.2 | 50.2 | 784.2 | 930.7 |
| J-3 | 170.0 | 216.9 | 5.5 | 78.3 | 957.6 | 1258.3 |
| J-4 | 170.0 | 64.1 | 10.2 | 140.4 | 775.0 | 989.7 |
| K-1 | 2.7 | 5.0 | 3.9 | 8.5 | 26.7 | 44.1 |
| K-2 | 2.7 | 4.4 | 0.0 | 6.2 | 36.6 | 47.2 |
| K-3 | 2.7 | 1.5 | 3.2 | 2.2 | 18.0 | 24.9 |
| K-4 | 2.7 | 1.7 | 2.2 | 3.9 | 24.5 | 32.3 |
| L-1 | 7.3 | 3.2 | 7.5 | 4.4 | 23.4 | 38.5 |
| L-2 | 7.3 | 0.5 | 0.0 | 4.4 | 8.3 | 13.2 |
| L-3 | 7.3 | 1.3 | 1.1 | 2.6 | 25.0 | 30.0 |
| L-4 | 7.3 | 0.0 | 1.0 | 2.1 | 11.6 | 14.7 |
| L-5 | 7.3 | 0.9 | 1.0 | 3.4 | 20.4 | 25.7 |
| Average | | 92.0 | 45.4 | 62.1 | 391.4 | 590.8 |
| Maximum | | 796.9 | 1635.2 | 411.0 | 2017.5 | 2912.5 |
| Minimum | | 0.0 | 0.0 | 2.1 | 8.3 | 13.2 |

[a] Units: ppb (mg creatinine/ml urine)$^{-1}$.

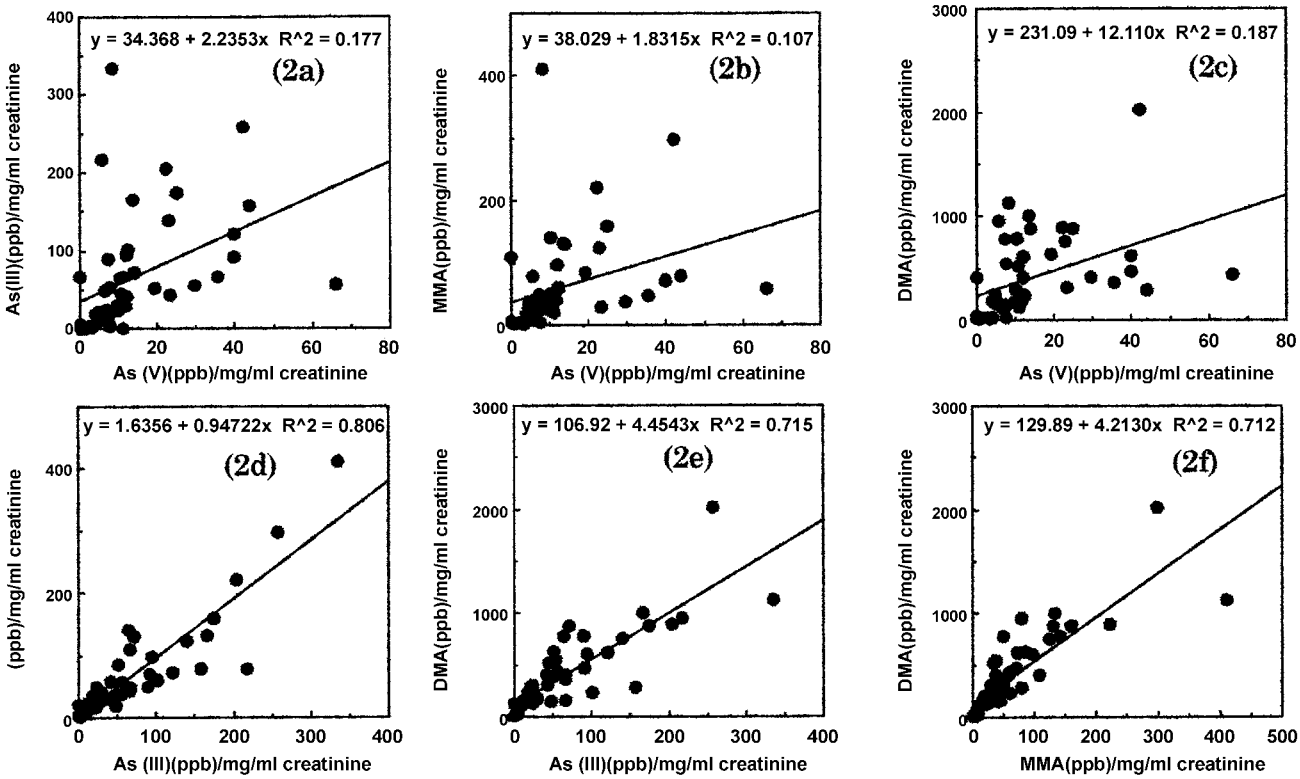


**Figure 2.** Relationship between each arsenic speciation in urine from families A–E and G–I.

ranged from 13.2 to 47.2 ppb (mg creatinine/ml urine)$^{-1}$. In the case of family F, the arsenic concentration of 23.9 ppb in tubewell water was comparable to the Indian guideline for arsenic in drinking water (<50 ppb). However, the total arsenic in their urine was at a high level, ranging from 403.9 to 2912.5 ppb (mg creatinine/ml urine)$^{-1}$. These results suggest the possibility of intake of arsenic from other sources. This may be from another arsenic-contaminated tubewell or from some other source. The urine from D-3 (boy, 3 years old) and F-3 (boy, 13 years old) contained high amounts of inorganic arsenic (As(III): 737.6 and 796.9 ppb (mg creatinine/ml urine)$^{-1}$; As(V): 21.1 and 1635.2 ppb (mg creatinine/ml urine)$^{-1}$). These values were quite abnormal with respect to the other values. When the data obtained from D-3 and F-3 are eliminated, urinary As(III) or As(V) ranged from 0 to 334.6 ppb (mg creatinine/ml urine)$^{-1}$ (average 64.4 ppb (mg creatinine/ml urine)$^{-1}$) or from 0 to 66.0 ppb (mg creatinine/ml urine)$^{-1}$ (average 13.5 ppb (mg creatinine/ml urine)$^{-1}$) respectively for all other cases. The urinary MMA and DMA ranged from 2.1 to 411.0 ppb (mg creatinine/ml urine)$^{-1}$ (average 62.6 ppb (mg creatinine/ml urine)$^{-1}$) and from 8.3 to 2017.5 ppb (mg creatinine/ml urine)$^{-1}$ (average 393.8 ppb (mg creatinine/ml urine)$^{-1}$) respectively.

We selected families A–E, G–I and J because these families used tubewell water which was above the Indian guideline for arsenic in drinking water. There were 37 persons in these families. The relationship between each arsenic species is demonstrated in Fig. 2. Figure 2a–c related As(V) to the three other species. Statistically, there was not a good correlation ($p > 0.05$) between As(V) and the other species. Figure 2d–f shows the relationship between As(III) and MMA, As(III) and DMA and MMA and DMA. Each relationship gave a good correlation. This evidence might reflect an arsenic metabolism such as the metabolic pathway of As(V) → As(III) → MMA → DMA.

We estimated the ratios of (MMA + DMA) to total arsenic or of MMA to (MMA + DMA). The ratios of (MMA + DMA) to total arsenic obtained from D-3 (boy, 3 years old) and F-3 (boy, 13 years old) were remarkably low against the average. Total arsenic from D-3 and F-3 were 1044.1 ppb (mg creatinine/ml urine)$^{-1}$ and 2912.5 ppb (mg creatinine/ml urine)$^{-1}$ respectively. However, G-2 to G-4, H-1, H-2, H-4, H-5 and I-3 also excreted high amounts of total arsenic into the urine. On comparing those urine sample having high levels of arsenic, the ratios (as a percentage) of (MMA + DMA) to total arsenic obtained from D-3 and F-3 were 27.3 of and 16.5% and differed from the ratio of 80.9 to 92.3% from G, H and I. So the result, suggest that D-3 and F-3 might have a damaged arsenic-methylating enzyme. The ratios of (MMA + DMA) to

 

**Table 4.** Arsenic metabolites and total arsenic in urine and the ratios MMA/(MMA + DMA), (MMA + DMA)/total arsenic and (MMA + DMA)/total arsenic

| | MMA + DMA[a] | Total arsenic[a] | MMA/(MMA + DMA)(%) | (MMA + DMA)/total As (%) |
|---|---|---|---|---|
| A-1 | 274.3 | 297.8 | 12.8 | 92.1 |
| A-2 | 196.7 | 219.4 | 8.2 | 89.7 |
| A-3 | 344.6 | 377.6 | 14.2 | 91.3 |
| A-4 | 461.4 | 515.4 | 12.7 | 89.5 |
| A-5 | 234.7 | 257.8 | 7.8 | 91.1 |
| B-1 | 158.5 | 188.5 | 11.0 | 84.1 |
| B-2 | 116.2 | 132.2 | 7.4 | 87.9 |
| B-3 | 139.2 | 151.1 | 14.9 | 92.1 |
| C-1 | 188.2 | 214.3 | 13.8 | 87.8 |
| C-2 | 358.2 | 559.1 | 21.8 | 64.1 |
| C-3 | 198.8 | 237.8 | 13.3 | 83.6 |
| D-1 | 230.0 | 271.9 | 17.8 | 84.6 |
| D-2 | 718.9 | 789.3 | 11.8 | 91.1 |
| D-3 | 285.4 | 1044.1 | 15.2 | 27.3 |
| D-4 | 168.6 | 222.5 | 10.7 | 75.8 |
| E-1 | 186.9 | 204.8 | 11.7 | 91.3 |
| E-2 | 230.0 | 252.5 | 7.7 | 91.1 |
| E-3 | 163.2 | 193.7 | 15.4 | 84.3 |
| E-4 | 179.1 | 202.3 | 14.9 | 88.5 |
| E-5 | 199.8 | 277.0 | 22.1 | 72.1 |
| F-1 | 337.1 | 403.9 | 8.6 | 83.5 |
| F-2 | 440.5 | 524.6 | 8.5 | 84.0 |
| F-3 | 480.4 | 2912.5 | 11.7 | 16.5 |
| F-4 | 549.5 | 605.2 | 5.9 | 90.8 |
| F-5 | 691.1 | 851.7 | 10.4 | 81.1 |
| G-1 | 698.9 | 805.1 | 13.8 | 86.8 |
| G-2 | 1138.0 | 1317.7 | 11.7 | 86.4 |
| G-3 | 1015.7 | 1100.5 | 12.9 | 92.3 |
| G-4 | 1116.0 | 1343.2 | 19.8 | 83.1 |
| H-1 | 1045.0 | 1243.4 | 15.3 | 84.0 |
| H-2 | 877.1 | 1039.3 | 14.2 | 84.4 |
| H-3 | 581.3 | 641.9 | 6.4 | 90.6 |
| H-4 | 1537.0 | 1880.0 | 26.7 | 80.9 |
| H-5 | 2315.9 | 2616.2 | 12.9 | 88.5 |
| H-6 | 294.1 | 407.7 | 20.6 | 72.1 |
| I-1 | 410.1 | 511.7 | 11.3 | 80.1 |
| I-2 | 492.6 | 615.0 | 11.9 | 80.1 |
| I-3 | 537.6 | 668.4 | 13.2 | 80.4 |
| J-1 | 520.0 | 586.4 | 20.9 | 88.7 |
| J-2 | 834.4 | 930.7 | 6.0 | 89.6 |
| J-3 | 1035.9 | 1258.3 | 7.6 | 82.3 |
| J-4 | 915.4 | 989.7 | 15.3 | 92.5 |
| K-1 | 35.2 | 44.1 | 24.2 | 79.9 |
| K-2 | 42.8 | 47.2 | 14.5 | 90.6 |
| K-3 | 20.2 | 24.9 | 10.8 | 81.2 |
| K-4 | 28.4 | 32.3 | 13.7 | 88.1 |
| L-1 | 27.8 | 38.5 | 15.9 | 72.2 |
| L-2 | 12.7 | 13.2 | 34.8 | 96.5 |
| L-3 | 27.6 | 30.0 | 9.4 | 91.9 |
| L-4 | 13.7 | 14.7 | 15.3 | 93.4 |
| L-5 | 23.8 | 25.7 | 14.3 | 92.5 |
| Average | 453.5 | 590.8 | 13.8 | 83.2 |
| Maximum | 2315.9 | 2912.5 | 34.8 | 96.5 |
| Minimum | 12.7 | 13.2 | 5.9 | 16.5 |

[a] Units: ppb/(mg creatinine/ml urine).

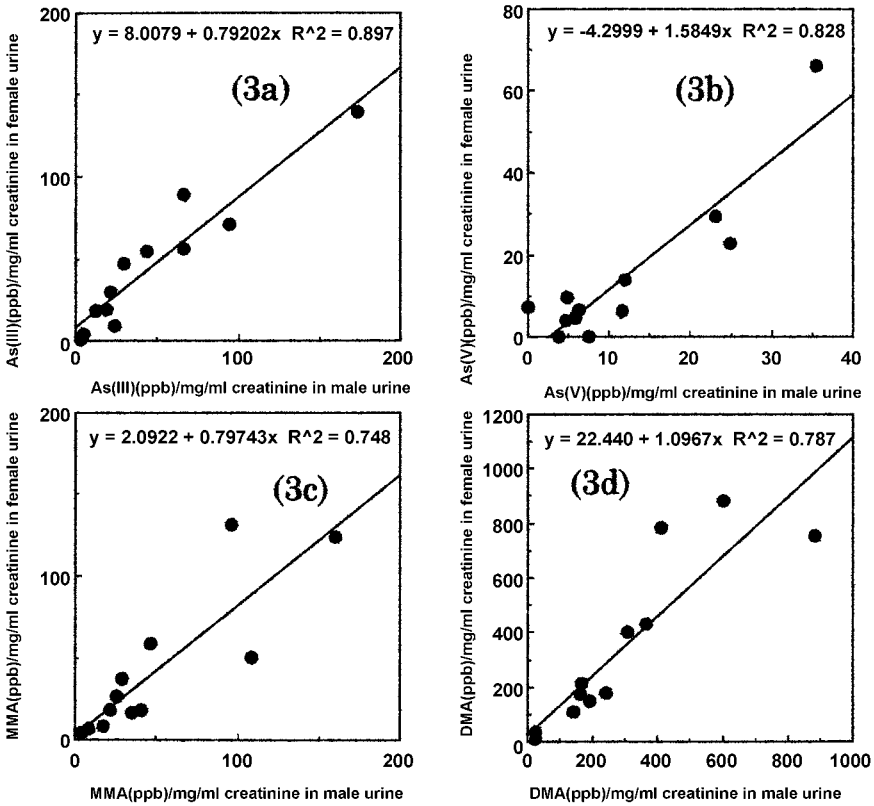


**Figure 3.** Relationship of arsenic speciation between each couple in families.

total arsenic obtained from 49 persons ranged from 72.0 to 96.5%. Del Razo *et al.*[11] investigated arsenic species in control urine (arsenic concentration of drinking water was 25 ppb) from Santa Ana, Cohuila, Mexico, and exposed urine (arsenic concentration of drinking water was an average 415 ppb) from Nazareno, Durango, Mexico, and reported that the ratios of (MMA + DMA) to total arsenic were 86.8% and 66.2% respectively. Their data correspond with our data except for

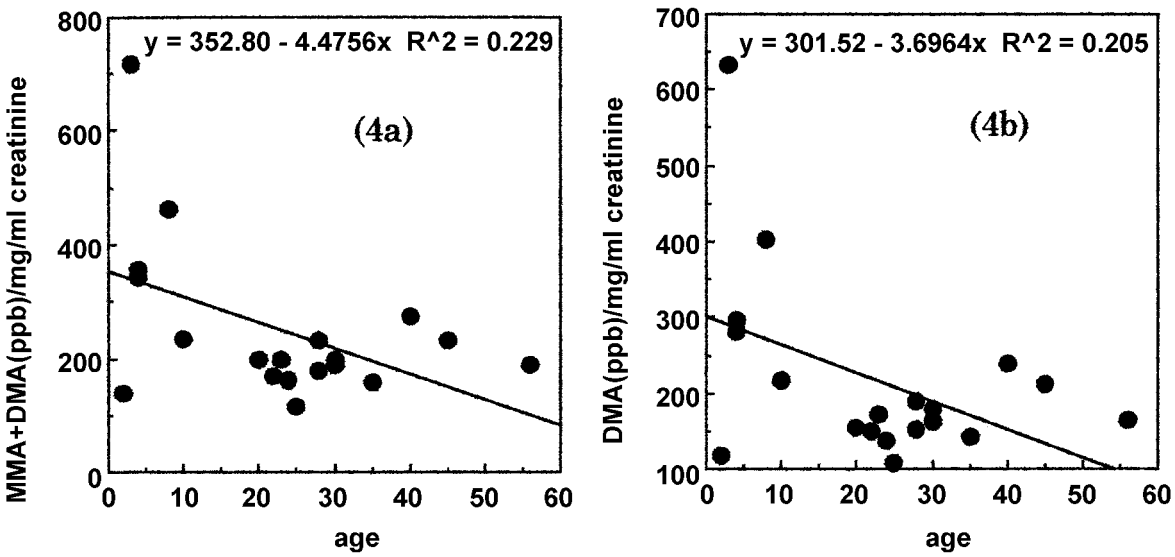


**Figure 4.** Relationship between age and arsenic species from families A–E.

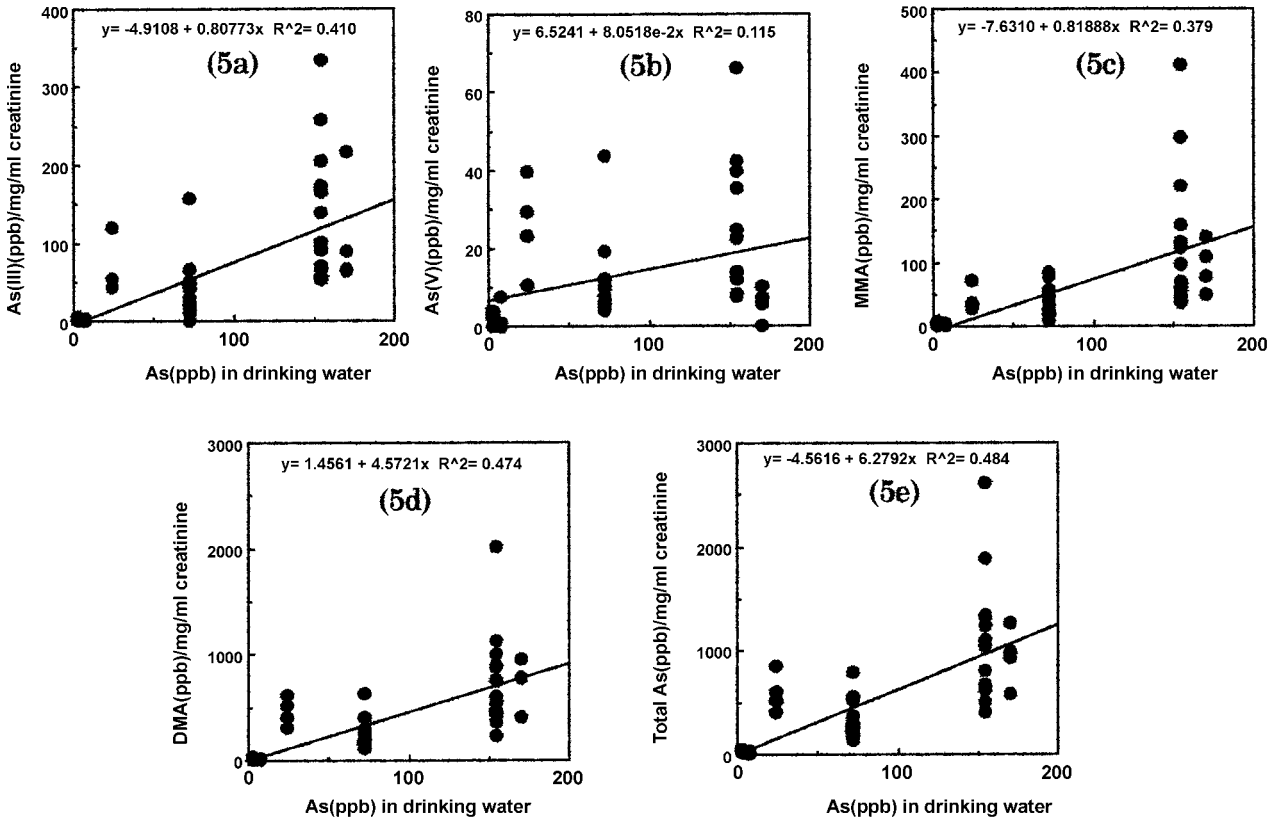


**Figure 5.** Relationship between arsenic in tubewell water and total arsenic in urine.

D-3 and F-3. The results suggested to us that 49 persons (except D-3 and F-3) maintained the normal arsenic-methylating capacity in their bodies.

In order to estimate the correlation between males and females and urinary arsenic species we selected couples in each family. Total arsenic in male and female urine ranged from 38.5 to 1243.4 ppb (mg creatinine/ml urine)$^{-1}$ and from 13.2 to 1100.5 ppb (mg creatinine/ml urine)$^{-1}$ respectively. The averages of total arsenic were 400.9 ppb (mg creatinine/ml urine)$^{-1}$ and 444.6 ppb (mg creatinine/ml urine)$^{-1}$ respectively. The relationships of arsenic species between male and female are shown in Fig. 3. There was a good correlation ($p < 0.05$) between males and females, as shown in Fig. 3a–d.

In order to estimate the correlation between age and urinary arsenic species we selected families A to E, because they used the same tubewell water for drinking, cooking and other household purposes. The relationship between the amounts of (MMA + DMA) and ages is shown in Fig. 4. There was a good correlation between the amounts of (MMA + DMA) and age.

We then estimated the correlation between urinary arsenic species and the arsenic concentrations in tubewell waters. The sample number of tubewell waters was six and it was difficult to evaluate statistically the correlation between the urinary arsenic species and the arsenic concentration in the tubewell waters. The correlations between the urinary arsenic species and the arsenic concentration in tubewell waters are shown in Fig. 5. The relationship between the As(III), MMA, DMA or total arsenic and the arsenic concentrations in tubewell waters are in good agreement, as shown in Fig. 5a and c–e.

## CONCLUSIONS

The results of our field surveys of tubewell waters and human urine obtained from the arsenic-affected district of Mushidabad, in West Bengal, India, were as follows.

(1) The arsenic concentrations in tubewell waters ranged from 2.7 to 170 ppb.
(2) The concentrations of As(III), As(V), MMA and DMA in urine obtained from 51 persons ranged from 0 to 796.9 ppb (mg creatinine/ml urine)$^{-1}$, from 0 to 1635.2 ppb (mg creatinine/ml urine)$^{-1}$, from 2.1 to 411.0 ppb (mg creatinine/ml urine)$^{-1}$ and from 8.3 to 2017.5 ppb (mg creatinine/ml urine)$^{-1}$ respectively. The average of total arsenic was 590.8 ppb (mg creatinine/ml urine)$^{-1}$.
(3) On comparison of the ratio of (MMA + DMA) to total

 

arsenic, the average was 83.2%, but the ratios (as a percentage) from two boys whose ages were 2 years and 13 years were 27.3% and 16.5% respectively. This result suggests that these two boys might have suffered damage to their arsenic methylating capacity.

(4) When estimating the arsenic species in urine obtained from families A–E, G–I and J, the relationships between As(III) and MMA, As(III) and DMA or MMA and DMA in urine were in good agreement ($p < 0.05$).
(5) The arsenic species in urine from couples showed the same profile in males as in females.
(6) On selecting 19 urine samples from families A to E, the relationship between the urinary (MMA + DMA) and the ages was in good agreement ($p < 0.05$).
(7) The relationships between As(III), MMA, DMA and total arsenic in urine and arsenic concentration in tubewell waters were in good agreement ($p < 0.05$).

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