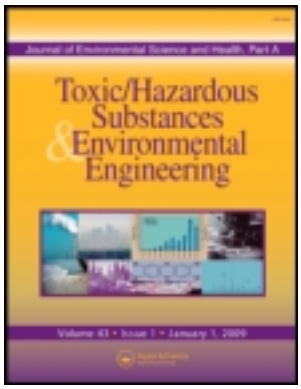

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Evaluation of dietary arsenic exposure and its biomarkers: A case study of West Bengal, India

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Evaluation of dietary arsenic exposure and its biomarkers: A case study of West Bengal, India

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Few reports are available that characterize daily arsenic exposure through water and diet among people living in groundwater-contaminated regions and correlate it with biomarkers. The present study describes the total individual arsenic exposure and arsenic level in urine and hair of such an arsenic-exposed population in West Bengal. Demographic characteristics and the total daily arsenic intake through water and diet were determined in 167 (Group-1 participants selected from arsenic endemic region) and 69 (Group-2 participants selected from arsenic non-endemic region) in West Bengal. Out of 167 Group-1 participants 78 (Group-1A) had arsenical skin lesions while 89 Group-1B) had no such lesion. Arsenic level in water samples as well as diet, urine and hair samples, collected from all the individual participants, were estimated. The mean value of estimated total arsenic content from water and diet was 349 (range: 20–1615) μg/day in 167 (Group-1) participants living in As endemic region [As in water: mean value 54 (range:BDL-326) μg/L] and 36 (range:12–120) μg/day in 69 (Group-2) participants living in As non-endemic region (As in water: below detection level (BDL), < 0.3 μg/L). Estimated mean arsenic level in urine in these two groups of participants was 116 (range: 6–526) μg/L and 17 (range: BDL-37) μg/L and in hair was 1.0 (range: 0.22–3.98) mg/Kg and 0.16 (range: 0.06–0.37) mg/Kg, respectively. Multiple regressions analysis in Group-1 participants showed that total arsenic intake was associated significantly with urinary and hair arsenic level. The estimated regression coefficient was 0.0022 (95% confidence interval, C.I: 0.0016, 0.0028; $P < 0.001$) and 0.0024 (95% C.I: 0.0021, 0.003; $P < 0.001$), respectively. In sub group analysis, higher median urinary arsenic value relative to arsenic intake through water and diet was observed in 78 Group-1A subjects with skin lesion compared to urinary arsenic value in 89 Group-1B subjects without skin lesions, though there was a marginal difference of median total arsenic intake in these two groups. This study showed that significant elevation of arsenic level in urine and hair was associated with elevated arsenic intake through water and diet in people living in arsenic endemic region (Group-1), while these values were low in people living in non-endemic region (Group-2). Those with skin lesions were found to have higher arsenic in urine and hair compared to those without skin lesion with similar arsenic intake through water and diet.

Keywords: Arsenic intake in exposed and unexposed population, arsenic in urine with skin lesion, arsenic in hair, total arsenic exposure.

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] There is increasing evidence of elevated rice grain arsenic levels in regions of West Bengal and Bangladesh where paddy fields are irrigated with arsenic-rich water.[2–5] On the other hand arsenic content in rice grown in region without groundwater contamination was found to be low.[6–8] Arsenic contamination of vegetables grown on soils irrigated with arsenic contaminated water has also been reported.[9–11] 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

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by many investigators.[6,9,12–14] However, few studies have reported the total arsenic intake among individuals living in regions without groundwater arsenic contamination in the region. Arsenic levels in urine, hair, and nail are important biomarkers of arsenic intake.

Total arsenic level in urine has been used as a biomarker of recent arsenic exposure, while that in hair and nails have served as biomarkers of chronic exposure.[15–21] Reports of the correlation of arsenic exposure through water and diet and arsenic level in biomarkers are available from regions with low arsenic content in drinking water.[22,23] To date, no report comparing data on total arsenic intake from water and diet and arsenic level in the urine and hair samples in participants living in regions with and without high arsenic contamination in groundwater has been available.

The current study was therefore undertaken to ascertain the total daily arsenic intake through drinking water and diet and correlate this value with the arsenic level in urine and hair samples in participants living in arsenic endemic (Group-1) and non- endemic (Group-2) regions of West Bengal. It also sought to determine whether total arsenic intake in Group-1 participants with and without skin lesion reflected similarly with the arsenic level in these biomarkers.

Materials and methods

Study design

The study was carried out in West Bengal, India. Two hundred and eight participants (Group-1) were recruited from six villages in two arsenic-affected blocks in Nadia district where groundwater was being used for irrigation purpose and 100 control participants (Group-2) were recruited from one arsenic non-contaminated village in Hoogly district (arsenic level in groundwater was below detection level). The 208 participants (Group-1) were selected from a population of 900 residents belonging to 212 households (4% of total households in the selected villages) who had been identified in a previous cross-sectional study. 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.[24]

The 100 Group-2 (control) participants were age and sex matched with those belonging to Group-1. Subjects of the first group consisted of 108 arsenicosis cases (Group-1A) with typical arsenical skin lesions of pigmentation and/or keratosis, and 100 individuals without arsenical skin lesions (Group-1B). The criteria for classifying keratosis and hyperpigmentation as arsenic-caused skin lesions were as follows. Keratosis had to involve 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.[21,25] 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 for 24 h was determined for each participant in both groups. Arsenic levels in biomarkers such as urine and hair were also determined for each participant. 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, Govt. of India.

Field study

Information from each participant was collected on demographic and social characteristics, occupation, (broadly grouped as in Gopalan et al.[26] into sedentary and moderate workers) and addiction to smoking, alcohol or usage of other tobacco products. In this study addiction was assessed by taking into account the history of smoking, drinking and tobacco chewing in field visits. Addiction was considered to be present if the participant had the daily habit of using any of these substances for prolonged period, failing to stop even they wanted to do so. 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, urine and hair

Water samples were collected from the present drinking and cooking water sources of each family, as well as those from previous water sources when available, in certified metal free containers. Total daily water consumption by a participant was determined from self-report on the number of glasses (250 mL capacity) of water the person consumed in a 24-h period. First morning void urine samples were also collected from participants in certified metal-free containers. Both the water and urine samples were kept in an icebox before leaving the field and stored at -20°C. In the collection of hair samples, a bunch of whole length hair was cut from the base of the scalp of each participant by a stainless steel blade and kept in a plastic packet. Participants with insufficient scalp hair were excluded from this study. All these samples were collected on the same day of diet collection and stored according to standard protocol of WHO (2005) until further analysis.[21]

Assessment of diet intake

Food samples were collected by duplicate portion sampling method.[13] 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-hour diet intake was formulated. Diet questionnaire was previously validated by similar dietary study on nutrient intake in arsenic exposed population in West Bengal.[27] 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 by the serving woman was recorded.

To estimate the amount of food consumed by a participant in the family, duplicate portions of the cooked items in each meal was collected in a measuring bowl of known 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 and collected together 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. Dry food items consisted of, a) dry cereals (puffed rice, flaked rice, biscuits, etc., b) fried pulses (motor, chola, etc.) and f) raw fruits (banana, mango, etc.).

Duplicate portions of each food category were taken in each meal and collected together in separate sampling packets by the dietitician. 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. Full 24-h diet samples could be collected from 167 out of 208 (Group-1) participants from the arsenic-contaminated region and from 69 out of 100 (Group-2) control participants living in the village without such contamination.

Total arsenic analysis

Arsenic levels in water, urine and hair samples were measured using an Atomic absorption spectrophotometer with a flow-injection hydride generation system (Perkin Elmer A Analyst 400, Waltham, MA, USA).[18] The lower limit of detection determined at the 90% confidence level was 0.3 μg/L. For dealing with the possibility for exogenous arsenic contamination, the hair samples were first washed with distilled water, then with de-ionized water and finally with acetone as recommended by the International Atomic Energy Agency.[28]

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.[29] For arsenic analysis, the dry samples were crushed and part of each sample (1.0 gm) 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 same way preparation of NIST 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 Atomic absorption spectrophotometer (Perkin-Elmer A-Analyst 400) equipped with a flow injection hydride generation system (FIAS-100, Perkin-Elmer). Quality assurance included same way preparation of NIST urine sample SRM-2670, in each time of sample batch run, recovery percentages varied from 95 to 97%. Coefficients of variation of analysis of arsenic was 50.84% in Group-1 and 49.08% in Group-2 in food; 118.02% in Group-1 and 472.48% in Group-2 in water; 90.21% in Group-1 and 64.36% in Group-2 in urine and for hair samples it was 68.99% in Group-1 and 38.08% in Group-2.

Statistical methods

The daily arsenic intake of each individual from drinking water, milk and cooked and dry food was estimated as follows. The arsenic intake from drinking water (μg/day) was calculated by multiplying the arsenic concentration in drinking water of the current drinking source (μg/L) by the water consumption rate (L/day). Arsenic intake from milk was similarly estimated. The daily arsenic intake from each cooked wet food category (μg/day) was calculated by multiplying the arsenic concentration in each wet food category (μg/Kg wet wt.) by the 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). Descriptive statistics were calculated, including a mean, standard deviation, median and interquartile ranges.

A multiple regression was fitted to urinary and hair arsenic with total arsenic intake as the exposure, occupation, presence of skin lesions, and BMI as potential confounders. All covariates (including age, sex and addictions) were screened to determine whether or not they were significant risk factors or confounders. These were initially included in the regression but later dropped as they did not appear to confound the association of interest. The urinary and hair arsenic values were log transformed as the data

was found to be positively skewed. Multiple R-squared and adjusted R-squared coefficients were used to study model fit. Analysis of variance tests were conducted to determine the explanatory power of various risk factors and confounders. The software package used for statistical analysis was R, version 2.13.[30]

Results and discussion

Study group characteristics

There was no difference in age, sex and occupation among the 167 (Group-1) participants living in regions with groundwater arsenic contamination and the 69 (Group-2) participants living in the region without such contamination in West Bengal (Table 1). Border line statistical significance was observed among the underweight participants between the two groups. There was no difference in smoking habit between the two groups. However, 18 participants belonging to Group-1 compared to two participants among the Group-2 had a history of tobacco chewing ($P < 0.05$).

Water and diet consumption

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' diets in both groups (56% in Group-1 and 65% in Group-2 participants) (Table 2). Similar observations were made by others who had carried out dietary studies on people living in the Indo-Bangladesh subcontinent.[9,13,14,31,32]

Table 1. Baseline characteristics of study participants living in arsenic endemic region with groundwater arsenic contamination (Group-1) and non-endemic region without such contamination (Group-2), West Bengal, India.

	(Group 1) (n = 167)		*(Group 2) (n = 69)*		
	n	*(%)*	*n*	*(%)*	*P-value*
Age in years:					
15–29	28	16.77	14	20.29	>0.05
30–44	70	41.92	31	44.93	>0.05
45–74	69	41.31	24	34.78	>0.05
Sex:					
Male	106	63.47	42	60.87	>0.05
Female	61	36.53	27	39.13	>0.05
Occupation					
[1]Sedentary	26	15.57	9	13.04	>0.05
[2]Moderate	141	84.43	60	86.96	>0.05
Addiction					
Smoking	48	28.74	24	34.78	>0.05
Tobacco chewing	18	10.78	2	2.90	<0.05
BMI Classification					
Underweight (<18.50)	55	32.93	14	20.29	0.05
Normal (18.50 - 24.99)	100	59.88	45	65.22	>0.05
Overweight (≥25)	12	7.19	10	14.49	>0.05

[1]Sedentary–Teacher, tailor, businessman, student, shopkeeper, hawker, housewife, clipmaker, cook, retired personnel.
[2]Moderate–Farmer, agricultural labour, industrial labour, van driver, temporary construction worker for hosting ceremony, mason.

Daily arsenic intake through drinking water and diet

Mean arsenic level in current drinking water source for the 167 Group-1 participants was 54 μg/L, (range:BDL-326 μg/L) while that of the 69 Group-2 participants was below the detection limit (BDL, < 0.3 μg/L).Total daily arsenic intake from diet was 165(range 20–479) μg/day in Group-1 while 36 (range:12–120) μg/day in Group-2 participants ($P < 0.001$) (Table 3). Arsenic intake from diet in Group-1 participants from Nadia in the present study was found to be similar to the values of arsenic intake through diet (171 and 189 μg /day) as reported in people living in two blocks in Murshidadad, another adjacent district of West Bengal with groundwater arsenic contamination.[9]

Total arsenic content in water and diet taken daily by Group-1 participants was 349 (range: 20–1615) μg/day. In comparison, total average arsenic intake through water and diet in an arsenic exposed population in Bangladesh was reported to be 174 μg/day in 47 women by Kile et al.[14] and 551 μg/day in 19 men and women by Watnabe et al.[12] The reason for such variation of results might be due to differences in the contribution of total arsenic intake through drinking water or due to regional variation of study populations. Ninety-four (43.7%) participants belonging to Group-1 were found to be drinking arsenic-safe (As in water,<50 μg/L, Permissible limit in India) [33] water currently. Total arsenic intake from water and diet combined in this Group was 177 (range: 20–441) μg/day of which arsenic from diet constituted 154 (range: 20–380) μg/day (data not shown).

Correlation of arsenic level in water and diet consumed by participants and arsenic level in biomarkers (urine and hair)

Estimated arsenic value in urine in the 167 Group- 1 participants was found to be high, (mean: 116,range: 6–526) μg/L, while it was low, (mean: 17, range: BDL-37μg/L) in the 69 Group-2 participants, ($P < 0.001$). Arsenic level in hair was also elevated (mean: 1.0, range: 0.22–3.98 mg/Kg) in the former group but low in the latter group (mean: 0.16, range: 0.06–0.37 mg/Kg), ($P < 0.001$). This study documented significantly low level of arsenic exposure through diet in population with low arsenic level in groundwater in West Bengal with corresponding low arsenic level in urine and hair. Mean arsenic level in urine and hair in 94 participants belonging to Group-1 drinking arsenic safe (<50 μg/L) water was 119 (range: 6–526.0) μg/L and 0.77 (0.18–3.0) mg/Kg, respectively [Data not shown]. Even

Table 2. Daily consumption rates of water and various food categories by Group-1 and Group-2 participants.

	Group-1, n = 167			*Group-2, n = 69*			
Food Items (g/Day)	*n*	*Mean*	*Range*	*n*	*Mean*	*Range*	*P-value*
Raw Rice (d. w.)	167	458	35–945	69	447	66–966	>0.05
Cooked Rice (w. w.)	167	1340	100–2900	69	1359	200–3000	>0.05
[1]Dry Cereals (d. w.)	49	24	5–55	31	42	5–200	<0.01
[2]Chapati (w. w.)	34	212	40–800	25	192	40–400	>0.05
[3]Cooked Pulses (w. w.)	55	141	5–500	8	105	60–120	>0.05
[4]Cooked Vegetables (w. w.)	167	281	20–740	69	272	60–710	>0.05
Milk (w. w.) (L/Day)	24	126	50–250	15	93	12–175	>0.05
Cooked Meat/Chicken (w. w.)	26	82	20–200	–	–	–	–
[5]Cooked Fish (w. w.)	72	48	2–165	31	40	15–110	>0.05
[6]Cooked Egg (w. w.)	32	39	5–90	19	23	10–60	>0.05
[7]Fruit (w. w.)	5	75	50–100	–	–	–	–
Avg. Water Intake (M)L/Day	106	3.86	1–6.5	42	3.64	2.5–5	>0.05
Avg. Water Intake (Fe)L/Day	61	2.62	1–4.7	27	3.14	2–5	>0.05

d. w: Dry wt.; w. w: Wet wt.
[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', 'chal kumra', 'kochu lati' '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.

after lowering arsenic level in drinking water to <50 μg/L (permissible limit in India),[33] significant arsenic exposure occurred through water and diet, reflected by elevated level of arsenic in urine, in people living in arsenic endemic region. One earlier report from West Bengal also showed fluctuations of arsenic content in urine even after supplying arsenic safe water for drinking for two years among a cohort of people living in the district of South 24 Parganas (West Bengal).[34]

Establishment of correlation of biomarkers with the skin lesions of arsenicosis

Among the 167 participants belonging to Group-1, 78 (Group-1A) had arsenical skin lesions while 89 (Group-1B) had no skin lesions. In sub group analysis, higher median urinary arsenic value relative to arsenic intake through water and diet was observed in 78 Group-1A subjects with skin lesions compared to urinary arsenic value in 89

Table 3. Summary statistics and percentile distribution of arsenic in drinking water, diet samples urine and hair samples in Group-1 and Group-2 participants studied.

Group-1, n = 167	*Mean*	*SD*	*Median*	*Min*	*Max*
Arsenic content of current water: (μg/L)	54	63.32	26	BDL	326
Daily As intake through water only:(μg/day)	185	227.73	100	0	1258
Daily As intake through Diet only :(μg/day)	165	83.73	150	20	479
Daily As intake through water & Diet:(μg/day)	349	261.12	292	20	1615
Total As intake /kg body wt/day :(μg/Kg/day)	6.93	4.99	6.05	0.39	28
Urine As level :(μg/L)	116	104.32	88	6	526
Hair As level::(mg/Kg)	1.00	0.69	0.84	0.22	3.90
Group-2, n = 69					
Daily As intake through water: (μg/day)	36	17.32	32.35	12	120
Daily As intake through water & Diet:(μg/day)	36	17.32	32.25	12	120
Urine As level :(μg/L)	17	10.70	17.00	BDL	37
Hair As level:(mg/Kg)	0.16	0.062	0.15	0.06	0.37

BDL = concentration below detection level.

Table 4A. Multiple regression of log urinary arsenic on total arsenic (water and food) exposure in participants in As Endemic Area ($n = 167$), West Bengal, India.

Parameters	*Reg. Coeff*	*95% LCI*	*95% UCI*	*P-value*
(Intercept)	4.9883	3.9737	6.0029	<0.001
Total arsenic intake	0.0022	0.0016	0.0028	<0.001
(Occupation = moderate)	0.0476	−0.3777	0.4729	>0.05
(Occupation = sedentary)	0.3976	0.0187	0.7765	<0.05
Skin disease present	1.056	0.7106	1.4014	<0.001
BMI	−0.0581	−0.1077	−0.0085	<0.05

Multiple R-squared: 0.3095, Adjusted R-squared: 0.2944.

Group-1B subjects without skin lesions, though there was only marginal difference in median value of total arsenic intake between these two Groups (Figs. 1a, 1b). The results from the multiple regressions conducted on the 167 Group-1 participants are reported in Table 4A and 4B.

Total arsenic intake was found to be significantly positively associated with urinary arsenic value (Table 4A). The estimated regression coefficient was 0.0022 (0.0016–0.0028; $P < 0.001$). Thus, a 10-unit increase in total arsenic intake resulted in a 1.022-fold increase in average urinary arsenic content. Other significant risk factors and confounders included were occupation and BMI. Those with a sedentary lifestyle excreted higher amounts of urinary arsenic as compared to those with an active lifestyle. Those with higher BMI reported lower arsenic excretion. Those with skin lesions were found to excrete significantly more arsenic in urine. The corresponding regression coefficient was 1.056 (0.7106–1.4014; $P < 0.001$). Thus, those with skin lesions excreted on average 2.9 [= exp (1.056)] (2.04–4.06) times as much urinary arsenic as compared to those without skin lesions with the same arsenic intake.

Total arsenic intake was also found to be significantly positively associated with hair arsenic content (Fig. 1c). The estimated regression coefficient was 0.0024 (0.0021– 0.0028; $P < 0.001$) (Table 4B). Thus a 10-unit increase in total arsenic intake resulted in a 1.024-fold increase in average hair

Table 4B. Multiple regression of log hair arsenic on total arsenic (water and food) exposure in participants in As Endemic Area ($n = 167$), West Bengal, India.

Parameters	*Reg. Coeff*	*95% LCI*	*95% UCI*	*P-value*
(Intercept)	−1.1412	−1.7501	−0.5322	<0.001
Total arsenic	0.0024	0.0021	0.0028	<0.001
(Occupation = moderate)	−0.1166	−0.3679	0.1347	>0.05
(Occupation = sedentary)	0.0623	−0.1632	0.2877	>0.05
Skin disease present	0.507	0.3016	0.7123	<0.001
BMI	0.0082	−0.0214	0.0379	<0.05

Multiple R-squared: 0.4857, Adjusted R-squared: 0.4743.

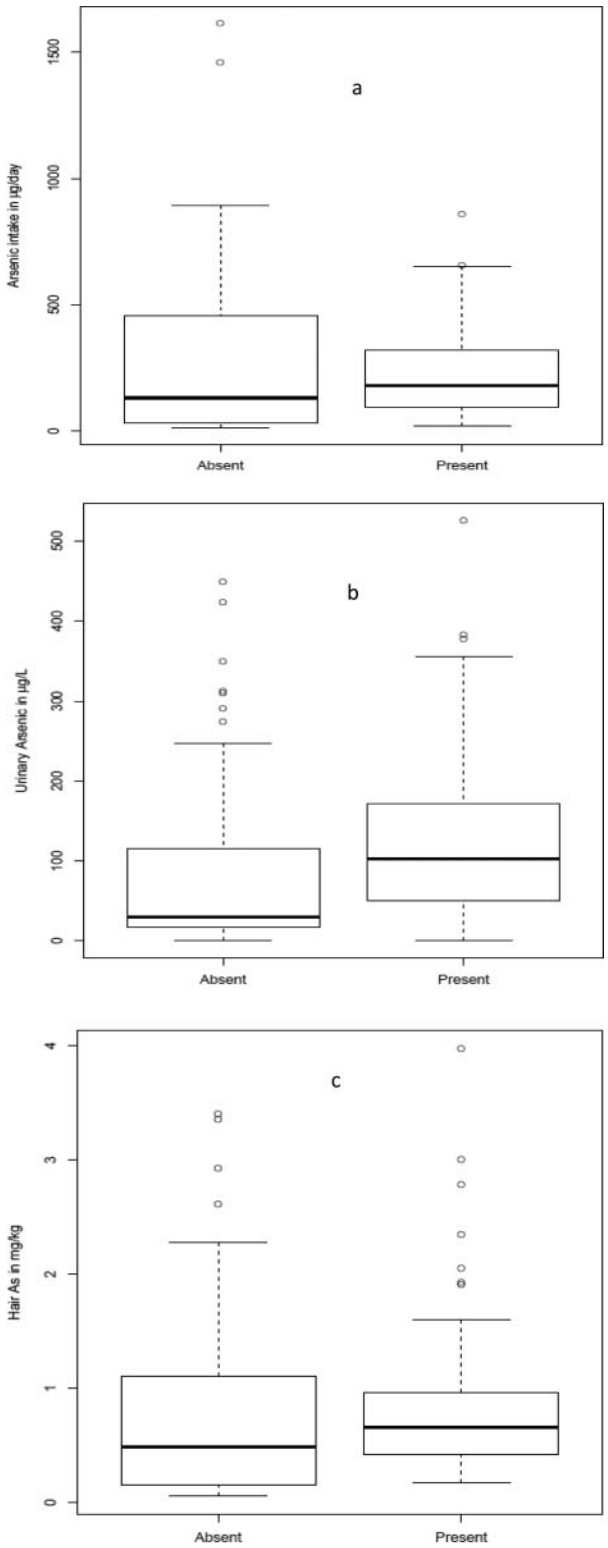

Fig. 1. Box plot: Differential arsenic value in urine (μg/L) and hair (mg/Kg) in relation to total arsenic exposure (μg/day) in arsenic exposed participants with and without skin lesion. a) Total Arsenic exposure in μg/day, b) Arsenic level in urine in μg/L, and c) Arsenic level in hair in mg/Kg, West Bengal, India, 2008–2009. Box, 1st and 3rd quartiles; line, median; whisker length, 1.5 (Q3-Q1); circles, outliers.

arsenic content. No significant variation of arsenic value in hair was found in regard to difference in occupation and BMI. Those with skin lesions were found to have significantly more arsenic in hair. The corresponding regression coefficient was 0.507 (0.3016– 0.7123; $P < 0.001$). Thus, those with skin lesions had on average 1.66 (1.35–2.01) times as much hair arsenic as compared to those without skin lesions for the same level of arsenic intake.

Total arsenic level in urine had often been used as a biomarker of recent arsenic exposure.[16,35,36] A report from Bangladesh highlighted that urinary arsenic may be a strong predictor of skin lesions than arsenic in drinking water in the population.[37] No data are available correlating arsenic intake through water and diet and arsenic in urine and hair in this region. In the present study, arsenic level in urine was found to be higher (>50 μg/L, value considered by WHO,[21] as evidence of recent As exposure) irrespective of whether the arsenic exposed people were currently drinking arsenic contaminated water or not.

However in the arsenic unexposed group (Group-2), urinary arsenic levels were low (BDL-37 μg/L). It was interesting to observe that participants with skin lesions excreted much higher amount of urinary arsenic compared to those without skin lesion relative to total arsenic exposure through water and diet. Further studies are needed to ascertain the reason for this differential arsenic excretion in urine in people with and without arsenical skin lesions in an arsenic endemic region.

Hair is an important biomarker of chronic arsenic exposure. In people with no known exposure to arsenic, the concentration of arsenic in hair is generally 0.02–0.2 mg/kg.[15,17] Arsenic level in hair in this study was found to be elevated in arsenic-exposed people (more with those with skin lesion) but low in unexposed subjects. In the only report correlating arsenic exposure through water and diet with biomarkers of chronic arsenic by Kile et al.[38], the average total daily arsenic intake was found to be significantly correlated with toenail arsenic concentration.

This is the first report in which two biomarkers—urine (indicator of current arsenic exposure) and hair (indicator of both present and past arsenic exposure) have been correlated with quantities of arsenic intake through water and diet in people living in arsenic endemic and non endemic region of West Bengal. In this study it was seen that even after lowering arsenic level in drinking water to <50 μg/L (permissible limit in India), significant arsenic exposure occurred through water and diet, reflected by elevated level of arsenic in urine, in people living in arsenic endemic region. Further, comparison of biomarker response has been made in people with and without arsenical skin lesion having similar exposure of arsenic through water and diet. Those with skin lesions had much higher level of arsenic in urine and hair compared to those without skin lesions with similar arsenic intake.

There was some limitation in the assessment of arsenic level in the diet in the current study as some of the families cooked their diet in arsenic contaminated water. This resulted in observation of higher values of arsenic in cooked diet in these participants. Further to estimate the arsenic intake from drinking water, we assumed that the subjects drank water only from the collected drinking water sources from home and neighborhood. This might have introduced error, probably underestimation, in the estimated arsenic intake through water. Further, in this study more people belonging to the exposed group were chewing tobacco compared to the unexposed group. However, this variation in the history of tobacco chewing would not possibly have a major influence on the effect of arsenic in diet and biomarker.

The strength of the study undertaken was that a good number of participants (belonging to both sexes) were included in estimating the total daily intake of arsenic through water and diet and in comparing the values in biomarkers such as urine and hair in people (age- and sex-matched) living in a region with and without groundwater arsenic contamination in West Bengal. Further, inclusion of a significant number of participants drinking arsenic safe and unsafe water and with and without skin lesion from arsenic endemic region helped us to find out biomarker response in these different subgroups. Studies are needed to understand the reason for our finding of higher concentration of arsenic in urine and hair in arsenic exposed people with skin lesion compared to those without skin lesion having similar arsenic exposure.

Conclusions

The current study showed significantly high dietary arsenic intake in people living in the Nadia district of West Bengal where contaminated groundwater was used for irrigation purpose, but significantly low in the region of Hoogly, where groundwater was uncontaminated. Even after lowering arsenic level in drinking water to <50 μg/L (permissible limit in India), significant arsenic exposure occurred through water and diet, reflected by elevated level of arsenic in urine in people living in the arsenic-endemic region studied. Those with skin lesions were found to have a higher level of arsenic in urine and hair compared to those without skin lesion.

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