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Arsenic Exposure in Pregnancy: A Population-based Study in Matlab, Bangladesh

Marie E. Vahter[1], Li Li[1], Barbro Nermell[1], Anisur Rahman[2,3], Shams El Arifeen[2], Mahfuzar Rahman[2], Lars Åke Persson[3], and Eva-Charlotte Ekström[1,3]

[1]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden, [2]ICDDR,B, GPO Box 128, Dhaka 1000, Bangladesh, and [3]International Maternal and Child Health, Uppsala University, Sweden

ABSTRACT

This study assessed the exposure of pregnant women to arsenic in Matlab, Bangladesh, an area with highly-elevated concentrations of arsenic in tubewells, by measuring concentrations of arsenic in urine. In a defined administrative area, all new pregnancies were identified by urine test in gestational week 6-8, and women were asked to participate in the assessment of arsenic exposure. Urine for analysis of arsenic was collected immediately and in gestational week 30. In total, 3,426 pregnant women provided urine samples during January 2002–March 2003. There was a considerable variation in urinary concentrations of arsenic (total range 1-1,470 µg/L, adjusted to specific gravity 1.012 g/mL), with an overall median concentration of 80 µg/L (25th and 75th percentiles were 37 and 208 µg/L respectively). Similar concentrations were found in gestational week 30, indicating no trend of decreasing exposure, despite the initiation of mitigation activities in the area. Arsenic exposure was negatively associated with socioeconomic classes and achieved educational level. There were marked geographical variations in exposure. The results emphasize the urgent need for efficient mitigation activities and investigations of arsenic-related reproductive effects.

***Key words*:** Arsenic; Arsenic exposure; Spatial variation; Pregnancy; Bangladesh

INTRODUCTION

There is an increasing number of regions world-wide with elevated concentrations of arsenic in groundwater used for drinking purposes (1,2). Without testing for arsenic, a large number of shallow tubewells have been installed, particularly in low-income countries, due to concerns for infectious diseases caused by waterborne pathogens. Many of those tubewells have later been found to contain water with high concentrations of arsenic. Exposure to inorganic arsenic is associated with various forms of cancer, skin-effects (hyperkeratosis, melanosis), respiratory effects, diabetes, hypertension, and liver- and neurotoxicity (1,3). Although arsenic is known to pass the placenta to the foetus (4), little information is available on foetal exposure levels of arsenic and its effects on early human development (5-9).

The present project concerns a population-based study of individual arsenic exposure by measuring concentrations of arsenic in urine of women in Matlab, Bangladesh, an area with high prevalence of arsenic-contaminated tubewell water (10). The project is part of our ongoing research in Bangladesh aimed at elucidating the effect of exposure to arsenic on early human development, using estimates of individual exposure. Bangladesh is one of the countries most severely affected by arsenic in groundwater, and it has been estimated that about half of the total 6-11 million tubewells that have been installed since the early 1970s are contaminated with arsenic above the World Health Organization's guideline value of 10 µg/L (11,12). While there are numerous studies of ar-

Correspondence and reprint requests should be addressed to:
Dr. Marie Vahter
Institute of Environmental Medicine
Karolinska Institutet
PO Box 210
17177 Stockholm
Sweden
Email: marie.vahter@ki.se

senic in tubewell water, data on the actual intake of arsenic in the population is largely missing. There are obvious advantages with urine-based assessment of arsenic exposure because of variations in concentrations of arsenic in tubewell water, considerable inter-individual variation in water intake, both for drinking and cooking purposes, and additional exposure via contaminated food (13). A further justification for the need of data on individual intake of arsenic is to enable evaluation of mitigation activities in the area (14). Therefore, we have determined individual arsenic exposure based on concentrations of arsenic in urine. Inorganic arsenic, the form of arsenic in groundwater, is metabolized in the body by methylation and excreted in urine as methylarsonic acid (MMA) and dimethylarsinic acid (DMA), besides a certain fraction of unmetabolized inorganic arsenic (15). The concentration of arsenic in urine is remarkably stable over time in the case of continuous exposure via drinking-water (16,17). This is most likely true for populations such as that in Bangladesh with limited intake of other fluids, e.g. bottled water or commercial soft drinks, besides domestic drinking-water.

MATERIALS AND METHODS

Study area and population

The study was carried out in Matlab, 53 km southeast of Dhaka, where the Meghna River joins the confluent streams of the Brahmaputra and Ganges rivers, and groundwater is highly affected by the historic natural sedimentation of arsenic-laden soils transported by the rivers from the mountains in the north. The project benefited from ICDDR,B: Centre for Health and Population Research, Bangladesh and its Health and Demographic Surveillance System (HDSS), covering 142 villages in Matlab with a population of 220,000. The HDSS records all vital events, such as births, deaths, marriages, pregnancies and different pregnancy outcomes, and in- and out-migrations. The databases are updated monthly based on information collected through home-visits by community health research workers. ICDDR,B has also a central health facility in Matlab and four sub-centres (in areas called block A, B, C, and D), all equipped to provide health services to the population and to support clinical and public-health research in the area covering a population of 110,000.

Our study is nested into a food and micronutrient-supplementation trial among pregnant women. All women living in the coverage area of the ICDDR,B-administered antenatal care (block A-D) and who became pregnant during November 2001–October 2003 were invited to be assessed for eligibility (n=5,880) to the supplementation trial. Arsenic exposure has, so far, been assessed for women who became pregnant during January 2002–March 2003. Socioeconomic data were excerpted from the HDSS databases. For socioeconomic status, a wealth index was constructed using asset ownership based on the model and relevant for assets in these rural settings (18). Each household asset with available information was assigned a weight or factor, and score was generated through principal component analysis. Household asset scores, divided into quintiles (1 representing the poorest and 5 the richest), were used for classifying the socioeconomic status of individuals. Asset scores and achieved education level expressed by number of years of formal schooling were used as stratifying variables for arsenic exposure.

Assessment of exposure to inorganic arsenic

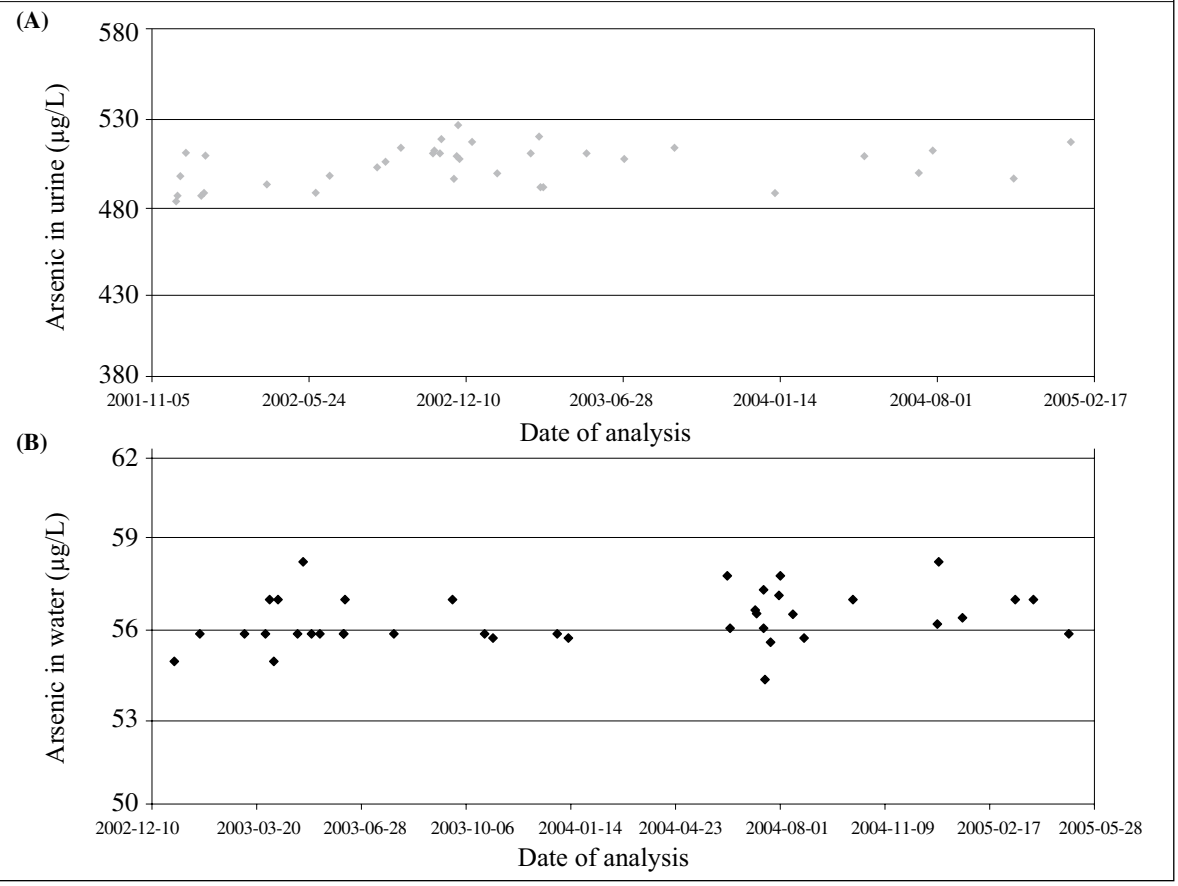
Concentrations of arsenic in drinking-water have been determined in a parallel research project, aimed at assessing the prevalence of arsenic-induced skin lesions in Matlab (10,19). In total, 166,934 individuals, including the women in the current project, were visited in their homes during January 2002–August 2003. After the screening for skin lesions had been completed in an area, concentrations of arsenic in tubewell water were tested using a field-kit (14). If water contained arsenic of more than 50 µg/L, the tubewell was painted red, and if <50µg/L, the tubewell was painted green. A second water sample was collected in 24-mL plastic vials (Zinsser Analytic GMBH, Frankfurt, Germany), checked to be free from arsenic contamination, and was analyzed for arsenic content by atomic absorption spectrophoto-metry (19).

Within the HDSS, women with amenorrhoea at the time of the monthly home-visit by the community health research workers were invited to an immediate urinary pregnancy test. Thus, in most cases, pregnancy was detected in week 6-8. In the case of positive pregnancy test, the woman was asked to donate a urine sample for the measurement of arsenic. This urine sampling was initiated in late January 2002. The women were invited to one of the health clinics for ultrasound investigation for confirmation of pregnancy and assessment of gestational age. Additional clinical investigations and sampling of urine were carried out in gestational weeks 14, 19, and 30. In this study, data on concentrations of arsenic in urine collected in early pregnancy (gestational week 6-8) and late (week 30) of women who became pregnant during January

2002–March 2003 were analyzed. In total, 3,426 women provided urine samples in week 6-8 and 2,025 in week 30. The estimated 41% loss of subjects between gestational week 6-8 and week 30 was mainly due to refusals or because they were unable to give consent to participate in the supplementation trial (24%), not eligible for supplementation due to exceeded gestational age-criteria (23%), abortions, or miscarriages (22%) and out-migration or could not be located (13%).

Spot-urine samples were collected in disposable plastic urine-collection cups (Papyrus, Gothenburg, Sweden) and were transferred to 24-mL plastic vials (Zinsser Analytic GMBH, Frankfurt, Germany). Samples collected in the women's home (early pregnancy) were kept in insulated bags with cooling blocks while being transported to the hospital laboratory freezers (-80 °C) at the end of the day, at the latest. At the health clinics, the urine samples were kept in a refrigerator until being transported to the hospital freezer. The frozen urine samples (500-1,000 at a time) were regularly transported to the laboratory in Sweden, where the sum of inorganic arsenic and the methylated metabolites, hereinafter referred to as arsenic in urine, were measured by hydride generation-atomic absorption spectroscopy (HG-AAS) (20). The detection limit of the AAS method was 1.3±0.27 µg/L. Because of the long time of urine sampling and the large number of samples, analyses of arsenic were carried out over more than 12 months. Therefore, certified reference materials (NIST 2670 urine HL with certified 480±100 µg/L of arsenic and NIST 1643d water with certified 56.02±0.73 µg/L of arsenic) were included in each analytical run (Fig. 1). As certified reference materials exist only for total arsenic, we also conducted inter-laboratory comparisons of arsenic metabolites in urine to verify the analytical accuracy (within the EU Ashram project). There was a good agreement between our results and the results of University of Graz, Austria, using HPLC-HG-ICPMS (correlation 0.997 within the concentration range of 10-150 µg/L, n=7).

Fig. 1. Results of analysis of reference materials: (A) NIST 2670 urine HL with certified 480±100 µg/L of arsenic and (B) NIST 1643d water with certified 56.02±0.73 µg/L of arsenic, over the time period of analysis of arsenic in collected urine samples



To compensate for variation in the dilution of the urine caused by variation in fluid intake due to temperature and physical activity, we adjusted the obtained concentrations by specific gravity (the average being 1.012 g/mL). Adjustment based on specific gravity is less dependent on muscle-mass and nutritional status than is adjustment by creatinine excretion (21,22). However, the observed low average specific weight of the collected urine samples (on average 1.012 g/mL) indicates that malnutrition, which is common among women in Bangladesh (23), influences the excretion of minerals to some extent. There were eight urine samples in week 6-8 and 76 in week 30 with a specific gravity of 1.001 g/mL, indicating highly-diluted urine samples. Because of the disproportion of low-density samples in early and late pregnancy, it seems possible that pregnancy also affected the dilution of urine as shown in previous studies (24). Therefore, we have excluded those samples (1.5% of the total number of samples) in further respective statistical analyses.

Statistical methods

For statistical computations, we used STATISTICA 7.1 (StatSoft Inc., Tulsa, OK, USA). Descriptive analysis of data included calculations of central tendency (mean/median) and variation (frequency distribution and percentiles). Concentrations of arsenic in water and urine were not normally distributed. To meet the assumptions of normality and equality of variances and to achieve approximately normal distribution for parametric statistical analysis, concentrations of arsenic in water were transformed to a power of 0.2, while natural log-transformation was applied to concentrations of arsenic in urine according to the tests for normal distribution. To evaluate the associations between concentrations in water (independent variable) and urine (dependent variable), Pearson's correlation was used. Mann-Whitney Rank Sum Test was used for testing the comparisons between groups. Observations more than 1.5 times the interquartile range (the height of the box in box-plot) beyond the upper or the lower quartile were defined as outliers, and three times the range as extremes. The statistical significance level was set at $p<0.05$.

Ethics

Because of the long time-period between collection of urine and analysis of arsenic, we could not inform the women about their arsenic exposure. However, the project is linked to a mitigation programme run in collaboration with BRAC, a non-governmental organization in Bangladesh (14). All functioning tube-wells in Matlab were screened for arsenic content, and those with water containing more than 50 µg/L—the national drinking-water standard for arsenic—were painted red to indicate unsafe water (10). A series of village-information meetings were held before starting the study. All households and individuals were informed and asked for consent to participate. Both Ethical Committee at the Karolinska Institute and Ethical Review Committee of ICDDR,B approved the study.

RESULTS

In total, 3,418 pregnant women at gestational week 6-8 and 1,944 at gestational week 30 were included in the assessment of arsenic exposure. The distribution of main individual characteristics (age, area of residence, education level, and socioeconomic status) was similar for the women participating in early and late pregnancy (Table 1). The mean age at the time of recruitment was 27 (range 14-49) years. Concentration of arsenic in urine collected in early pregnancy (gestational week 6-8) varied from 1 to 1,470 µg/L, the mean being 152 µg/L. In total, 47 samples showed less than 10 µg/L. As shown in Figure 2, the distribution of concentrations of arsenic was skewed, and the overall median urinary concentration of arsenic (80 µg/L) was considerably lower than the mean concentration. The 25th and 75th percentiles were 37 and 208 µg/L respectively. There was no association between urinary concentrations of arsenic and age of the women. Arsenic in urine varied markedly between different areas (blocks) in Matlab. Block C showed the highest median concentration of arsenic (156 µg/L, $p<0.001$ compared to all other blocks) and block D the lowest (52 µg/L, $p<0.001$ compared to all other blocks). There were similar urinary concentrations of arsenic in Block A (88 µg/L) and Block B (68 µg/L; $p=0.19$).

For 2,330 women, we were able to match concentrations of arsenic in drinking-water (WAs) used at the time of pregnancy with concentrations in urine (UAs). There was a significant association between arsenic in urine and drinking-water (linear regression: $\ln UAs=3.0+0.70\ WAs^{0.2}$, $R^2=0.39$, $p<0.001$, (Fig. 3), but there was a considerable variation among individuals. Urinary concentration of arsenic at zero µg/L arsenic was 20 µg/L when it was calculated from the equation. The ratio between the median of arsenic in urine and water for women whose concentrations of arsenic in water were >10 µg/L was 0.7, while the ratio for those whose concentrations of arsenic in water were ≤10 µg/L was 76. Also, arsenic in drinking-water varied between the different blocks in Matlab (Table 2). There were similar concentrations of arsenic in water in Block C (median 180 µg/L) and Block A (me-

Table 1. Main individual characteristics of studied pregnant women at gestational weeks 6-8 and 30, Matlab, Bangladesh

Characteristics	Gestational week 6-8		Gestational week 30	
	No.	%	No.	%
Age (years)				
<20	526	15	300	15
20-30	1,826	53	1,106	57
≥30	969	28	522	27
Unknown	97	3	16	1
All	3,418	100	1,944	100
Block*				
A	1,036	30	561	29
B	998	29	536	28
C	717	21	453	23
D	644	19	391	20
Unknown	23	1	3	0
All	3,418	100	1,944	100
Education level†				
None	1,003	29	560	29
Primary	954	28	555	29
Secondary	1,112	33	677	35
Higher	43	1	19	1
Unknown	306	9	133	7
All	3,418	100	1,944	100
Socioeconomic status‡				
Poorest	735	22	404	21
Second	797	23	405	21
Middle	625	18	371	19
Fourth	583	17	371	18
Richest	653	19	404	21
Unknown	25	1	3	0
All	3,418	100	1,944	100

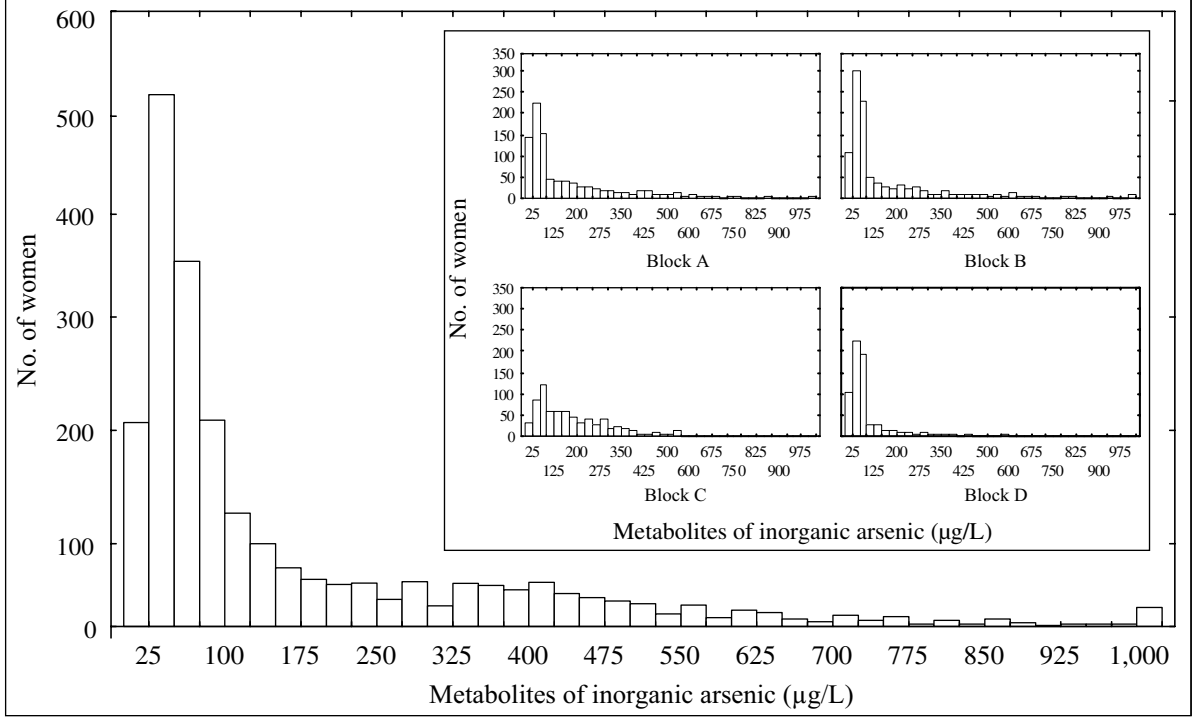
*Block A, B, C, and D=Four administrative areas in Matlab; †Educational levels: expressed by the number of years of formal schooling (None=0 year, Primary=1-6 year(s), Secondary=7-12 years, and Higher=>12 years); ‡Based on household asset scores which were divided into quintiles from the poorest to the richest

dian 160 μg/L; p=0.08). Block B showed much lower concentrations of arsenic in water (median 55 μg/L; p<0.01 compared to all other blocks). Block D had the lowest median concentrations of arsenic in water (7 μg/L; p<0.001 compared to all other blocks).

In general, concentrations of arsenic in urine in gestational week 30 (n=1,944) were similar to those in early pregnancy (paired analysis, urine samples at both early and late pregnancy of 1,944 women, Table 3). The mean difference in urinary arsenic between late pregnancy and early pregnancy was 14 μg/L (median 2 μg/L). However, there was a substantial variation in the changes. In 535 women, urinary concentrations of arsenic increased by ≥50 μg/L between gestational week 6-8 and 30, while, in 412 women, it decreased by ≥50 μg/L. Further, in 346 women, urinary concentrations of arsenic increased by ≥100 μg/L between gestational week 6-8 and 30, while it decreased by ≥100 μg/L in 271 women.

There was no significant change in concentrations of arsenic over the study period. The variation in urinary arsenic (week 6-8) over time in each block

Fig. 2. Frequency distribution of concentrations of metabolites of inorganic arsenic in urine of all studied pregnant women at gestational week 6-8 and by four different study areas (blocks) in Matlab, Bangladesh. Concentrations exceeding 1,000 µg/L—total 32 samples—not included in the figure



is shown in Figure 4. Exposure to arsenic showed a negative association with education and socioeconomic class, as defined by household asset score. Urinary concentration of arsenic (week 6-8) decreased with increasing level of achieved education: the median concentrations were 90, 85, 75, and 36 µg/L for no

Fig. 3. Comparison of metabolites of inorganic arsenic in urine (µg/L) of pregnant women (gestational week 6-8) and inorganic arsenic in drinking-water (µg/L) in Matlab, Bangladesh

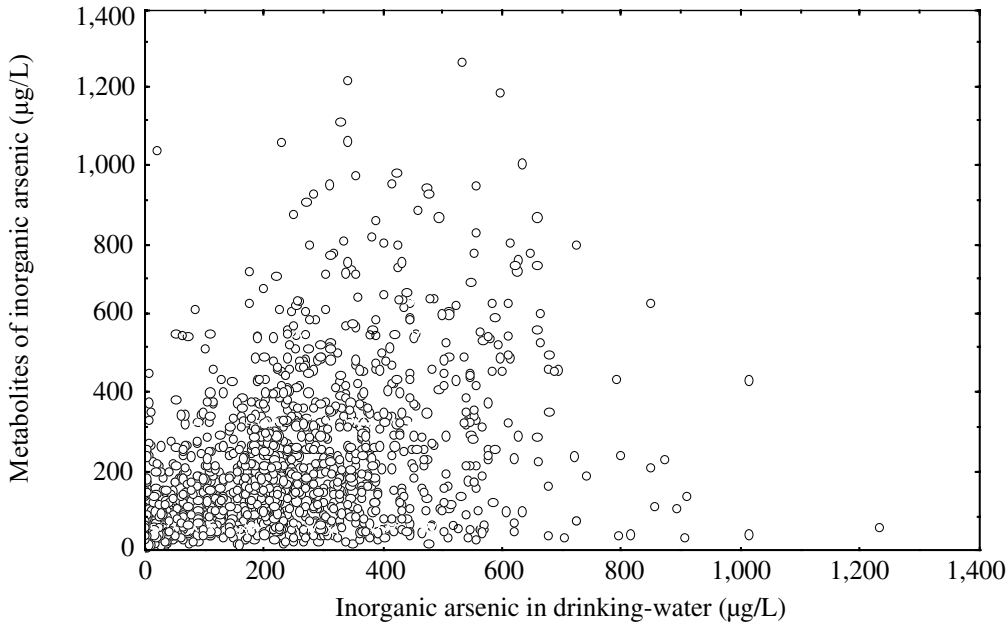


Table 2. Concentrations of arsenic in drinking-water (μg/L) used by studied pregnant women at gestational week 6-8, by blocks, Matlab, Bangladesh

Water arsenic (μg/L)	Block				Unknown	All
	A	B	C	D		
No. of pregnant women	639	699	549	429	14	2,330
Mean	202	160	160	72	231	156
SD	208.9	196.9	115.6	141.1	193.0	180.4
Range	0.5-1234	0.5-1015	0.5-610	0.5-742	0.5-616	0.5-1234
10th percentile	1	1	1	1	1	1
25th percentile	2	5	58	1	4	2
Median	160	55	180	7	283	78
75th percentile	362	273	236	50	299	263
90th percentile	506	437	302	270	466	410

SD=Standard deviation

Table 3. Urinary concentrations of arsenic (sum of inorganic arsenic metabolites) (μg/L, adjusted to specific gravity 1.012 g/mL) of 1,944 women with urine samples collected at both gestational week 6-8 and 30, Matlab, Bangladesh

Urinary arsenic (μg/L)	Gestational week	
	6-8	30
Mean	150	164
Standard deviation	171	187
Range	1-1211	3-1212
10th percentile	23	27
25th percentile	36	42
Median	81	83
75th percentile	205	225
90th percentile	371	415

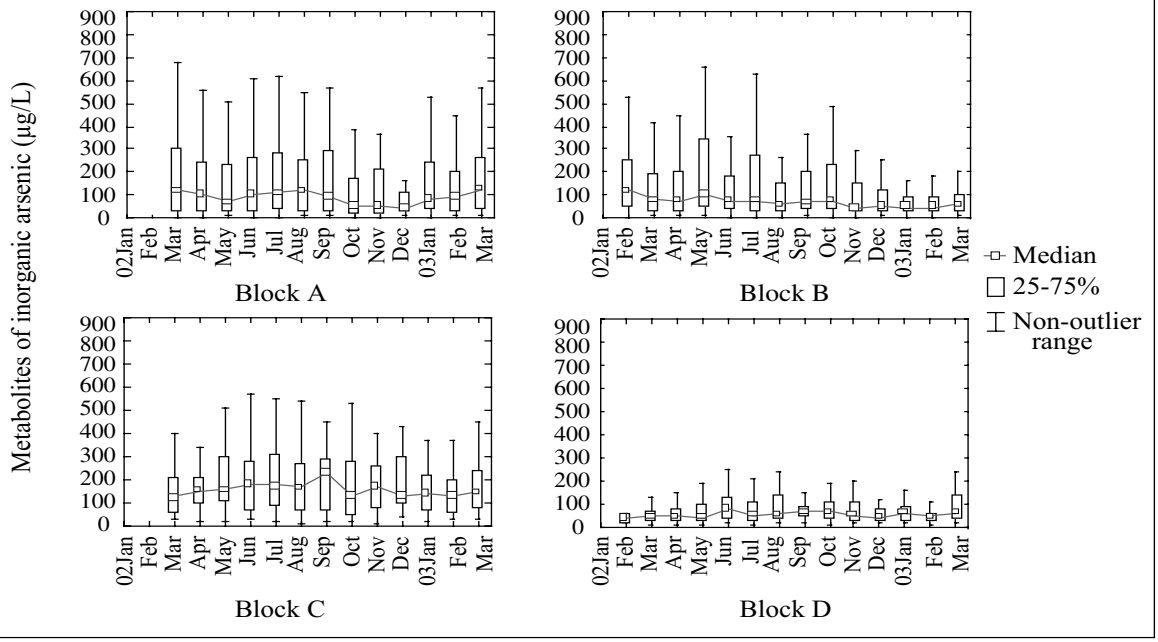
formal education (n=1,002), primary (954), secondary (1,112), and higher education (43) respectively ($p<0.01$). Furthermore, urinary concentrations of arsenic decreased significantly ($p<0.01$) at the highest socioeconomic quintile (median values 89, 94, 88, 74, and 57 μg/L respectively).

DISCUSSION

Our results showed that pregnant women in Matlab, Bangladesh, were highly exposed to inorganic arsenic. The median concentration of arsenic metabolites in urine was 80 μg/L, with a 90th percentile of almost 400 μg/L, which should be compared to a background concentration of less than 5-10 μg/L in people without particular exposure to arsenic (25). Only 47 (1.4%) of the urine samples had concentrations of arsenic in urine below 10 μg/L, which shows that a few women were unaffected by arsenic in water. Arsenic exposure was about the same in early pregnancy and late pregnancy, indicating that the foetuses were exposed during the entire intrauterine life. Arsenic is a potent toxicant and carcinogen, and there is reason to believe that the developing child is particularly sensitive (26,27). Therefore, we have initiated studies to elucidate to what extent the arsenic exposure interferes with foetal and child survival and development.

Households with pregnant women in Matlab and individuals with arsenic-related skin lesions were prioritized for mitigation activities (14). Such activities were initiated in a parallel project, in which all the functioning tubewells in Matlab were screened for arsenic during January 2002–August 2003 (10). A series of village-information meetings were held before starting of the survey, and tubewells with concentrations of arsenic in water exceeding 50 μg/L, as tested by field-kits, were instantly painted red to indicate that people should avoid taking drinking-water from those wells, but preferably collect drinking-water from nearby green-painted tubewells with arsenic in water below 50 μg/L. Further advice and practical assistance concerning alternative sources of water are being provided by BRAC. Although our parallel survey of lifetime arsenic exposure indicates that people in Matlab have started shifting to water with less arsenic (10), there was no tendency of decreasing arsenic exposure during collection of urine among the pregnant women in the present study, i.e. January 2002 (first urine sampling of women in gestational week 6-8) to October 2003 (last sampling gestational week 30). Also, there was no tendency of decreasing urinary concentration of arsenic between early pregnancy and late pregnancy,

Fig. 4. Temporal variations in concentrations of arsenic in urine (µg/L) of pregnant women at gestational week 6-8 in Matlab, Bangladesh



which indicates that mitigation of arsenic is a slow and complex process. In rural Bangladesh, it is mainly the women who collect water. Obviously, collecting water from far-away green-painted tubewells may not always be feasible, especially during pregnancy.

The exposure of the pregnant women to arsenic was negatively associated with education and socioeconomic class, as defined by household asset score, which may indicate that higher socioeconomic groups have started shifting to drinking-water with less arsenic. Similar findings were obtained in our parallel arsenic exposure survey, which also showed that higher socioeconomic groups had been taking the lead in installing tubewells in the 1970s and 1980s (10). Thus, arsenic exposure in relation to socioeconomic groups has changed over time.

Although, in Bangladesh, exposure to arsenic occurs mainly from drinking-water, people may be additionally exposed to arsenic via food, which has not yet been considered in mitigation activities. In the present study, we used concentration of arsenic metabolites in urine as a measure of total individual exposure to inorganic arsenic, i.e. from both drinking-water and food. The high ratio of arsenic between urine and water among individuals whose concentration of arsenic in water was ≤10 µg/L and the calculated urinary concentration of arsenic of 20 µg/L at zero µg/L in water indicates exposure via food. Rice, the staple food in Matlab, often eaten with some vegetables, lentils (*dhal*), and sometimes a little fish, may be heavily contaminated by arsenic due to the use of tubewell water for irrigation, especially in the dry winter season (13,28). On average, Bangladeshi rice contains about 150 µg arsenic/kg dry-weight (13), which may result in a daily intake of approximately 10-50 µg of arsenic. Vegetables may also take up arsenic from contaminated soil (29). Obviously, cooking rice and vegetables in arsenic-rich water will increase concentration of arsenic in food even more (30,31). To enable proper mitigation activities, it is essential to evaluate further the contribution of various foods to the total arsenic exposure. It is also essential to follow the concentration of arsenic in urine of women after specific mitigation activities.

The marked geographical variation found in arsenic exposure is mainly related to variations in concentration of arsenic in tubewell water. As in other areas of Bangladesh (11), there are large variations in concentrations of arsenic in tubewells in Matlab, both locally and between larger areas. There was a fairly small fluctuation in urinary concentration of arsenic over time. Whether this is due to variation in concentration of arsenic in tubewells, or to other factors, is currently under investigation. Temporal variations in concentrations of arsenic in groundwater have not been extensively studied. Recent small-scale studies in Bangladesh, Argentina, and the USA also indicate

fairly stable concentrations of arsenic in groundwater over time (11,32-35).

In summary, the pregnant women in Matlab, Bangladesh, were highly exposed to inorganic arsenic via drinking-water and, possibly, food. The overall exposure was about the same during pregnancy and during the entire study period, i.e. January 2002–October 2003, despite initiation of mitigation activities. The results emphasize the urgent need for further mitigation activities, particularly among low socioeconomic groups. It is also essential to investigate the presence of arsenic-related health effects.

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