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Dietary arsenic exposure with low level of arsenic in drinking water and biomarker: A study in West Bengal

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The authors investigated association of arsenic intake through water and diet and arsenic level in urine in people living in arsenic endemic region in West Bengal supplied with arsenic-safe water (<50 μg L^{-1}). Out of 94 (Group-1A) study participants using water with arsenic level <50 μg L^{-1}, 72 participants (Group-1B) were taking water with arsenic level <10 μg L^{-1}. Multiple regressions analysis conducted on the Group-1A participants showed that daily arsenic dose from water and diet were found to be significantly positively associated with urinary arsenic level. However, daily arsenic dose from diet was found to be significantly positively associated with urinary arsenic level in Group-1B participants only, but no significant association was found with arsenic dose from water in this group. In a separate analysis, out of 68 participants with arsenic exposure through diet only, urinary arsenic concentration was found to correlate positively ($r = 0.573$) with dietary arsenic in 45 participants with skin lesion while this correlation was insignificant ($r = 0.007$) in 23 participants without skin lesion. Our study suggested that dietary arsenic intake was a potential pathway of arsenic exposure even where arsenic intake through water was reduced significantly in arsenic endemic region in West Bengal. Observation of variation in urinary arsenic excretion in arsenic-exposed subjects with and without skin lesion needed further study.

Keywords: Arsenic in water, arsenic in diet, arsenic in urine, biomarker, arsenicosis.

Introduction

Arsenic contamination in drinking water has been reported from many countries in the world, but the severity of this contamination in India and Bangladesh is unprecedented.[1] The main focus of attention, until recently, has been exclusively on exposure of arsenic through drinking of arsenic contaminated groundwater and human health. However, since arsenic contaminated groundwater is also used extensively for crop irrigation in the arsenic belt of West Bengal and Bangladesh, the possibility of a buildup of arsenic concentration in agricultural soils and agronomic produce resulting in arsenic exposure through food chain has also been considered. Sufficient information is now available regarding high arsenic buildup in soil and high level of arsenic in rice and vegetables grown in arsenic contaminated irrigated region in Bengal basin.[2,3]

Several reports are available describing raised levels of arsenic in rice grain in arsenic endemic regions of West Bengal and Bangladesh because of use of arsenic contaminated groundwater for irrigation purpose.[2,4–6] Arsenic contamination of vegetables grown in soil irrigated with arsenic contaminated water has also been reported.[7–9] Significant quantities of total daily arsenic intake through water and diet have been reported in people living in arsenic-exposed regions of India and Bangladesh by many investigators.[7,10–13] Arsenic level in urine is an important biomarker of arsenic exposure. Total arsenic level in urine has frequently been used as a biomarker of recent arsenic intake.[14,15]

As drinking of arsenic contaminated groundwater has been considered to be the main source of arsenic exposure

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in West Bengal, many villagers are currently using water with low level of arsenic (<50 μg L^{-1}, permissible limit in the region) for drinking and cooking purposes in recent years due to the wide awareness.[16] But, in spite of drinking water with low level of arsenic, there is a possibility that significant arsenic exposure occurs through diet in these people because of report of intake of arsenic-contaminated rice grain and vegetables by people in this region. The current study was therefore undertaken to ascertain the biological effect of arsenic intake through diet, as reflected by the arsenic level in urine, a biomarker of current arsenic exposure, in people living in this region using water with low level (<50 μg L^{-1}) of arsenic.

Materials and methods

Study design

The present study describes dietary arsenic exposure and exposure from low level of arsenic (<50 μg L^{-1}) (the permissible limit in India)[17] in drinking water and correlates with arsenic level in urine in 94 people living in arsenic endemic region in West Bengal. The study participants were selected from a source population of 167 participants who were exposed to arsenic in drinking water both above and below 50 μg L^{-1} and were studied earlier for assessment of total individual arsenic exposure and arsenic level in urine.[18] The source population was recruited from a population of 900 residents belonging to 212 households (4% of total households in the selected villages), which had been identified in a previous cross-sectional study carried in six villages in two arsenic-affected blocks of Chakdah and Haringhata in Nadia district (Fig. 1). The households in a sample village were selected by systematic sampling with a random start procedure after preparing a village map and listing of households.[19] Water samples used for drinking and cooking purpose as well as diet and urine samples were collected from all 94 individual participants, and arsenic levels were estimated. Out of 94 (Group-1A) participants who were using water with arsenic level <50 μg L^{-1}, 72 (Group-1B) participants were using water with arsenic level <10μg L^{-1} (the WHO safe limit).[20]

Among the 94 (Group-1A) study subjects, 60 cases had typical arsenical skin lesions of pigmentation and/or keratosis (Arsenicosis), while 34 individuals had no such skin lesions. The criteria for classifying keratosis and hyperpigmentation as arsenic-caused skin lesions were as follows. Keratosis was characterized by diffuse bilateral thickening of the palms and/or soles with or without nodules of various shapes and sizes. Hyperpigmentation was identified as areas of mottled dark brown pigmentation distributed bilaterally on the trunk. Hyperpigmentation was frequently present on the limbs and sometimes alongside spots of depigmentation, but these characteristics were not regarded as essential for the diagnosis.[19,21]

All patients were examined in the field by one of two physicians (DNGM, AG) who have had many years of experience in diagnosing arsenic-caused skin lesions in West Bengal. For the assessment of total individual arsenic exposure, the total arsenic level in drinking water and diet samples taken in 24 h was determined for each participant in both groups. All subjects included in this study gave written consent for their participation. Approval of the study protocol was obtained from the Ethical Committee of the DNGM Research Foundation, fulfilling the Helsinki criteria and recommendation of the Indian Council of Medical Research, Government of India.

Field study

In the field study, information was collected on demographic and social characteristics, occupation, and addiction from each participant. Addiction was assessed by taking into account the history of smoking, drinking and tobacco chewing in field visits. Weight and height were measured and used to calculate Body Mass Index (BMI, i.e., weight in Kg/height in m^2).

Collection of water and urine

Water samples were collected in certified metal-free containers from the current drinking and cooking water sources of each family. Total daily water consumption of a participant was determined from a self-report on the number of glasses (250-mL capacity) of water the person consumed in a 24-h period. First morning void urine sample was also collected from each participant in certified metal free container. Both the water and urine samples were kept in an icebox before leaving the field and stored at −20°C. All these samples were collected on the same day of collection of diet sample and stored according to standard protocol of the WHO until further analysis.[20]

Assessment of diet intake

Food samples were collected using the duplicate portion sampling method.[12] The "senior" woman (mother or eldest daughter-in-law of the family) involved in preparation of food for the family was interviewed. A detailed questionnaire for collecting information on participants' 24-h diet intake was formulated. The diet questionnaire was previously validated by similar dietary study on nutrient intake in arsenic-exposed population in West Bengal.[22] The participating women were questioned about each meal, from the previous day's afternoon meal to lunch on the following day. The quantity of each diet category administered in each meal to each participant was recorded. To estimate the amount of food consumed by a participant in the family, duplicate portions of the cooked items in each meal were collected by the dietician in a measuring bowl of known

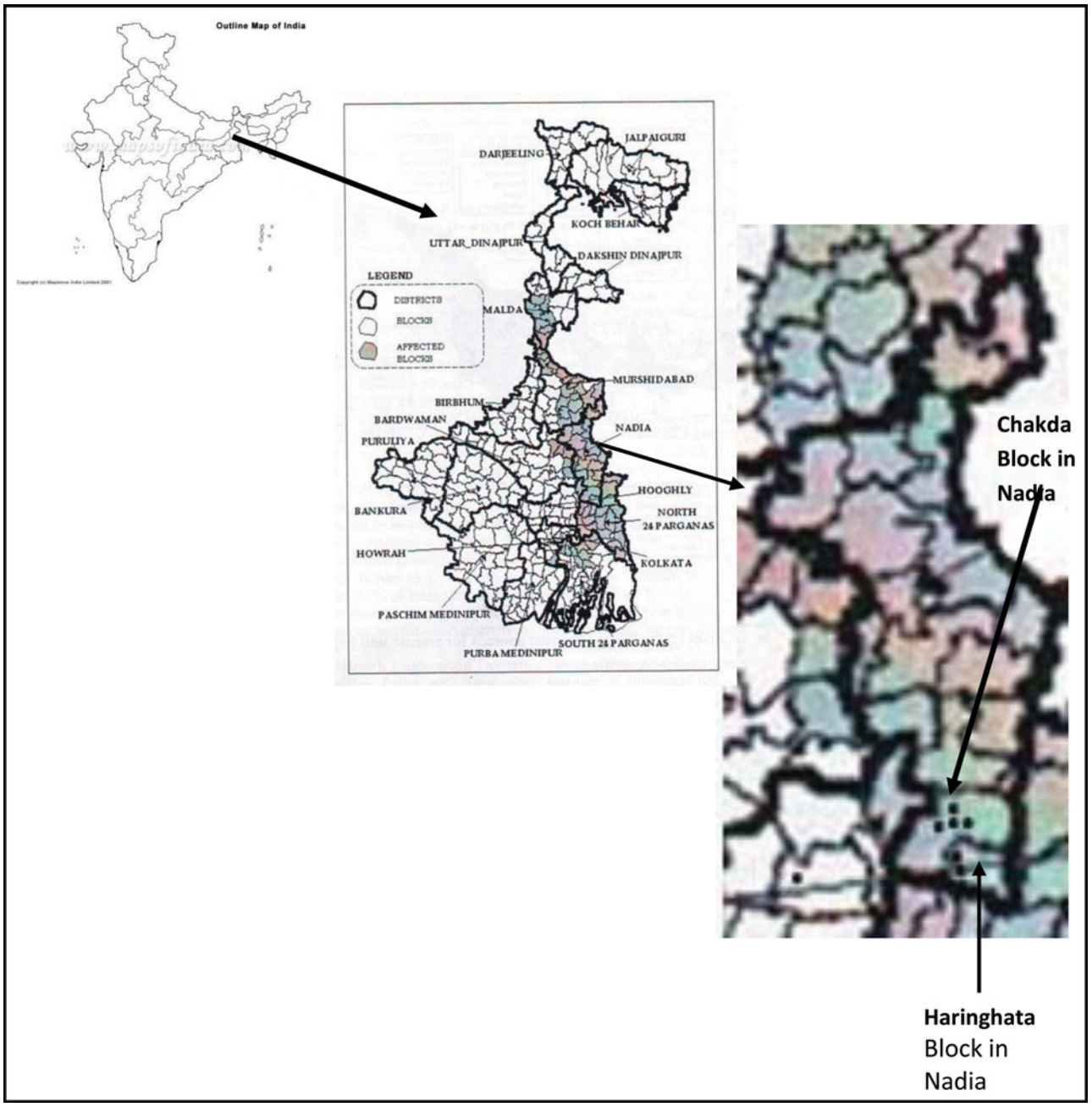

Fig. 1. Map of India, arsenic affected areas in West Bengal and district of Nadia with locations of study villages.

volume, and the wet weight was taken. Similarly, dry cooked food items were collected and their dry weight was taken.

All wet cooked food items of meals were categorized into: (a) cooked cereals (cooked rice, khichuri, suji, payes, semai etc.) and chapati; (b) cooked pulses (lentil, mug dal, motor dal, kalai dal etc.); (c) cooked vegetables (curry made of different vegetables); (d) cooked curry of animal food (fish/egg/meat); and (e) milk. All these items were collected together. Dry food items consisted of: (a) dry cereals (puffed rice, flaked rice, biscuits, etc.); (b) fried pulses (motor, chola, etc.); and (c) raw fruits (banana, mango, etc.). Raw rice samples used in the preparation of cooked food was also collected from each family for comparison of arsenic content in both raw and cooked rice. All diet samples collected were kept in a well-zipped polyethylene pack, carried from the field in an ice bucket, and stored at -20°C until analysis.

Total arsenic analysis

Arsenic levels in water and urine samples were measured using an atomic absorption spectrophotometer with a flow-injection hydride generation system (Perkin-Elmer A Analyst 400, Waltham, MA, USA).[23] The lower limit of

detection determined at the 90% confidence level was 0.3 μg L^{-1}. For urine analysis one portion of urine was digested for total arsenic estimation following Das et al.[23] In this procedure 1 mL of the raw urine sample was digested with an acid mixture (500 μl HNO_3, 200 μL $HCLO_4$ and 200 μl H_2SO_4, Merck, Darmstadt, Germany) in a Kjehldahl flask. The sample was heated using a small funnel at the top of a sand-bath until it became clear. If necessary, a 200-μL aliquot of HNO_3 was subsequently added.

Again the sample was heated until fumes of SO_3 evolved. The solution was cooled and made up to a volume of about 10 mL. Total arsenic analysis of these digest was performed using Atomic Absorption Spectrophotometer equipped with a flow injection hydride generation system (FI-HG-AAS, A-Analyst 400, Perkin-Elmer).[18] Quality assurance included same methods of preparation and analysis of urine standard reference materials (NIST, USA, SRM 2670a) in each sample batch run; recovery percentages varied from 95 to 97% (certified value 220 ± 10 μg/L; obtained concentration range 209–213 μg L^{-1}).

In the case of mixed food categories, the samples were wet- weighted (in the case of cooked food) or dry-weighted (in case of raw food) and oven dried until constant weight in 60°C and percentage moisture was calculated. Samples were digested separately following block digestion procedure.[24] For arsenic analysis, the dry samples were crushed and part of each sample (1.0 g) was transferred to a 100-mL digestion flask pre-wetted with a mixture of nitric acid, perchloric acid, and sulphuric acid (10:4:1) and kept at room temperature. The following day, these samples were heated in a block digestion chamber at 110–120°C until a clear solution of about 1.0 mL was obtained.

Quality assurance included the same NIST preparation method of rice sample SRM 1568a, recovery percentages varied from 96 to 98% in the case of diet. For total arsenic analysis of urine, sample was acid digested, and arsenic analysis was performed using an atomic absorption spectrophotometer (Perkin-Elmer A-Analyst 400) equipped with a flow injection hydride generation system (FIAS-100, Perkin-Elmer). Quality assurance included the same preparation method for NIST urine sample SRM-2670, in each sample batch run; recovery percentages varied from 95 to 97%. Coefficients of variation of analysis of arsenic were 50.84% in food; 118.02% in water and 90.21% in urine.

Statistical methods

The daily arsenic intake of each individual from drinking water, milk, cooked and dry food was estimated as follows. The arsenic intake from drinking water (μg day^{-1}) was calculated by multiplying the arsenic concentration in drinking water of the current drinking water source (μg L^{-1}) by the water consumption rate (L day^{-1}) in a day. Arsenic intake from milk was similarly estimated. The daily arsenic intake from each cooked wet food category (μg day^{-1}) was calculated by multiplying the arsenic concentration in each wet food category (μg/Kg wet wt) by the daily consumption rate (Kg wet wt/day) of that food category. The arsenic intake from dry food category was similarly estimated from arsenic concentration as μg/Kg dry wt. Estimation of total dietary arsenic intake was based on the sum of arsenic ingested from each cooked wet and dry food category consumed during the 24-h period by each participant. Daily total arsenic intake was the sum of total daily arsenic intake from drinking water and diet. Dividing each participant's daily arsenic intake by their body weight determined the daily arsenic dose (μg/Kg bodyweight/day).

Frequency distributions of characteristics such as age, sex, addiction, BMI and skin lesion status of both Group-1A and Group-1B were calculated. Descriptive statistics were presented on the data including median and range in regard to quantity of dietary items taken, a mean (SD), median and range in regard to arsenic content in current drinking water source and in urine and daily arsenic intake through water and diet of both the group of participants. A multiple regression model was fitted to urinary arsenic with average arsenic intake from water and diet as the exposure and age, sex and presence of skin lesions as potential confounders for Group-1A (94) participants.

All covariates were screened to determine whether or not they were significant risk factors or confounders. The urinary arsenic and daily arsenic intake and daily arsenic dose values were log transformed as the data was found to be positively skewed. Adjusted R-squared coefficients were used to study model fit. Three exposure scenarios, tube well arsenic concentration (μg L^{-1}, daily arsenic intake (μg day^{-1}) and daily arsenic dose (μg/kg body wt/day), were evaluated to determine which model best predicted the urinary arsenic concentrations. The multiple regression model in Group-1B participants was similarly fitted to evaluate the direction of association of urinary arsenic concentration with any source(s) of arsenic intake for 72 participants using arsenic-safe water (<10 μg L^{-1}, WHO safe limit) for drinking and cooking purpose.

Further, arsenic exposure was found to occur mainly through diet in 68 participants who were drinking water below detection limit (<0.3 μg L^{-1}). Out of these, arsenical skin lesions were found to be present in 45 and absent in 23 participants. Scatter plots of arsenic value in urine (μg L^{-1}) and arsenic intake from diet (μg kg^{-1} day^{-1}) were constructed for participant with and without skin lesion and a regression line was fitted for both the groups. The software package used for statistical analysis was R, version 2.13.[25]

Results

Study group characteristics

There was no difference in age, sex, addiction and Body mass index (BMI) among the Group-1A and Group-1B

Table 1. Baseline characteristics of study participants living in arsenic endemic region with groundwater arsenic contamination taking water with As <50 μg L^{-1} (Group-1A = 94) and <10 μg L^{-1} (Group-1B = 72) in West Bengal.

	Group-1A (n = 94)		*Group-1B (n = 72)*		
	n	*(%)*	*n*	*(%)*	*P value*¥
Age in years:					
15–29	17	18	14	20	0.3
30–44	42	45	34	47	0.86
45–74	35	37	24	33	0.37
Sex **:**					
Male	58	62	44	61	0.91
Female	36	38	28	39	0.93
Addiction					
Smoking	26	28	18	25	0.82
Tobacco chewing	10	11	7	10	0.94
BMI Classification					
Under Weight (<18.50)	37	39	30	42	0.80
Normal (18.50–24.99)	56	60	42	58	0.84
Overweight (≥ 25)	1	1	0	0	
Arsenical skin lesion present	60	64	48	67	0.74
Arsenical skin lesion absent	34	36	24	33	0.81

¥Two-tailed.

participants using water with arsenic level <50 μg L^{-1} and <10 μg L^{-1}, respectively. About 45% of participants belonging to Group-1A were aged between 30–44 years, majority (62%) being males (Table 1). Thirty-nine percent of the Group-1A participants were underweight, history of smoking and tobacco chewing being present in 28% and 11% of subjects, respectively. None of the participants had any history of alcohol intake.

Water and diet consumption and arsenic content in water and food categories

There was no significant difference in quantity of intake of water and various dietary items taken by the two groups (Table 2). Of the various dietary constituents, cooked rice and cooked vegetables were taken by all the participants. Cooked rice constituted the major bulk of the participants' diet in both groups (56% in Group-1A and 65% in Group-1B participants), (Table 2).That rice constituted the major bulk of diet in people living in the Indo-Bangladesh subcontinent had also been reported by others.[7,12,13,26,27]

There was no significant difference in arsenic content in various food categories taken by the two groups of subjects (Table 3). Median arsenic content of cooked rice of Group-1A and Group-1B participants was 83 μg kg^{-1} and 74 μg kg^{-1} respectively. A few data are available on arsenic content in cooked rice in India Bangladesh subcontinent. In one report from arsenic contaminated areas of West Bengal arsenic content in cooked rice was reported as 65 (range: 33–138) μg kg^{-1},[28] while it was reported as 100 (range: 28–600 μg kg^{-1}) from another study from Bangladesh.[11] Median arsenic content of cooked vegetables taken by the two groups in the present study was 81 and 75 μg kg^{-1}, respectively.

Daily arsenic intake through drinking water and diet and arsenic in urine

Total daily arsenic intake through drinking water ranged from <0.3 to 139 μg day^{-1} in 94 Group-1A participants, while ranging from <0.3 to 23 μg day^{-1} ($P < 0.001$) in 72 Group-1B participants. The range of dose of daily arsenic intake through drinking water was <0.3–3.09 μg/kg body wt/day for the former, while it was <0.3–0.42) μg/kg body wt/day for the latter ($P < 0.001$). Median value of total daily arsenic intake from diet was 136 (range: 20–380) μg day^{-1} in Group-1A while 124 (range: 20–380) μg day^{-1} in Group-1B participants ($P = 0.14$). Median dose of total daily arsenic intake from diet was 3.01 (range: 0.39–8.00) μg/kg body wt /day in the former, while 2.66 (range: 0.39–7.61) μg/kg body wt/day in the later ($P = 0.16$). Median arsenic level in urine in Group-1A participants was 64 (range: <0.3–526) μg L^{-1}, while its value in Group-1B participants was 51(range: <0.3–449) μg L^{-1} ($P = 0.12$). Median arsenic value in urine was 156 (range <0.3–526) μg L^{-1} in 22 out of 94 Group-1A participants who were drinking water with arsenic level > 10 μg L^{-1} (WHO safe limit) but <50 μg L^{-1} (permissible limit in India).

Sixty-eight people out of 72 participants belonging to Group-1B were exposed to arsenic mainly through diet as they were drinking water with arsenic level below detection limit (<0.3 μg L^{-1}). Median value of total daily arsenic intake from diet was 130 (range: 20–380) μg day^{-1}. Median value of arsenic level in urine in these 68 participants was 55 (range <0.3–449) μg L^{-1}. Amongst these, 45 participants were having features of arsenicosis characterized by arsenical skin lesion of pigmentation and keratosis, while 23 participants had no such lesion. The urinary arsenic concentration was found to correlate positively ($r = 0.573$), (Fig. 2) with dietary arsenic level in the 45 participants who had arsenical skin lesions, while this correlation was insignificant ($r = 0.007$), (Fig. 3) in 23 participants without skin lesions.

The results of multiple regressions analysis conducted on the 94 Group-1A participants drinking water with

Table 2. Daily consumption rates of water and various food categories by 94 (Group-1A) and 72 (Group-1B) study participants.

	Group-1A n = 94				*Group-1B n = 72*				
Food items (g day^{-1}) / Water (Liter day^{-1})	*n*	Median	Min	Max	*n*	Median	Min	Max	*P value*[¥]
Raw Rice (d. w.)	94	439	35	945	72	413	35	887	0.48
Cooked Rice (w. w.)	94	1300	100	2900	72	1250	100	2700	0.46
[1]Dry Cereals (d. w.)	24	22	5	50	21	25	5	50	0.98
[2]Chapati (w. w.)	13	160	50	800	13	160	50	800	1.00
[3]Cooked Pulses (w.w.)	34	100	20	500	31	100	20	500	0.79
[4]Cooked Vegetables (w. w.)	94	260	20	717	72	255	20	640	0.46
Milk (w. w.) (L day^{-1})	5	125	125	250	5	125	125	250	1.00
Cooked Meat/Chicken (w. w.)	14	50	20	200	14	50	20	200	1.00
[5]Cooked Fish (w. w.)	32	27	2	165	15	45	8	165	0.18
[6]Cooked Egg (w. w.)	19	40	5	90	18	40	10	90	0.78
[7]Fruit (w. w.)	5	75	50	100	4	72	50	100	0.90
Water Intake(Male) L day^{-1}	58	3.5	1	6.3	44	3	1	6.3	0.28
Water Intake (Female) L day^{-1}	36	2.5	1	4	28	2	1	4	0.3

d. w: dry weight; w. w: wet weight.
[1]Dry cereals: Puffed rice, flaked rice, biscuits, etc.
[2]Chapati: Made from wheat flour, bread, etc.
[3]Cooked pulses: Lentil, mug, matar, Bengal gram, kalai, green peas, soya bean nugget, 'bari' (made from any pulses), etc.
[4]Vegetables: (i) Roots and tubers: Potato, carrot, radish, sweet potato, colocasia, 'oal', 'thor', onion; (ii) GLV: Cabbage, cauli flower, spinach, 'sajna sag', nate, 'pumpkin sag', 'lau sag', 'kochu sag'; (iii) Other vegetables: Tomato, onion stalk, brinjal, papaya, 'sajne data', parwar, cluster beans, beans, 'jhinga', pumpkin, bitter gourd, bottle gourd, ladies finger, plaintain green, 'kakrol', 'chalkumra', 'kochulati' 'mocha', pumpkin flower, 'chichinga', 'echor,' green mango.
[5]Fish: Rohu, mrigel, hilsa, puti, pona, bata.
[6]Egg: Hen, duck and poultry.
[7]Fruit: Banana, mango, coconut, jack fruit.

arsenic level <50 μg L^{-1} are presented in Table 4. The exposure assessment showed that daily arsenic dose from water and diet explained the most variability of urinary arsenic level. Doses of daily arsenic intake from water and diet, both, were found to be significantly positively associated with urinary arsenic level (Table 4). Thus, a one-unit increase in daily arsenic dose from water resulted in a 0.344-fold increase in average urinary arsenic content, and one unit increase in daily arsenic dose from diet resulted in a 0.154-fold increase in average urinary arsenic content. Another significant risk factor and confounder observed were skin lesions. Those with skin lesions were found to excrete significantly more arsenic in urine (β =0.865, $P < 0.001$). Thus, those with skin lesions excreted on an average 0.865 as much urinary arsenic, as compared to those without skin lesions with the same dose of

Table 3. Arsenic content in various food categories taken by 94 (Group-1A) and 72 (Group-1B) study participants.

	Group-1A n = 94				*Group-1B n = 72*				
As conc. of different Food items (μg kg^{-1})	*n*	Median	Min	Max	*n*	Median	Min	Max	*P value*[¥]
Raw Rice (d. w.)	58	276	26	621	45	276	26	621	0.98
Cooked Rice (w. w.)	58	83	30	164	45	74	30	164	0.31
[1]Dry Cereals (d. w.)	18	185	49	437	16	185	49	437	0.79
[2]Chapati (w. w.)	11	171	46	222	11	171	46	222	1.00
[3]Cooked Pulses (w. w.)	23	31	4	61	20	30	4	49	0.68
[4]Cooked Vegetables (w. w.)	60	81	13	210	46	75	13	210	0.44
Milk (w. w.) (L day^{-1})	4	60	60	60	4	60	60	60	
Cooked Meat/Chicken (w. w.)	7	100	20	200					
[5]Cooked Fish (w. w.)	21	96	30	174	10	96	40	108	0.85
[6]Cooked Egg (w. w.)	14	191	7	191	14	191	7	191	
[7]Fruit (w. w.)	4	20	20	20	4	20	20	20	

[¥]Two-tailed, d. w: dry weight, w. w: wet weight.

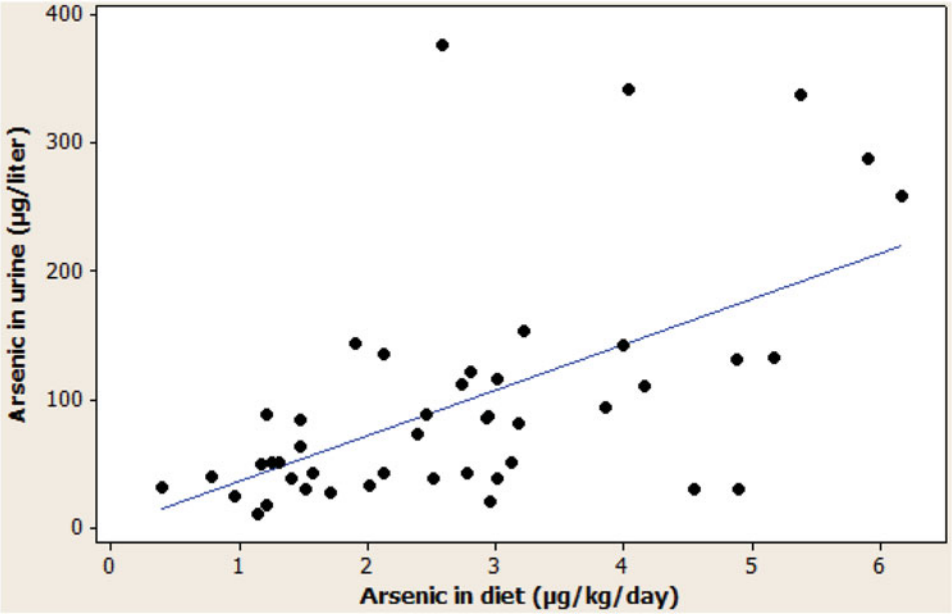

Fig. 2. Correlation of urinary arsenic concentration (µg L^{-1}) with daily dietary arsenic intake (µg kg^{-1} day^{-1}) for participant drinking water with arsenic level <0.3 µg L^{-1} having skin lesion ($n = 45$), West Bengal, India (r = 0.573).

arsenic intake. The adjusted R-squared coefficient was 0.189.

Further, results of multiple regression analysis conducted on the 72 (Group-1B) participants who were drinking water with arsenic level < 10 µg L^{-1} are presented in Table 5. In this case also, the daily dose of arsenic from water and diet explained the greatest variability of urinary arsenic exposure as in the previous case. However, daily doses of arsenic intake from water was no longer significantly associated with urinary arsenic, whereas daily dose of arsenic from diet was still found to be significantly positively associated with urinary arsenic value (Table 5). Furthermore, one unit increase in daily dose of arsenic from diet resulted in a 0.436-fold increase in average urinary arsenic content. Moreover, skin lesions were still a significant risk factor and confounder of urinary arsenic and excreted

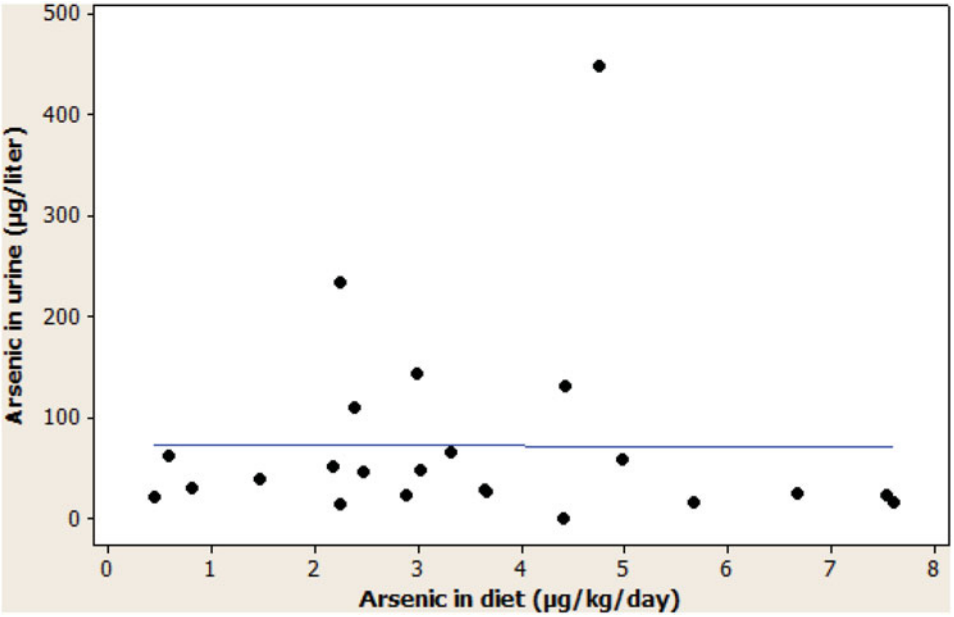

Fig. 3. Correlation of urinary arsenic concentration (µg L^{-1}) with daily dietaryarsenic intake (µg kg^{-1} day^{-1}) for participant drinking water with arsenic level <0.3 µg L^{-1} having no skin lesion ($n = 23$), West Bengal, India (r = 0.007).

Table 4. Results for linear regression model evaluating different exposure scenarios for log $_{10}$ urinary arsenic in 94 participants (Group-1A) taking water with As <50 μg /L^{-1}.

Parameter	*Estimate*	*SE*	*P-value*
Model- I: Log Tube-well water As content (μg/Liter)			
Intercept	3.844	0.472	< 0.001
Tube well conc. (μg L^{-1})	0.536	0.191	0.006
Age	−0.004	0.010	0.694
Sex	−0.295	0.237	0.217
Skin lesion	0.784	0.244	0.002
Adj $R^2 = 0.124$			
Model– II: Log Total daily intake from diet + Log Av Total daily intake from water (μg day^{-1})			
Intercept	3.589	0.498	< 0.001
Average total daily intake from water (μg day^{-1})	0.007	0.003	0.031
Average total daily intake from diet (μg day^{-1})	0.003	0.001	0.103
Age	−0.006	0.010	0.558
Sex	−0.445	0.248	0.068
Skin lesion	0.900	0.248	< 0.001
Adj $R^2 = 0.150$			
Model- III: Log Av Total daily dose from diet + Log Av Total daily dose from water (μg/Kg-day)			
Intercept	3.575	0.481	< 0.001
Average total daily dose from water (μg/kg body wt/day)	0.344	0.141	0.017
Average total daily dose from diet (μg/kg body wt/day)	0.154	0.070	0.031
Age	−0.010	0.010	0.366
Sex	−0.370	0.229	0.111
Skin lesion	0.865	0.237	< 0.001
Adj $R^2 = 0.189$			

significantly more arsenic in urine ($\beta = 0.797$, $P < 0.01$). Thus, those with skin lesions excreted on average 0.797 as much urinary arsenic as compared to those without skin lesions with the same arsenic intake. The adjusted R-squared coefficient was 0.206.

Discussion

The current study highlighted that even when people were using arsenic-safe water (< 50 μg L^{-1}, permissible limit in India) for drinking and cooking purposes, daily doses of arsenic intake from both water and diet were significantly positively associated with urinary arsenic level in people living in an arsenic endemic region. When the arsenic level in drinking water was further reduced to < 10 μg L^{-1} (WHO safe limit), the dose of arsenic exposure from diet was still found to be associated with significant urinary arsenic excretion but no significant association was found with arsenic dose from water in this group. Median arsenic level in urine in people using arsenic safe water (<50 μg L^{-1}) was 64 (range: <0.3–526) μg L^{-1} while its value in participants drinking water with arsenic level <10 μg L^{-1} was 51 (range: <0.3–449) μg L^{-1}. In another separate study carried by us in three other different villages in northern Nadia, the risk of arsenic exposure was assessed in a cohort of 157 participants (male: 68; female: 89) among the people supplied with arsenic safe drinking water for the past few years.[29] The determination of urinary arsenic concentration of the participants who were drinking arsenic-safe water (<50 μg L^{-1}), showed that despite low intake of arsenic from drinking water, the concentration of arsenic in urine was considerably high (range: <0.3 μg L^{-1}–753 μg L^{-1}; median: 42.3 μg L^{-1}). By assessing the risk of arsenic exposure at three regions of West Bengal, Mondal et al. [30] have also reported that the risk of arsenic exposure from rice consumption predominates in the area where arsenic concentration in drinking water was low.

Data of total individual arsenic exposure and arsenic level in urine of 169 arsenic-exposed people in Nadia, West

Table 5. Results for linear regression model evaluating different exposure scenarios for log $_{10}$ urinary arsenic in 72 participants (Group-1B) taking water with As <10 μg L^{-1}.

Parameter	*Estimate*	*SE*	*P-value*
Model- I: Log Tube-well water As content (μg L^{-1})			
Intercept	4.41	0.491	< 0.001
Tube well conc. (μg L^{-1})	−0.081	0.100	0.420
Age	−0.017	0.011	0.117
Sex	−0.222	0.241	0.362
Skin lesion	0.708	0.250	0.006
Adj $R^2 = 0.135$			
Model- II: Log Total daily intake from diet + Log Av Total daily intake from water (μg/day)			
Intercept	2.574	1.003	0.013
Average total daily intake from water (μg day^{-1})	−0.072	0.183	0.693
Average total daily intake from diet (μg day^{-1})	0.394	0.191	0.043
Age	−0.019	0.011	0.078
Sex	−0.291	0.238	0.228
Skin lesion	0.816	0.250	0.002
Adj $R^2 = 0.185$			
Model- III: Log Av Total daily dose from diet + Log Av Total daily dose from water (μg/Kg-day)			
Intercept	4.12	0.490	< 0.001
Average total daily dose from water (μg/kg body wt/day)	0.224	0.425	0.601
Average total daily dose from diet (μg/kg body wt/day)	0.436	0.183	0.020
Age	−0.021	0.010	0.052
Sex	−0.251	0.233	0.287
Skin lesion	0.797	0.244	0.002
Adj $R^2 = 0.206$			

Bengal, who were using water with arsenic level both above and below 50 μg L^{-1}, and which constituted the source population of this study, were reported earlier.[18] Total daily arsenic intake from diet was higher (median: 165, range 20–479 μg day^{-1}) in that group compared to the total daily dietary arsenic intake (median: 136, range: 20–380 μg day^{-1}) as observed in 94 (Group-1A) participants of our presented study group who were using arsenic- safe water (arsenic level <50 μg L^{-1}) for drinking and cooking purposes.

It was interesting to note from our present study that in people exposed to arsenic through diet only, significant correlations of arsenic levels in urine occurred in people showing clinical features of arsenical skin lesion, whereas no such correlation was found in people who didn't show such skin lesions. Out of the 68 participants with arsenic exposure mainly through diet, urinary arsenic concentration was found to correlate positively ($r = 0.573$) with dietary arsenic intake in the 45 participants with skin lesions (Fig. 2), while this correlation was insignificant ($r = 0.007$) in 23 participants without skin lesions (Fig. 3). Higher median urinary arsenic value relative to arsenic intake through water and diet was also reported earlier in 78 subjects with skin lesions compared to urinary arsenic value in 89 subjects without skin lesions amongst 167 participants studied earlier, although there was a marginal difference of median total arsenic intake through water and diet in these two groups.[18]

The variation in urinary arsenic excretion in arsenic-exposed subjects with and without skin lesions might be due to difference in bio-accessibility of arsenic in the system from the gut after oral intake or due to altered metabolism or differential bio-elimination of arsenic in urine after absorption in people having presence and absence of skin manifestations with similar arsenic exposure. The pathophysiologic reason for this differential behavior of arsenic excretion in urine in arsenic-exposed people with and without arsenical skin lesion needs further study.

From a public health perspective, our findings are particularly important given the fact that millions of people are exposed to arsenic in drinking water in India-Bangladesh subcontinent where arsenic contaminated groundwater is used for irrigation purposes, and there is a relevance of reduction of safe limit of arsenic in drinking water. Our study further suggested that the supply of arsenic-safe water with WHO standard (<10 μg L^{-1}) to the population in rural Bengal alone was not enough to reduce the biological effects of chronic arsenic exposure. Dietary intake was another potential pathway of arsenic exposure that must also be considered. Introduction of policies for sustainable agricultural practices that minimize the transfer of arsenic from groundwater to soils to the human food chain was urgently needed. It should also be mentioned that when arsenic from diet contributed significantly to the total daily arsenic intake, an effort should be made to reduce the safe limit of arsenic in water from 50 μg L^{-1} to 10 μg L^{-1} in India's Bangladesh subcontinent.

Conclusion

This study highlights that dose of daily arsenic intake from both water and diet are significantly positively associated with urinary arsenic levels in an arsenic-endemic region of West Bengal, even when people are using arsenic-safe water (<50 μg L^{-1}) for drinking and cooking purposes. When arsenic levels in drinking water were further reduced to <10 μg L^{-1} (WHO safe limit), dose from diet was still found to be associated with urinary arsenic excretion significantly. But no significant association was found with arsenic dose from water in this group. Further, when exposed to arsenic through diet only, urinary arsenic concentration was found to correlate positively with dietary arsenic in participants having skin lesions only, while this correlation was insignificant in participants without skin lesion. It can be concluded that, in rural Bengal, any mitigation of chronic arsenic toxicity needed integrated approaches of attempting to reduce arsenic entry into the food chain on the one hand and reduction of safe limits of arsenic in drinking water on the other.

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